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Evaluation of molecular techniques in prediction and diagnosis of cytomegalovirus disease in immunocompromised patients

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Abstract

Evaluation of molecular techniques in prediction and diagnosis of cytomegalovirus disease in immunocompromised patients

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Objectives: To evaluate selected molecular tests in diagnosis and screening of cytomegalovirus (CMV) infection in immunosuppressed patients.

Design: Clinical and cost-effectiveness were assessed through a prospective two-stage trial of CMV screening regimes in a routine service setting. Different molecular test results were fed back to clinicians in each stage, plus antigenaemia results. The technical performance of the molecular methods was assessed through an independent masked comparison of each molecular test against the established (antigenaemia) test. Scientists performing a particular test were blind to the other test results for that sample. Diagnostic and therapeutic impact were recorded prospectively for all tests, to include any effect on diagnostic certainty, changes to CMV therapy and any other reported impact on patient management. The cost of each test was estimated under different laboratory conditions. Prospective patients undergoing CMV screening were compared with consecutive historical controls in the same unit. Towards the end of the study, a survey of all UK virology laboratories was undertaken to identify current CMV screening practice and test preferences. In addition, all UK renal transplant surgeons and haematology transplant centres were surveyed in order to identify current clinical practice and perceptions of the benefits of CMV screening.

Setting: Study patients were recruited from University Hospital Wales (UHW), Cardiff. Staff in the Cardiff Public Health Laboratory Service virology laboratory performed the tests.

Participants: A consecutive series of transplant patients was recruited to the prospective study over a 42-month period, totalling 98 renal and 140 haematology patients. A consecutive series of historical controls was identified, with 199 renal and 136 haematology patients who underwent transplants in the UHW during the 29 months prior to the prospective CMV screening trial.

Interventions: A predefined CMV screening protocol was applied to all patients in the prospective trial. Renal patients were tested every 4 weeks until 16 weeks post-transplant (five tests in total). Haematology patients were tested every 2 weeks until 12 weeks post-transplant, and then every 4 weeks until 24 weeks (10 tests in total). The assays used for CMV screening were as follows: non-molecular test, (1) pp65 antigenaemia assay; molecular tests, semi-quantitative in-house polymerase chain reaction (PCR), (2) single-round (PCR1) and (3) two-round, nested (PCR2); and qualitative commercial tests, (4) Roche Amplicor Assay (Amplicor) and (5) pp67 NASBA assay (NASBA).

Main outcome measures: Test failure rates, sensitivity/specificity values and positive predictive value (PPV) and negative predictive value (NPV) were measured for each assay. The laboratory cost of undertaking various CMV tests was measured and other NHS costs associated with false-positive or false-negative test results were estimated. The likelihood of CMV disease and the likely impact of positive or negative test result on therapy and further investigations were recorded. On receipt of the test result, interim outcome measures were recorded to include the impact of test result on diagnostic certainty, changes to planned patient management (e.g. therapy, investigations) and perceived benefit. All definitive diagnoses of CMV disease, prescribing of CMV therapy and interim patient outcome at the end of the screening period were recorded.

Results: In haematology and renal transplant patients, all tests had a similar NPV (0.976–0.997 and 0.935–0.995, respectively) when used in CMV screening. PCR1 is the least expensive molecular test

(£7.80–13.70). Commercial tests, NASBA and Amplicor, are both more expensive (£22.50–34.70 NASBA; £23.20–29.20 Amplicor). Antigenaemia costs £12.50–27.40 depending on staff grade and batch size. Quantitative PCR (COBAS) is the most expensive at around £50 per sample. No clear link between screening test results and CMV prescribing was detected; clinicians appear to consider screening results in the context of other factors. There was no evidence that the introduction of CMV screening led to reductions in CMV deaths or improved transplant success rates. For cost per positive test result, PCR1 was the most cost-effective screening test on this indicator (renal patients £116 per true positive, haematology patients £518). Antigenaemia was the least cost-effective screening test (renal patients £643 per true positive, haematology patients £2475). Cost-effectiveness analysis and cost per 'beneficial result' (as judged by clinicians) confirmed that PCR1 remained the most cost-effective test. Modelling outputs for targeted screening protocols also supported this.

Conclusions: The study findings offer some evidence that a CMV screening regime is more cost-effective than diagnostic testing alone, based on the cost per true positive detected and interim outcome such as changes in patient management. However, the study was unable to demonstrate any benefits in terms of longer term patient outcomes. If CMV screening is introduced, the use of antigenaemia pp65 is clearly less cost-effective than the use of molecular tests. The study identified the optimum test for CMV screening as an in-house molecular test (single-round PCR test). This test was less costly to perform and also resulted in lower costs linked to false positives and negatives than other tests. The in-house, semi-quantitative test was two to three times more cost-effective than the commercial molecular tests assessed; however changes to European Union legislation may mean that it may not be feasible to use in-house tests. The use of targeted screening (limiting CMV screening to high-risk transplants) as opposed to universal screening offers a significant improvement in the cost-effectiveness ratio for haematology transplant patients, but has limited impact in the case of renal transplants. Economic analyses could be expanded to model the cost-effectiveness of more frequent screening tests (as reported nationally), and screening in other 'at risk' groups. Subgroup specific disease groups should be investigated across a larger population to allow more accurate modelling of the impact of CMV screening on disease progression. Further studies of CMV screening programmes should address a range of outcome measures, including patient outcomes.

Contents

List of abbreviations

BMT	bone marrow transplant
CI	confidence interval
CMV	cytomegalovirus
CT	computed tomography
DEAFF	detection of early antigen immunofluorescent foci
GVHD	graft versus host disease
HAART	highly active antiretroviral therapy
ICER	incremental cost-effectiveness ratio
IVIg	intravenous immunoglobulins
LOS	length of stay
MHRA	Medicines and Healthcare Products Regulatory Agency
MRI	magnetic resonance imaging
mRNA	messenger RNA
MUD	matched unrelated donor
NASBA	nuclei acid sequence-based amplification
NCCHTA	National Coordination Centre for Health Technology Assessment
NPV	negative predictive value
PBSCT	peripheral blood stem cell transplant
PCR	polymerase chain reaction
PHLS	Public Health Laboratory Service
PML	polymorphonuclear leucocytes
PPV	positive predictive value
QS	quantitative standard
RT	reverse transcribed
SCT	stem cell transplant
STARD	Standards for Reporting of Diagnostic Accuracy
UHW	university Hospital of Wales
UWCM	university of Wales College of Medicine

All abbreviations that have been used in this report are listed here unless the abbreviation is well known (e.g. NHS), or it has been used only once, or it is a non-standard abbreviation used only in figures/tables/appendices in which case the abbreviation is defined in the figure legend or at the end of the table.

Executive summary

Background

In individuals who have severely reduced immunity, cytomegalovirus (CMV) can cause serious and even fatal infection. Those at greatest risk from CMV infection include renal transplant recipients and patients who receive stem cell harvests or are bone marrow transplant recipients. Asymptomatic reactivation of CMV may occur with low levels of virus replication and no tissue damage. The difficulty for clinicians is to distinguish this type of innocuous presence of persistent virus from its active replication and disease production. The value of screening and diagnostic tests for CMV in different 'at risk' patient groups, and the best use of screening assays in predicting CMV disease and enabling pre-emptive therapy, represent an important area for health technology assessment.

Objectives

The objectives were to evaluate selected molecular tests in diagnosis and screening of CMV infection in immunosuppressed patients by

- measuring technical performance (test failure, sensitivity/specificity and turn-around time) for molecular methods versus the most commonly used non-molecular test (antigenaemia)
- determining the impact of CMV screening tests on diagnostic certainty and clinical management
- assessing the cost-effectiveness of CMV screening using molecular versus non-molecular tests and alternative testing protocols for the early identification of CMV infection.

Design

Clinical and cost-effectiveness were assessed through a prospective two-stage trial of CMV screening regimes in a routine service setting. Different molecular test results were fed back to clinicians in each stage, plus antigenaemia results. The technical performance of the molecular methods was assessed through an independent masked comparison of each molecular test against the established (antigenaemia) test. Scientists performing a particular test were blind to the other test results for that sample. Diagnostic and therapeutic impact were recorded prospectively for all tests, to include any effect on diagnostic certainty, changes to CMV therapy and any other reported impact on patient management. The cost of each test was estimated under different laboratory conditions.

Prospective patients undergoing CMV screening were compared with consecutive historical controls in the same unit.

Towards the end of the study, a survey of all UK virology laboratories was undertaken to identify current CMV screening practice and test preferences. In addition, all UK renal transplant surgeons and haematology transplant centres were surveyed in order to identify current clinical practice and perceptions of the benefits of CMV screening.

Setting

Study patients were recruited from University Hospital Wales (UHW), Cardiff. Staff in the Cardiff PHLS virology laboratory performed the tests.

Participants

A consecutive series of transplant patients was recruited to the prospective study over a 42-month period, totalling 98 renal and 140 haematology patients. It was planned to also recruit 40 patients with advanced HIV infection (CD4 $<100/mm^3$), but only seven were recruited owing to the success of new therapy [highly active antiretroviral therapy (HAART)]. Recruitment of AIDS patients was discontinued in agreement with the HTA programme.

A consecutive series of historical controls was identified, with 199 renal and 136 haematology patients who underwent transplants in the UHW during the 29 months prior to the prospective CMV screening trial.

Interventions

A predefined CMV screening protocol was applied to all patients in the prospective trial. Renal

patients were tested every 4 weeks until 16 weeks post-transplant (five tests in total). Haematology patients were tested every 2 weeks until 12 weeks post-transplant, and then every 4 weeks until 24 weeks (10 tests in total).

The assays used for CMV screening were as follows: non-molecular test, (1) pp65 antigenaemia assay; molecular tests, semi-quantitative in-house polymerase chain reaction (PCR), (2) single-round (PCR1) and (3) two-round, nested (PCR2); and qualitative commercial tests, (4) Roche Amplicor Assay (Amplicor) and (5) pp67 NASBA assay (NASBA).

Main outcomes measured

Diagnostic accuracy
Test failure rates, sensitivity/specificity values and positive predictive value (PPV) and negative predictive value (NPV) were measured for each assay.

Test costs
The laboratory cost of undertaking various CMV tests was measured and other NHS costs associated with false-positive or false-negative test results were estimated.

Clinical effectiveness
The likelihood of CMV disease and the likely impact of positive or negative test result on therapy and further investigations were recorded. On receipt of the test result, interim outcome measures were recorded to include the impact of test result on diagnostic certainty, changes to planned patient management (e.g. therapy, investigations) and perceived benefit. All definitive diagnoses of CMV disease, prescribing of CMV therapy and interim patient outcome at the end of the screening period were recorded.

Results

Diagnostic accuracy

Haematology transplant patients
All tests had a similar NPV (0.976–0.997) when used in CMV screening. Antigenaemia had the highest PPV (0.900), but a 25% failure rate. In-house PCR (first-round) had the highest PPV for a molecular test (0.815) and test failures were <1%.

Renal transplant patients
All tests had similar NPV (0.935–0.995). In-house PCR (first-round) had the highest PPV (0.965), with test failures <1%. Antigenaemia had a PPV of 0.939 in this patient group, with a 12% test failure rate.

Test costs
PCR1 is the least expensive molecular test (£7.80–13.70). Commercial tests, NASBA and Amplicor, are both more expensive (£22.50–34.70 NASBA; £23.20–29.20 Amplicor). Antigenaemia costs £12.50–27.40 depending on staff grade and batch size. Quantitative PCR (COBAS) is the most expensive at around £50 per sample.

Clinical effectiveness

Prospective study
Prospective data were collected via structured questionnaires completed by clinicians (2554 pre- and post-test). Clinical signs/symptoms when a screening test was requested were not related to CMV disease status, except for pyrexia ($p < 0.05$).

Renal clinicians were more likely ($p < 0.01$) to report that CMV screening results had been of benefit than were haematologists (72% vs 63%).

Ex ante haematology clinicians were significantly ($p < 0.01$) more likely to report that positive results would lead to a repeat CMV test request and other investigations (e.g. X-rays, CT/MRI, bronchoscopy); and to prescribing of CMV therapy ($p < 0.01$).

Recorded impacts on diagnostic certainty and patient management were relatively uncommon, but significant differences were observed between patient groups. Increases in diagnostic certainty were more likely in haematology patients: 13% haematology, 4% renal results ($p < 0.01$). Changes in patient management were even rarer, associated with <5% of test results. Initiation of CMV therapy was reported following 4% of results; further investigations following 3% results (significantly more likely ($p < 0.01$) for haematology patients); and avoidance of planned CMV therapy following <0.5% test results.

No clear link between screening test results and CMV prescribing was detected; clinicians appear to consider screening results in the context of other factors. For renal patients, 25% with CMV disease identified by screening tests were not prescribed ganciclovir and 10% with no disease received ganciclovir. For haematology patients, all those with CMV disease identified by screening were prescribed ganciclovir; 5% of negative patients also received ganciclovir. This pattern mirrors national survey responses, indicating that other factors are considered by clinicians (see national surveys below).

Historical controls
There was no evidence that the introduction of CMV screening led to reductions in CMV deaths or improved transplant success rates; one CMV-related death occurred during the screening period (haematology patient). No significant differences were detected in level of CMV disease (historical groups, 13% renal and 2.2% haematology; prospective study, 13% renal and 3.6% haematology). A significant increase was observed in the number of CMV diagnostic tests requested during the prospective screening trial.

Cost-effectiveness analysis (CEA)
Cost per positive test result
PCR1 was the most cost-effective screening test on this indicator (renal patients £116 per true positive, haematology patients £518). Antigenaemia was the least cost-effective screening test (renal patients £643 per true positive, haematology patients £2475). Antigenaemia diagnostic testing was less cost-effective than molecular (PCR1) screening on this parameter (renal patients £130 per true positive, haematology patients £1287).

When wider NHS costs were included, PCR1 remained the most cost-effective screening test (renal patients £116 per true positive, haematology £727). The nested in-house test (PCR2) was the least cost-effective of all tests owing to the high costs associated with false positives.

Incremental cost-effectiveness analysis
This confirmed that PCR1 remained the most cost-effective CMV screening test for renal and haematology patients.

Sensitivity analysis confirmed that PCR1 was the most cost-effective test for CMV screening.

Based on this outcome measure, CMV screening was more cost-effective in renal than haematology patients.

Cost per interim outcome measure
The cost per change in diagnostic certainty (laboratory costs and associated costs included) was £284 for renal and £134 for haematology patients. The cost per change in patient management was £993 for renal and £507 for haematology patients. Hence, based on these outcome measures, CMV screening appears to be more cost-effective in haematology than renal patients.

Cost per 'beneficial result' (as judged by clinicians)
PCR1 remained the most cost-effective test on this outcome measure. Cost-effectiveness ratios were calculated to be much more favourable for this measure: £16.54 per beneficial result for renal patients and £26.54 for haematology patients.

Value of screening
It was not possible to judge from these analyses whether the use of screening assays *per se* is worthwhile in either patient group.

National surveys
Laboratory testing
UK laboratories reported annual (2001) CMV test throughputs of 18–6776 samples; screening tests represented ≥ 75% of laboratory CMV workload. Some 28% of laboratories used antigenaemia and the remainder used PCR-based tests (one-third real-time quantitative PCR). Only 16% of laboratories expressed a preference for antigenaemia; the remainder preferred PCR tests, and were equally divided between real-time, other quantitative and qualitative PCR tests.

CMV screening protocols
Those reported nationally by laboratories and clinicians were similar to those introduced in the study, although testing was more frequent. For renal patients, weekly CMV screening tests (as opposed to 4-weekly) were undertaken for a period of 12–24 weeks post-transplant. For haematology patients, weekly or twice-weekly tests (as opposed to 2–4-weekly) were undertaken for 12–24+ weeks post-transplant.

Prescribing protocols
Fewer than half of renal transplant centres had a formal protocol to guide prescribing following CMV screening test results; most specify intervention if CMV disease is clinically suspected. Almost all (90%) haematology transplant centres reported a formal protocol, most requiring two positive tests before prescribing.

Individual clinician behaviour
If a patient tests positive after previously testing negative for CMV, two in three renal clinicians would prescribe for R–D+ transplant patients, one in three for other transplants (R+D–; R+D+; R–D–) [CMV serostatus (+/–); R = recipient, D = donor]. Haematologists would nearly all (80%) prescribe for an allograft patient, but only 20% for autografts.

CMV prophylaxis
Most renal clinicians (90%) would give prophylaxis to R–D+ transplants, fewer than one-quarter would prescribe for R+D+ or R+D– and none for R–D– transplants. No haematology transplant

centre reported giving prophylaxis to autografts or to R–D– allografts, but 20% might give prophylaxis to other allografts.

Targeted CMV screening
Only one in three renal centres target CMV screening (all screen R–D+ transplants); 90% of haematology centres limit CMV screening (all exclude autologous transplants, 60% do not screen R–D– allogeneic transplants and the remainder screen all allogeneic transplants).

Cost-effectiveness of targeted screening protocols
Modelling outputs for the following targeted screening regimes (as reported in national surveys) show that PCR1 remains the most cost-effective test in both types of patient:

- renal patients – screening of R–D+ group only
- haematology patients – screening of allogeneic transplants only, excluding R–D–.

The impact of targeted screening in renal patients is calculated to be limited (cost per true positive will fall from £116 to £98); a greater effect is predicted in haematology patients (cost per true positive falling from £727 to £170).

Conclusions

The study findings offer some evidence that a CMV screening regime is more cost-effective than diagnostic testing alone, based on the cost per true positive detected and interim outcome such as changes in patient management. However, the study was unable to demonstrate any benefits in terms of longer term patient outcomes.

If CMV screening is introduced, the use of antigenaemia pp65 is clearly less cost-effective than the use of molecular tests.

The study identified the optimum test for CMV screening as an in-house molecular test (single-round PCR test). This test was less costly to perform and also resulted in lower costs linked to false positives and negatives than other tests. The in-house, semi-quantitative test was two to three times more cost-effective than the commercial molecular tests assessed.

The use of targeted screening (limiting CMV screening to high-risk transplants) as opposed to universal screening offers a significant improvement in the cost-effectiveness ratio for haematology transplant patients, but has limited impact in the case of renal transplants.

Implications for the health service

CMV screening using antigenaemia pp65, as reported by a number of UK laboratories, is clearly less cost-effective than the use of molecular tests. The use of targeted screening for haematology patients, as reported by the majority of UK centres, is clearly worthwhile. For renal transplant patients, targeted (as opposed to universal) screening offers limited improvements in cost-effectiveness. Although in-house tests are more cost-effective than the commercial molecular tests assessed, it may not be feasible to use them. Owing to changes in European Union legislation, in-house molecular assays used by the NHS must be CE marked if, as in the present case, molecular diagnostic units test screening samples are sent from patients in other hospitals and primary care settings. In the future, health technology assessments may need to be confined to commercially available CE-marked *in vitro* diagnostic kits. It will be a challenge for NHS providers to develop any in-house assays to a point where they can be assessed.

Recommendations for further research

Economic analyses could be expanded to model the cost-effectiveness of more frequent screening tests (as reported nationally), and screening in other 'at risk' groups. Subgroup specific disease groups should be investigated across a larger population to allow more accurate modelling of the impact of CMV screening on disease progression. Further studies of CMV screening programmes should address a range of outcome measures, including patient outcomes.

In a rapidly changing area such as this, health technology assessment requires careful thought. A 'fast track' assessment approach may be required, otherwise advances in technology may compel the use of CMV assays for which clinical and cost-effectiveness data are unavailable.

Because of changes in European legislation, widespread use of in-house molecular assays in the NHS may be difficult in the future. Thought should therefore be given to including funding for CE marking of in-house assays that are found to be cost-effective in any future health technology assessments.

Chapter 1
Introduction and background

Introduction

Failure to diagnose cytomegalovirus (CMV) infection correctly in immunocompromised patients may lead to patient disability and even death, in addition to unnecessary costs for the NHS. Many diagnostic tests are slow and there is no 'gold standard' technique for accurate diagnosis. Manufacturers are now marketing a number of molecular tests for the detection of CMV nucleic acid (either DNA or RNA). Increasing numbers of NHS laboratories are considering the introduction of these molecular tests to replace or complement non-molecular methods. In addition, there is widespread use by laboratories of locally developed in-house molecular methods that have not necessarily been subjected to rigorous evaluation but which are less expensive than manufactured 'kit-based' assays.

There is incomplete research information on the technical performance of these various molecular tests compared with non-molecular tests, and inadequate information on their value in a service setting both in the diagnosis of infection and in predicting disease.

Cytomegalovirus

Human CMV is a member of the herpesvirus family. The virus is ubiquitous in the human population and persists throughout life after primary infection. Throughout much of the world CMV infection is acquired subclinically during childhood, but in some of the more affluent communities it tends to be delayed until an age when it is capable of doing considerably more damage. Primary infections during pregnancy can lead to severe congenital abnormalities in the foetus.

CMV infection is associated with significant disease and may be life-threatening in immunocompromised individuals; of all the herpesviruses, CMV is the one responsible for most morbidity and mortality in immunocompromised hosts[1]. Those at greatest risk from CMV infection include HIV-infected individuals, renal transplant recipients, patients who receive stem cell harvests and bone marrow transplant (BMT) recipients for the treatment of malignant disease, usually haematological. Opportunistic CMV infection following bone marrow transplantation is associated with particularly high morbidity and mortality rates.

Pathogenesis and epidemiology of cytomegalovirus infection and disease

Once infected with CMV, an individual carries the virus for life and may shed it intermittently in saliva, urine, semen, cervical secretions and/or breast milk. Up to 10% of people may be found to be shedding virus at any time, especially young children. The intermittent nature of CMV shedding and the fluctuations observed in antibody levels suggest that asymptomatic exacerbations occur occasionally throughout life. For example, reactivation occurs during pregnancy, rising markedly as term approaches. Hormonal factors may be at work here, but immunosuppression is generally the most powerful trigger.

CMV historically has been one of the commonest causes of blindness in AIDS patients in addition to pneumonitis and death in recipients of grafts, especially following allogeneic bone marrow transplantation. The virus can be isolated from >90% of patients profoundly immunosuppressed for organ or tissue transplantation. Such infections generally involve reactivation of a latent (or low-level chronic) infection that has been lying dormant in cells of either the donor or the recipient of the graft.

Relatively little is known about the pathogenesis of CMV infection and the mechanism of latency. During the viraemia (virus circulating in the blood) observed in acute infection, whether primary or reactivated, virus can be recovered from monocytes, polymorphs and, to a lesser extent, T lymphocytes. That these and other cells are potentially permissive has been confirmed by *in vitro* cultivation of CMV in such cells. However, it is almost impossible to reactivate CMV by cocultivation of leucocytes from healthy carriers with susceptible fibroblasts *in vitro*.

In summary, it is not yet clear which cell types constitute the principal reservoir of the viral

TABLE 1 *Outcome of CMV infection in transplant recipients*

Transplant type	Reported outcome
Solid organ	Direct symptomatology (e.g. febrile illness and hepatitis, gastrointestinal symptoms) Augmented immunosuppressive state Allograft dysfunction Decreased survival of graft and patient
Bone marrow and peripheral blood stem cell	Direct symptomatology (e.g. febrile illness and hepatitis) Immunopathology (e.g. pneumonitis) Bone marrow suppression
Adapted from Fox *et al*. (1995)[4] and Emery *et al*. (2000)[5]	

genome, or whether persistence is maintained by a continuous low-level chronic productive infection or by true latency in which episome (viral DNA not integrated into the host cell chromosomal DNA) copy numbers are maintained but expression of most genes is restricted, and no viral proteins made until immunosuppression releases immunological control.

Cytomegalovirus disease in immunocompromised patients

In healthy individuals, CMV virus causes little or no clinically significant infection, despite repeated reactivation. However, in individuals who have severely reduced immunity, CMV can cause serious and even fatal infection. *Table 1* highlights some of the reported outcomes for transplant patients and *Tables 2* and *3* illustrate reported CMV disease rates for different types of kidney and haematology transplants.

Detection of CMV disease

Clinical detection of CMV disease relies upon presentation of non-specific symptoms (e.g. pyrexia, leukopaenia, malaise) which may lead to inappropriate use of antivirals. As the anti-CMV drugs widely used are both toxic and expensive, their inappropriate use is unjustifiable as it can lead to possible loss of the transplant, prolonged hospital stay, delay in engraftment and systemic toxicity affecting particularly the bone marrow and liver.

In such circumstances, laboratory tests can clearly inform patient management. Traditional methods for laboratory diagnosis of CMV infection include serology, virus culture, modified culture (shell vial assay) and antigen detection. Culture may take up to 21 days to produce a positive result and is a relatively insensitive method whereas serology results are difficult to interpret, especially in immunocompromised patients whose ability to mount an immune response is, by definition, abnormal. Rapid diagnostic CMV methods such as the pp65 antigenaemia assay (which looks for CMV antigen inside circulating white blood cells) and amplification methods based on polymerase chain reaction (PCR) or nucleic acid sequence-based amplification (NASBA) for the detection of viral genome or messenger RNA (mRNA) are more sensitive than traditional methods.

Such sensitive methods may be used to detect virus switch from latency to replication prior to onset of disease and may prove beneficial for monitoring 'at risk' individuals to make the use of pre-emptive therapy possible. Pre-emptive therapy is the use of therapy to prevent the development of severe disease by stopping CMV replication at an early stage of the reactivation process. Equally important may be the value of a negative result and its use in prevention of inappropriate use of antivirals.

There is very little consistency in the molecular-based methods and strategies utilised by different UK centres for CMV screening and diagnosis in immunosuppressed patients.

In the three main groups of patients at risk of severe CMV disease (renal and stem cell transplant recipients and patients with AIDS), asymptomatic

TABLE 2 *Likelihood of CMV disease versus CMV status of donor (D) and recipient (R) (kidney transplant patients)*

Type of transplant	CMV status	Likelihood of CMV disease (%) (95% CI)
Kidney	R–D–	0 (0 to 15)
	R–D+	44[a] (29 to 61)
	R+D–	6 (2 to 16)
	R+D+	25[a] (17 to 37)

[a] Significantly different from D–R–.
Adapted from Fox *et al.* (1995)[4] and Emery *et al.* (2000).[5]

reactivation of CMV may occur with low levels of virus replication and no tissue damage. The difficulty for clinicians is to distinguish this type of innocuous presence of persistent virus from its active replication and disease production.

Study patients

The present project was commissioned by the NHS Health Technology Assessment (HTA) programme towards the end of 1998. The aim of the study was to provide reliable evidence, based on which clinicians, laboratory scientists and managers could make more rational choices about the use of CMV tests for diagnosis and screening in immunosuppressed patients. Three groups of patients were selected for examination:

- renal transplant patients
- patients who had received BMT or a peripheral blood stem cell transplant (PBSCT)
- patients with advanced HIV infection.

Although all these patient groups have a higher probability of developing CMV disease than other patient groups, the causes and likelihood of clinically significant CMV infection, and the periods during which individuals are particularly at risk, will differ depending on the group, as will the effect of any delay, or failure, in detecting infection. Details of these are presented below for each of the three patient groups. In turn, these factors will influence the potential value of improved CMV diagnosis and screening.

Renal transplant patients

In renal transplant patients, severe CMV disease is usually due to primary infection of the recipient from the donor kidney. Approximately 50% of donors will have a latent CMV infection, and a similar percentage of recipients will have been exposed to the infection when healthy. Transplant surgeons may decide to accept mismatching for CMV status of the donor and recipient when planning a transplant (because it allows them to match tissue more closely), assuming that they will be able successfully to detect and treat any CMV-related disease which may occur.

The period over which renal patients are particularly at risk of infection is reported to be the first 4 months post-transplant, with some controversy about the relative risk at the start and end of this period and a recognition that some risk of disease persists for many months post-transplantation in high-risk patients; in one summary of 16 studies of 1276 renal transplant patients, the rate of infection after transplantation varied from 59 to 100%, with a mean of 70% of patients.[1–4]

Primary infections, which have the most severe consequences, both for survival of the graft and the patient, have been reported in 83% of CMV seronegative (no previous exposure to CMV) patients who received kidneys from CMV seropositive (previously infected with CMV) donors. More recently reported data[4,5] on the likelihood of CMV disease in kidney transplant patients show variations depending on the CMV status of the donor (D) and recipient (R), as illustrated in *Table 2*.

Prior to the introduction of specific therapies for CMV, most primary infections led to loss of the transplanted organ. The most feared development is CMV pneumonitis, which has a mortality of up to 50% in renal transplant patients. Even in cases where the recipient has pre-existing immunity to the virus (CMV seropositive), infection has been reported to lead to loss of the kidney and even systemic infection with a fatal outcome.[1–4]

Bone marrow/stem cell transplant patients

For patients who receive a BMT or PBSCT, mostly for haematological malignancy, CMV has long been recognised as a major cause of morbidity after transplantation.[6–8] Transplants may be of two

TABLE 3 *Reported likelihood of CMV disease versus. CMV status of donor (D) and recipient (R) (haematology transplant patients)*

Type of transplant	CMV status	Likelihood of CMV disease (%) (95% CI)
Bone marrow	R–D– R–D+ R+D– R+D+	3 (1 to 11) 4 (1 to 21) 44[a] (27 to 63) 12 (7 to 28)

[a] Significantly different from D–R–.
Adapted from Fox *et al*. (1995)[4] and Emery *et al*. (2000)[5]

main types – allogeneic or autologous – and it is allogeneic transplant patients who are at most risk.

Recipients of an autologous transplant have their own stem cells infused into their blood. These cells are harvested during a period of disease-free remission and are stored so that later, if the patient relapses, they can undergo ablation of their leukaemic cells and regraftment with their own stored cells.

In contrast, patients who need an allogeneic transplant are those for whom remission cannot be induced and there are no leukaemia-free cells to transplant. These patients are treated in such a way as to kill all circulating blood cells, malignant and normal. The patient then receives healthy cells from another person, usually a related or 'matched' donor. Because the transplanted cells are from another person, the patient needs much more severe immunosuppression after their transplant to prevent 'graft versus host disease', a life-threatening condition in which the graft cells attack and destroy the normal healthy cells of the recipient.

CMV infection has been reported to occur in up to 40% of allogeneic transplant patients, depending on previous exposure to the virus of both the donor and recipient of the graft. For autologous transplant patients, the likelihood of infection is lower as these patients do not require long-term immunosuppression.

Table 3 presents some recent statistics[4,5] on the likelihood of CMV disease developing in allogeneic BMT patients.

In allogeneic patients, most severe disease is experienced by CMV seropositive recipients of a graft from a CMV naive donor (seronegative or no previous exposure to CMV). CMV reactivation in the patient is followed by a vigorous primary immune response by the donor cells, which can then cause tissue damage. This clinical situation is often made worse by exacerbated graft versus host disease (GVHD) as the donor cells become immunologically active, presenting clinically as fever, leukopaenia, hepatitis, oesophagitis, enteritis, retinitis or pneumonitis.[6,9] GVHD is a serious condition; as already described, increasing the level of immunosuppression in the recipient can treat it, but this is a dangerous strategy as it prevents the graft from establishing and reconstituting the patient's immune function. Most recipients of allogeneic stem cells will get some level of GVHD and the clinical spectrum is very wide, from minor skin and gastrointestinal symptoms to overwhelming organ failure and death.

The period over which bone marrow transplant patients are reported to be at particular risk of CMV disease is 2–6 months post-transplantation, with a much lower chance of developing CMV prior to that time.[7,8] CMV disease is considered and can occur at almost any time after transplantation, but the risk diminishes with time and new disease is rare more than 1 year post-transplant. Furthermore, it has been reported that in up to one-third of marrow transplant patients who develop CMV infection, the infection will progress to CMV pneumonia with an 85% mortality rate.[8,10,11]

Patients with advanced HIV infection

Patients with advanced HIV infections develop AIDS, at which time their immune system has been overwhelmed by the HIV virus and individuals have little or no ability to combat infection. Many of the most dangerous infections are those which arise from within the infected individual; an example is CMV, which has been latent and previously innocuous but which reactivates and causes disease as the person's ability to control the virus is compromised.

In such patients, the level of white blood cells is permanently low so that the increased risk of CMV

is constant once end-stage AIDS is reached. More than 25% of AIDS patients experience CMV disease during the progression of immunodeficiency, and post-mortem studies have shown that CMV disease occurs in 50–90% of AIDS patients. Disease presents as colitis, oesophagitis, hepatitis, encephalitis or particularly retinitis, which can lead to rapid blindness.[9,12,13] In addition to leading to blindness (if not treated promptly), CMV infection can lead to increased length of hospital stay, especially if generalised disease develops.

Since 1996 in the UK the use of highly active antiretroviral therapy (HAART) has changed the pattern of clinical disease seen in patients with longstanding HIV infection. Therapy has delayed progression to AIDS and the incidence of CMV disease has fallen. Deaths due to AIDS have fallen steadily during recent years.

Treatment of suspected CMV disease in immunocompromised patients

If CMV is suspected, the therapies available for treating the disease in immunocompromised patients need to be used with great care since they are both toxic and costly. Appropriate use of therapy should be guided by access to sensitive, specific and timely diagnosis of clinically significant CMV infection. However, because of the severe consequences resulting from infection, and the questionable reliability of current laboratory tests and other diagnostic investigations, clinicians may sometimes choose to treat patients based primarily on clinical symptoms. Balanced against this, inappropriate treatment for suspected CMV in the immunocompromised patient may have important consequences for patients' health and for healthcare costs.

Ganciclovir treatment

Ganciclovir is the most widely used drug for treating suspected CMV disease; it is also the most costly at about £2000 for a 3-week course of treatment. For the transplant patients studied, the treatment pattern used will vary depending on the type of transplant and other patient characteristics. Most patients receive at least 10 days of treatment, usually a 2- or 3-week course, and some patients require ganciclovir therapy for many weeks because of unresolving CMV disease. Treatment costs can, therefore, vary.

For renal transplant patients, treatment is usually 10–14 days in length with the dosage level dependent on renal function, and with a lower dose if renal function is poor. After 10–14 days, therapy then ceases. For haematology transplant patients, treatment is again usually 10–14 days in length but this is then followed by maintenance therapy (e.g. once a day) for a set time.

The route by which ganciclovir is administered will also vary, depending on the physical state of the patient and the type of transplant. For renal transplants, if the patient is physically unwell, ganciclovir is administered in an intravenous form; if the patient is well, oral ganciclovir is used. For haematology transplants, intravenous ganciclovir only is used.

Foscarnet treatment

The second-line drug for treatment of CMV disease is foscarnet, which costs about £1800 for a 3-week course of treatment. Foscarnet drug has a similar range of significant side-effects to those of ganciclovir, particularly gastrointestinal, cardiological, psychiatric, liver and renal toxicity. Acute renal failure can also occur, and the drug is also toxic to the bone marrow. Foscarnet is rarely used in Cardiff, most patients respond to ganciclovir. In non-responding patients, Foscarnet is usually added to ganciclovir rather than replacing it, so the cost and toxicity are additive. This salvage therapy has been successful in a very small number of patients.

CMV prophylaxis

Rates of CMV disease and patient outcome may be influenced by the use of prophylaxis or pre-emptive treatment regimens. It is well recognised that antiviral prophylaxis may alter the timing of related disease.

In Cardiff, a protocol for prophylaxis with ganciclovir was introduced for renal transplant patients towards the end of this study. For haematology transplant patients, prophylaxis was not used during the study period. These protocols therefore did not significantly influence the management of patients in the study.

Screening for CMV disease

In general, laboratory tests are requested by clinicians for one of three main reasons:

- As a screening test: to identify whether an infection is present although the patient exhibits no symptoms of the disease.

- As a diagnostic test: to confirm the presence of disease when the clinician suspects disease because of other clinical signs and symptoms.
- As a monitoring test: to monitor the progress of a disease and/or the effect of therapy once the disease has been identified by a diagnostic/ screening test.

In the case of CMV disease in immunocompromised patients, a fourth use for laboratory tests might be identified as follows:

- As a predictive test: to predict the **likelihood** that a patient will develop a disease.

The last mode of test use is similar to screening tests, in that the patient does not exhibit symptoms of the disease. However, unlike screening, the aim is not to identify disease in an asymptomatic patient, but instead to provide evidence on the risk of disease developing. In the present study the term 'screening' is used to describe both of these types of CMV tests carried out as part of a predefined surveillance protocol in post-transplant patients (i.e. screening and predictive tests), since these can be difficult to distinguish because they are only differentiated by the level of diagnostic certainty.

In all three groups of patients included in the study, asymptomatic reactivation of CMV can occur with low levels of virus replication and no tissue damage. The challenge for clinicians, therefore, is to distinguish this type of innocuous presence of persistent virus from its active replication and disease production, particularly in haematology and HIV patients where clinical consequences are rapid and severe if CMV disease is not detected and treated.

Because of the poor outcome, even with treatment, of established CMV disease in transplant patients, when the study commenced UK clinicians were beginning to screen renal and haematology transplant patients for evidence of CMV reactivation. Considerable effort was being invested in order to identify those patients who were in the early stages of clinically significant CMV infection so that early preventative therapy could be instituted. Frequency of screening varied from centre to centre, with less frequent sampling as the patient's immune system recovered owing to satisfactory establishment of the transplanted graft.[7,8,11,14,15]

As pointed out earlier, manufacturers are currently marketing a number of molecular tests to screen for CMV and NHS laboratories are also developing local in-house molecular methods to try to address this issue. However, in terms of both the diagnosis of infection and predicting disease, clinicians still need to depend on a combination of clinical symptoms, laboratory test results and other diagnostic investigations. Currently, there is no gold standard test for definitive diagnosis of CMV disease (other than post-mortem examination).

Clinical signs and symptoms

Diagnosis of CMV infection based solely on clinical presentation is unreliable because many of the presenting symptoms (e.g. pyrexia >38°C, malaise, shortness of breath, abdominal pain) are non-specific and may be due to a wide variety of causes. Similarly, signs based on routine parameters monitored following transplant (falling platelet count, falling white blood cell count or leukopenia, chest radiograph changes) may be due to recurrence of underlying disease, bacterial or fungal infection and, in allograft patients, graft versus host disease. Viruses other than CMV such as influenza, parainfluenza and respiratory syncitial virus can also cause a similar clinical picture.

Traditional laboratory techniques for detecting CMV disease

A number of traditional methods are available for the diagnosis of CMV infection, but their applicability in immunocompromised patients is limited. Traditional techniques include

- virus culture
- modified culture (shell vial assay)
- serology
- antigen detection.

The most basic traditional techniques involve growing the CMV virus in cell culture. Such culture-based tests are slow and no 'gold standard' technique for accurate diagnosis has been identified. Furthermore, culture may take up to 21 days to produce a positive result, which is not suitable for transplant patients, and it is also a relatively insensitive method.

A modified, more rapid culture-based method (shell vial culture) has been developed, called 'detection of early antigen immunofluorescent foci' (DEAFF). The DEAFF test can be completed in 24 hours and involves looking for early CMV antigen production by cells in tissue culture that have clinical samples centrifuged on to the cell surface to 'force' CMV into the cells. Early antigens are detected after 16–24 hours by

indirect immunofluorescence antibody. However, the DEAFF test is recognised as being unreliable, especially for transplant patients, since samples after transplant may contain drugs (such at cytotoxics, antibiotics, antifungals and immunomodulators) that are toxic to the cells used to culture CMV.[16–19] Even so, the DEAFF test was still being used in some laboratories when the study started.

Laboratory techniques that are based on serological diagnosis (i.e. the formation of specific antibody) are inappropriate for immunocompromised patients because these individuals cannot make antibody normally. Serology results are therefore difficult to interpret in patients whose ability to mount an immune response is, by definition, abnormal.

Tests used to detect CMV disease in immunocompromised patient groups have therefore tended to be based on direct detection of the virus, its protein antigens or nucleic acid to determine infection.

Direct detection using non-molecular methods

It is possible to detect CMV directly in peripheral blood, and therefore considerable effort has been directed over the last decade towards developing non-molecular methods for this purpose. The best method available when the study started was detection of pp65 antigenaemia in circulating peripheral blood leucocytes.[20–29]

Because the test depends on the presence of circulating white blood cells, in severely immunosuppressed patients who have very few circulating leucocytes (e.g. post BMT, PBSCT or AIDS), its negative predictive value is compromised and it may be of doubtful reliability.[28] Positive predictive value may also be low (45%). This can be improved by quantitating the number of circulating CMV positive cells[23,27] and attributing clinical significance only when a high threshold is reached. At the time this study commenced, however, the threshold level at which clinical significance could be assumed had not been established.

The pp65 antigenaemia assay (DiaSorin, Saluggia, Italy) was the technique routinely used in the Cardiff Public Health Laboratory Service (PHLS) for CMV diagnosis when the study commenced. This was therefore the non-molecular test chosen for inclusion as a comparator in the present study.

- Formal culture is slow
- Modified culture is problematic on blood samples
- Serological results difficult to interpret in 'at risk' individuals
- Antigenaemia assay does not work well in all patient groups
- Molecular assays are likely to be very sensitive

FIGURE 1 *Why use molecular tests for prediction/diagnosis of CMV disease?*

Molecular tests for CMV detection (new and emerging tests)

When the study started, there was little consistency evident in the molecular-based methods and strategies utilised by different UK centres for CMV screening and diagnosis in immunosuppressed patients. At the same time, the existing evidence clearly demonstrated a need for improved methods that might possibly include molecular tests. *Figure 1* illustrates this situation. These methods promised improvements on existing non-molecular techniques in terms of speed of test result and sensitivity/specificity. Some of the molecular tests made use of the PCR method to amplify small amounts of CMV DNA so that CMV disease could be detected. Other molecular tests made use of NASBA in order to detect CMV messenger RNA (mRNA) in whole blood samples.

Since rapid amplification methods based on PCR or NASBA are more sensitive than traditional methods, they might also be used to detect virus switch from latency to replication prior to onset of disease. This could prove beneficial for monitoring 'at risk' individuals to make the use of pre-emptive therapy possible. However, in all immunocompromised patients at risk of severe CMV disease, asymptomatic reactivation of CMV may occur with low levels of virus replication and no tissue damage, which is difficult to distinguish from active replication and disease production. Molecular tests were being introduced for this purpose at the time of embarking on this study, but there was little research information on the technical performance of these tests or their value in a service setting compared with current non-molecular tests, both in diagnosing infection and predicting disease.

Aims of the research

The present study aimed to provide data on three important molecular techniques, compared with the most reliable and widely used 'non-molecular'

TABLE 4 *Three main molecular tests included in the evaluation*

Molecular test type	Detection method	Source of test	Type of sample
Test 2: Single-round and nested in-house PCR	Semi-quantitative	In-house[a] (Cardiff)	Whole blood
Test 3: Roche Amplicor Assay	Qualitative	Commercial (Roche)	Plasma
Test 4: NASBA, Organon Teknika	Qualitative	Commercial (Organon Teknika)	Whole blood

[a] Primers used for this assay were developed by Professor V Emery.

method (pp65 antigenaemia assay). Within this overall aim, the objectives of the research were

- To establish the reliability, sensitivity/specificity and turn-around time for selected molecular methods in the diagnosis of existing CMV disease, and for the prediction of development of CMV disease, versus the most commonly used non-molecular test.
- To estimate the cost of these tests under various service conditions (e.g. different throughputs, staff grades, test methods).
- To measure the impact of results on diagnosis of CMV disease and clinical management, including the avoidance of inappropriate, toxic and costly treatment (e.g. ganciclovir).
- To assess the cost-effectiveness of molecular screening for CMV versus existing non-molecular tests, and to model the costs and benefits of different protocols for the early identification of CMV infection.

In this way, the technical performance, costs and benefits of molecular and non-molecular tests could be compared.

Tests evaluated

Four tests (three molecular) were selected for assessment. These differed in the detection approach and stage of acceptance:

- Test 1 (*non-molecular: accepted*): CMV *pp65 antigenaemia assay*.
- Test 2 (*molecular: already diffusing*): Semi-quantitative cell-associated CMV DNA detection by PCR (*single-round and nested in-house PCR*).
- Test 3 (*molecular: new/emerging*): Qualitative cell-free CMV DNA detection by PCR (*Roche Amplicor Assay*).
- Test 4 (*molecular: new/emerging*): Qualitative NASBA analysis of late CMV mRNA (*pp67 NASBA Assay, Organon Teknika*).

An overview of the molecular tests selected for evaluation is shown in *Table 4*.

Two of the molecular tests (Tests 2 and 3) made use of the PCR method to amplify small amounts of CMV DNA so that CMV disease could be detected. They differed in the type of sample used (whole blood or plasma) and in whether a quantitative or qualitative test result was obtained. PCR is a versatile and widely used amplification technique through which even the smallest amount of a defined DNA target can be amplified to provide quantities that are detectable and identifiable. Amplification utilises the action of an enzyme (DNA polymerase) which catalyses the rapid synthesis of new strands of DNA from an original strand using a primer. The PCR-based CMV tests can utilise either a single-round PCR or 'nested PCR' (two rounds of amplification). A second round may be necessary in order to amplify the small amounts of CMV DNA in the samples used and thus ensure optimal detection of CMV disease.

A third molecular test (Test 4) made use of NASBA in order to detect CMV mRNA in samples. This test used whole blood.

In addition, in the final year of the study a fifth test was added, but this only underwent technical evaluation:

- Test 5 (*molecular: new/emerging*): Quantitative cell-free CMV DNA detection by nested quantitative PCR (*COBAS Amplicor Monitor Assay by Roche*).

More details on these molecular tests and the process of selecting tests for inclusion in the health technology assessment is provided in Appendix 1.

Study design

The study design was a prospective observational study of CMV screening introduced in the service setting. This involved a phased introduction of different molecular tests with the aim of determining the extent to which routine use of molecular screening tests is reliable, effective and cost-effective compared with existing diagnostic testing using pp65 antigenaemia assay.

The study involved two stages. In the first stage (months 1–14), the technical performance of the three molecular tests (Tests 2–4) was assessed in the laboratory setting through an independent masked comparison of each molecular test against the established (antigenaemia) test. Only Test 2 (already diffusing) molecular results were fed back to clinicians during this stage of the study, along with antigenaemia results. In the second stage of the study (months 15–32), one of the emerging molecular tests (Test 3 or 4) was to be selected, based on its performance in stage one, and the results of this test would be provided to clinicians along with the established (antigenaemia) test results.

Predicted and reported impact on patient management in the service setting was recorded in both stages through structured questionnaires completed by clinicians at the point of requesting a screening testing and on receipt of the test result. Information was collected on clinical status, current drug therapy, *ex ante* likelihood of CMV disease, *ex ante* likely impact of positive or negative test result on therapy and further investigations, impact of result on diagnostic certainty, actual changes to planned patient management (e.g. therapy and investigations) and perceived benefit.

The study patients were recruited from the University Hospital of Wales (UHW), Cardiff; University of Wales College of Medicine (UWCM) staff working in the Cardiff PHLS virology laboratory performed the tests.

The evaluation framework used in the study was based on the hierarchy formulated by Fineberg and colleagues for diagnostic technologies[30] and an adapted form was developed for CMV testing with the following six levels:

- Technical capacity: does the test perform reliably and deliver accurate (i.e. precise) information?
- Diagnostic accuracy: does it contribute to accurate diagnosis and/or prediction of CMV disease?
- Diagnostic impact: does the test replace other diagnostic tests or procedures?
- Therapeutic impact: does the test result influence the selection and delivery of treatment?
- Patient outcome: does the test contribute to improved health for the patient?
- Cost-effectiveness: does use of the molecular test improve the cost-effectiveness of healthcare compared to alternative interventions?

Appendix 2 provides more detail on the stages of the study.

Conclusions

Background leading to the research

Prior to 1996, the virology laboratory at Cardiff offered CMV serological diagnostic tests for use in diagnosis of CMV disease in immunocompetent individuals. CMV isolation in cell culture was available and particularly useful for the diagnosis of congenital CMV in neonates. These babies excrete so much CMV that it grows very quickly and therefore an answer is available within 1 week; since there is no agreed treatment for such infants, speed of diagnosis is less of an issue.

For immunocompromised patients, assays were not appropriate and instead the laboratory offered pp65 antigenaemia tests done on a 'same day' basis. Although costly, this test performed well on samples from renal transplant patients who have only a moderate degree of immunosuppression and plentiful functioning circulating white blood cells.

In contrast, haematology patients post stem cell transplant are much more immunosuppressed and at the time of risk of CMV have very few circulating white blood cells. In addition, the cells that are present are immature and not functionally normal. Under these circumstances, pp65 antigenaemia becomes very unreliable. A negative test result was regarded with lack of confidence by both laboratory and clinical staff. This led to clinicians adding ganciclovir to already complex, costly and toxic treatment regimes in this group of patients 'in case' CMV disease was present but not diagnosed. Patients with end-stage HIV–AIDS were in a similar difficulty. They too have very few circulating white blood cells, and any that are present may not be functional so, again, a negative pp65 antigenaemia test was regarded as unreliable. In both patient groups, a positive pp65 antigenaemia test was, however, accepted as a true result and led to therapy initiation.

Pressures to introduce molecular tests for CMV screening and diagnosis

Clinicians and laboratory scientists were aware of the growing use of molecular tests to detect CMV DNA, particularly in centres with a strong track record of CMV research. Increasingly, clinicians wished to have such tests available to their patients; they particularly wanted access to PCR, which had become almost a talisman of best practice. There was little recognition or understanding of the range and variety of available molecular tests, or that few had been shown to have rigorous clinical utility in predicting CMV disease. Screening well patients for low-level CMV replication was being used to allow pre-emptive therapy in some centres without robust evidence that low-level viraemia led to overt CMV disease.

There was, and is, very little laboratory standardisation of methods in areas so close to new research, and centres tended to use whatever tests their local laboratory offered. Virtually no comparison had been made between methods or even between different technological approaches to molecular assays. There was no validated external quality assessment scheme at the time and results from different laboratories were not comparable.

All molecular assays were and are costly, and those groups relying on laboratory tests of extreme sensitivity saw more toxicity from ganciclovir than CMV disease. There was lack of consensus and considerable concern about how best to manage these vulnerable patients.

Finally, there were and are issues relating to patient access to high-technology diagnostic and screening assays. As more patients are discharged early from tertiary referral hospitals, district general hospitals and primary care teams are increasingly asked to provide appropriate monitoring.

Molecular monitoring is usually only available at the major teaching hospitals; patients returning to their homes may not continue to benefit from this aspect of care. By evaluating the role of molecular monitoring in patient outcome, the study was designed to show whether this lack of access represents a real disadvantage to such patients.

Chapter 2

Patients recruited to study and CMV testing patterns

Introduction

Patients recruited to the study fell into three groups:

- renal transplant patients
- bone marrow/stem cell transplant patients
- patients with advanced HIV infection.

It was planned to recruit a total of 260 consecutive patients, with an equal number of patients to be recruited in stages one and two of the study. All patients were to be screened for CMV over a defined period using agreed testing protocols.

CMV screening test protocols

CMV screening test protocols were designed to cover the period of highest risk for each patient group. Separate testing protocols were therefore developed for each group of patients based on:

- available evidence from the literature
- pragmatic assessment of the costs of CMV screening in the service setting
- ability of laboratory staff to deliver test results in a timely fashion
- ensuring that sample collection did not unduly inconvenience patients, therefore maximising recruitment and compliance levels.

Renal transplant patient CMV screening protocol

Renal transplant patients were to be screened for CMV during the first 4 months following transplant, the period of concern being the first 3–4 months with the highest risk of disease between weeks 8 and 16.[1–4] This period would cover the average inpatient stay of 2–3 weeks and 3–3½ months following discharge.

There was no consensus in the literature on the most appropriate testing interval for CMV screening tests, but transplant recipients in Cardiff usually attended the outpatient clinic regularly in the first few weeks following discharge, eventually attending every 4 weeks. A testing protocol with screening samples collected at 4-weekly intervals was therefore agreed with clinicians (as shown in *Table 5*).

Bone marrow/stem cell transplant patient CMV screening protocol

Bone marrow/stem cell transplant patients were to be screened over a longer period of 6 months following transplant, the period of highest risk being the first 20 weeks.[6] It was expected that this 6-month period would cover the average inpatient stay and 3–5 months following discharge. PBSCT patients, mostly autograft recipients, spend an average of 4 weeks as inpatients and then attend outpatients regularly for several months following discharge, at first weekly and then every 2 weeks. For BMT patients, mostly allogeneic transplant recipients, the inpatient stay is longer (12 weeks on average), followed by a similar pattern of outpatient attendances.

Once again, there was no consensus in the literature on the most appropriate interval between CMV screening tests. It was decided that samples would be collected at 2-weekly intervals for the first 3 months and then every 4 weeks (see *Table 5*).

Prior to transplantation, all renal patients received immunosuppressive therapy, consisting of methylprednisolone with tacrolimus or neoral. All haematology patients had undergone a regimen of total body irradiation and cyclophosphamide before transplantation. Prophylaxis for GVHD, consisting of ciclosporin and methotrexate, was

TABLE 5 *CMV screening test protocols for transplant recipients*

Patient group	Total screening tests per patient	Sample time points (weeks post-transplant or enrolment)
Renal	5	0, 4, 8, 12, 16
BMT and PBSCT	10	0, 2, 4, 6, 8, 10, 12, 16, 20, 24

administered to allogeneic transplant recipients only. Immunosuppressive therapy in these patients consisted of ciclosporin and prednisolone.

Monitoring of patients with advanced HIV infection

Patients with advanced HIV infection were to be monitored continuously, rather than for a fixed period, since the risk of CMV disease remains constant. A testing protocol with screening samples collected every 12 weeks was therefore agreed to coincide with outpatient visits for viral load measurement.

Patients recruited to study

All patients undergoing renal or bone marrow/stem cell transplants at UHW were invited to take part in the study; invitation for these patients was at the time of transplantation. For HIV patients, recruitment was at a regular outpatient visit. The objectives of the study were explained to the patient and written consent was obtained. Patients were able to withdraw from the study at any point.

In total, 238 consecutive transplant patients were recruited to the study over 2 years 7 months; this compared reasonably well with the target number of 260 patients in 2 years. The numbers recruited to each of the three patient groups were, however, slightly different from those originally planned, as were the numbers recruited to the two stages of the study.

Recruitment rates

Renal transplant patients recruited

In the initial plan, we aimed to recruit 40 consecutive renal transplant patients to each phase of the study; a total of 80 patients were to be recruited over a period of 12 months (the last 6 months of stage one and first 6 months of stage two). Recruitment of renal patients was initially slow owing to the abnormally low number of transplants performed during the first quarter of 1999. Subsequently, recruitment improved and in total 98 patients were recruited to the study over a 33-month period (30 March 1999–11 December 2001). In stage one, 47 patients were recruited (30 March 1999–13 November 2000), with the remaining 51 patients recruited in stage two (26 November 2000–11 December 2001). No patient requested to be withdrawn from the study. Study patients were screened for CMV over a period of 4 months following transplant so data collection was completed for the last renal patient in April 2002. A 'final' outcome was recorded for all patients at this stage, together with any longer term outcome information available at the end of the data collection period. In some cases, a complete set of screening test samples was not received from a patient because their care was transferred to a distant hospital following discharge.

Bone marrow/stem cell transplant patients recruited

The aim was to recruit 70 consecutive bone marrow/stem cell transplant patients to each stage of the study. It was expected that recruitment would be high because this group is highly motivated. In the event, the target 140 patients were successfully recruited, although the planned recruitment period had to be extended from 2 years to 3 years 6 months (transplants 12 November 1998–8 May 2002). A total of 83 patients were recruited over an extended stage one (12 November 1998–22 December 2000), exceeding the study target of 70; the remaining 57 patients were recruited over a slightly less extended stage two (10 January 2001–8 May 2002). All patients were screened for CMV over a period of 6 months following transplant so data collection was completed for the last haematology patient in November 2002. One patient requested to be withdrawn from the study after 6 weeks and another patient ceased to be screened after 3 weeks; both patients were included in analyses (with values recorded as missing where appropriate). A 'final' outcome was recorded for all patients at 6 months, as was any longer term outcome information available at the end of the data collection period.

HIV-infected patients recruited

It was planned to recruit 40 consecutive patients in stage one of the study with advanced HIV infection (CD4 < 100/mm^3). These same patients would continue to be monitored using the CMV screening test protocol throughout the course of the study (stages one and two). Patients were invited to participate in the study if, at an outpatient visit for viral load measurement, their CD4 count fell below 100/mm^3. All patients who fulfilled the entry criterion for the study were invited to participate and only one refused.

However, recruitment of HIV-infected patients proved to be much slower than anticipated because of a significant reduction in the number of patients with advanced AIDS. The decline in the number of such patients is a consequence of the success of HAART. In the event, only seven patients were

TABLE 6 *Haematology transplants: allogeneic patient characteristics*

Parameter	Number
Sex	25 male, 16 female
Clinical diagnosis	6 ALL, 9 AML, 7 CML, 19 other (e.g. NHL, MDS)
Donor type	39 sibling, 2 non-sibling
Cells transplanted	19 BM, 1 BM + PBSC, 21 PBSC
Outcome of transplant	18 successful, 23 died

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CML, chronic myeloid leukaemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma.

recruited during stage one of the study. After consideration, we concluded that it was unlikely that a sufficient number of patients with advanced HIV disease could be recruited in Cardiff, even with an extended recruitment period. In the absence of sufficient local patients who fulfilled the study entry criterion, an attempt was made to recruit participants from a second centre. However, the success of HAART had improved the health of the UK cohort of HIV-infected patients elsewhere and recruitment of another centre proved impossible. Because it was highly unlikely that this situation would change during the study period, recruitment of HIV patients was discontinued at the end of stage one, in agreement with the HTA programme. Furthermore, since the problem of CMV disease in this patient group is a consequence of advanced AIDS, the need for a cost-effective screening and diagnostic assay for CMV in HIV-infected patients was considered to be of reduced priority. The seven HIV patients recruited provided a total of only 36 samples and therefore these samples were excluded from the analyses.

Characteristics of patients recruited to study

Renal transplant patients

The majority of renal patients were male (58 male, 40 female) with an age range of 18–77 years (mean age 45.9 years). All patients had end-stage renal failure and maintenance haemodialysis was required for all. Sixteen (16%) renal transplants were from live donors and 82 (84%) were cadaveric transplants. At the time of transplantation, 40 donors (41%) were CMV seronegative (R–) and 58 (59%) CMV seropositive (R+) [CMV serostatus (+/–); R = recipient, D = donor]; the seropositive figure for live transplant donors was 62.5% and for cadaveric 58%. Hence >50% of patients would be considered at 'high risk' for CMV disease.

Bone marrow/stem cell transplant patients

The majority of patients were male (86 male, 54 female) with an age range of 16–70 years (mean age 46.5 years). Within this population, 41 patients (29%) received an allogeneic transplant and 99 (71%) an autologous transplant. At the time of transplantation, 61 patients (44%) were CMV R– and 79 (56%) CMV R+. Hence >50% of patients would be considered at 'high risk' for CMV disease.

An overview of patient characteristics for allogeneic transplants is presented in *Table 6*.

Observed CMV testing patterns

At the start of the study, clinicians in Cardiff could only request a CMV test if they suspected a patient had the disease (i.e. diagnostic tests). There was no protocol-based screening of patients for evidence of CMV infection prior to any clinical symptoms. Under the study protocol, clinicians continued to be able to request diagnostic tests at any time if they suspected CMV disease was present. However, they were also asked to send regular screening samples (as shown in *Table 5*) to the laboratory for indications of CMV activation or disease.

CMV testing patterns for renal transplant patients

According to the agreed testing protocol, each renal transplant patient was to receive five CMV screening tests over a period of 16 weeks (112 days). For the 98 patients entered in the study, this should have equated to 490 samples over a period of about 36.5 months, including 4-month follow-up; for the laboratory this would represent an average 3.1 renal samples per week. In fact, a total 664 samples were received from these patients by 11 April 2002 (the end of the 4-month follow-up period); this equated to an average 6.8 samples per patient, or 4.2 samples per week for the laboratory.

Table 7 shows that during this period, CMV testing patterns were similar across the different risk

TABLE 7 *Renal transplants: CMV testing patterns (all tests)*

CMV status	No. of patients (%)	Probability of CMV[a] (%)	Median no. of tests per patient	No. of tests per patient (range and quartiles)				Median follow-up (days) (weeks)
				Min.	Lower quartile	Upper quartile	Max.	
R–D–	22 (22.5%)	0	6	5	5	7	9	146.5 (21 weeks)
R–D+	28 (28.6%)	44[b]	8	5	6	9.5	14	159.0 (23 weeks)
R+D–	18 (18.4%)	6	7	5	6	7	9	168.5 (24 weeks)
R+D+	30 (30.6%)	25[b]	6	1	5	7	9	145 (21 weeks)
Total [protocol]	98 (100%)		6 [5]	1	6	8	14	22 weeks [16 weeks]

D, donor; R, recipient.
[a] Likelihood of CMV infection in patient group.
[b] Significantly different from D–R–.
Data from Fox *et al*. (1995)[4] and Emery *et al*. (2000).[5]

groups, with only a slightly higher level of testing reported for the highest risk group (R–D+). The median number of tests carried out also did not vary significantly between patient groups, with the median for the whole group of patients being six, slightly higher than the five tests expected from the protocol. Any impact of the few patients whose care was transferred to a distant hospital following discharge in terms of reduced test numbers was therefore not significant.

The follow-up period over which these tests were carried out differed somewhat from the protocol. The median period of follow-up observed was 22 weeks and 75% of patients' samples were received within 28 weeks; the protocol, however, required a 16-week follow-up period. Appendix 3 shows that this pattern remains unchanged if cases are divided further by the type of transplant donor (i.e. live or cadaveric).

Because *Table 7* (and Appendix 3) present data for **all** CMV tests requested during the study period, the observed testing patterns may include some diagnostic tests. *Table 8* (and Appendix 3) excludes any tests which clinicians identified as being requested for diagnostic purposes. *Table 8* also excludes patients who did not survive long enough to complete the protocol follow-up period, thus more truly demonstrating *screening* patterns. The test patterns are largely similar to those shown above, although the overall range (1–10) is lower than for all tests (1–14, see *Table 7*). The follow-up period during which patients received screening tests remains longer than the 16 weeks specified in the protocol (i.e. median 21 weeks).

CMV testing patterns for bone marrow/stem cell transplant patients

The testing protocol required that each haematology patient receive 10 CMV screening tests over a period of 24 weeks (168 days). For the 140 patients in the study this would equate to 1400 samples over a period of ~48 months, including 6-month follow-up; for the laboratory this would represent an average of 6.7 samples per week. In total, 1077 samples were received from these patients by 8 November 2002 (the end of the 6-month follow-up period). This equated to an average of 7.7 samples per patient, or 5.2 samples per week for the laboratory, lower than expected.

Table 9 shows that these low figures are largely attributable to the difference between the observed CMV testing patterns for autologous transplants (median six tests) and the higher risk allogeneic transplant patients who received significantly more screening tests (median 8–10.5). The period over which tests were carried out, once again, differed for individual patients; the median period of follow-up was 19 weeks and 75% of patients' samples were received within 28 weeks; the protocol required a 24-week follow-up period.

TABLE 8 *Renal transplants: CMV testing patterns (screening tests) (based on the patients who survived and had screening tests only)*

CMV status	No. of patients (%)	Probability of CMV[a] (%)	Median no. of tests per patient	No. of tests per patient (range and quartiles)				Median follow-up (days) (weeks)
				Min.	Lower quartile	Upper quartile	Max.	
R–D–	15 (27.8%)	0	6	5	5	6	7	135 (19 weeks)
R–D+	10 (18.5%)	44[b]	6	5	6	7	10	146.5 (21 weeks)
R+D–	11 (20.4%)	6	7	5	6	7	7	168 (24 weeks)
R+D+	18 (33.3%)	25[b]	6	1	5	6	7	139.5 (20 weeks)
Total [protocol]	54 (55%)		6 [5]	1	5	6	10	21 weeks [16 weeks]

D, donor; R, recipient
[a] Likelihood of CMV infection in patient group
[b] Significantly different from D–R–.
Data from Fox *et al*. (1995)[4] and Emery *et al*. (2000).[5]

Table 9 includes **all** CMV tests ordered for the haematology study patients, and therefore the data reported in the table will include some diagnostic tests. The data also include patients who died before the end of the follow-up period; this was a much more common occurrence for haematology transplant patients than for renal transplant patients (43 versus two deaths).

Table 10 excludes patients for whom diagnostic tests were requested and those who did not survive through the protocol follow-up period. The resulting patterns remain largely similar to those observed in *Table 9* with the median number of screening tests overall slightly lower (6 rather than 6.5). However, the follow-up period is slightly longer at 21 weeks and, therefore, closer to the protocol period of 24 weeks. The range of number of tests per patient once those receiving diagnostic tests or who died are excluded (1–13) is also much lower than the spread observed for all patients (1–30).

Conclusions

The target numbers of renal and haematology transplant patients were successfully recruited to the study. Recruitment of HIV-infected patients was extremely slow because of the success of new antiretroviral therapy, and recruitment of HIV patients was therefore discontinued in agreement with the HTA programme.

It was planned to recruit a total of 80 consecutive renal transplant patients in 12 months. In the event, a total of 98 patients were recruited, but over an extended period of 33 months.

A total of 140 consecutive bone marrow/stem cell transplant patients were to be recruited in 24 months. The target recruitment was achieved, although over an extended period of 3 years 6 months.

Renal patients were to undergo five screening tests over a period of 4 months following transplant; haematology patients were to receive 10 screening tests over a period of six months following transplant. These CMV screening protocols were agreed with clinicians in advance of the study. They were based on best available evidence and pragmatic decisions relating to cost and likely compliance. A 'final' outcome was recorded for all patients at 4 or 6 months, together with any longer term outcome information available at the end of the data collection period. Follow-up of renal patients was completed in April 2002 and for haematology patients in November 2002.

The number of screening tests requested for renal patients (median value six tests) was close to that

TABLE 9 *Bone marrow/stem cell transplants: CMV testing patterns (all tests)*

CMV status	No. of patients (%)	Probability of CMV[a] (%)	Median no. of tests per patient	No. of tests per patient (range and quartiles)				Median follow-up (days) (weeks)
				Min.	Lower quartile	Upper quartile	Max.	
Allogeneic transplant								
R–D–	15 (10.7%)	3	10	3	6	16	25	140 (20 weeks)
R–D+	4 (2.9%)	4	10.5	4	5	22.5	30	112.5 (16 weeks)
R+D–	8 (5.7%)	44[b]	8	2	4.5	16.5	23	91 (13 weeks)
R+D+	14 (10%)	12	9.5	2	4	15	21	114.5 (16 weeks)
Autologous transplant								
R–	42 (30%)	0	6	2	4	7	12	130 (19 weeks)
R+	57 (41%)	5	6	1	4	9	13	143 (20 weeks)
Total [protocol]	140 (100%)		6.5 [10]	1	4	10	30	19 weeks [24 weeks]

D, donor; R, recipient
[a] Likelihood of CMV infection in patient group.
[b] Significantly different from D–R–.
Data from Fox *et al*. (1995)[4] and Emery *et al*. (2000).[5]

TABLE 10 *Bone marrow/stem cell transplants: CMV testing patterns (screening tests) (based on the patients who survived and had screening tests only)*

CMV status	No. of patients (%)	Probability of CMV[a] (%)	Median no. of tests per patient	No. of tests per patient (range and quartiles)				Median follow-up (days) (weeks)
				Min.	Lower quartile	Upper quartile	Max.	
Allogeneic transplant								
R–D–	3 (4.5%)	3	10	6	6	11	11	140 (20 weeks)
R–D+	0 (0%)	4	–	–	–	–	–	–
R+D–	0 (0%)	44[b]	–	–	–	–	–	–
R+D+	0 (0%)	12	–	–	–	–	–	–
Autologous transplant								
R–	31 (47%)	~0	6	2	4	7	12	137 (20 weeks)
R+	32 (48%)	5	6	2	4	8	13	150 (21 weeks)
Total [protocol]	66 (47%)		6 [10]	2	4	8	13	21 weeks [24 weeks]

D, donor; R, recipient.
[a] Likelihood of CMV infection in patient group.
[b] Significantly different from D–R–.
Data from Fox *et al*. (1995)[4] and Emery *et al*. (2000).[5]

agreed in the protocol (five tests); see *Table 8*. However, tests were carried out over a longer period (median 21 weeks) than the follow-up period required in the protocol (16 weeks). More screening samples than were required by the protocol were received from some renal clinicians if they were unclear precisely when a patient's screening trial period ended. Junior medical staff changed on the renal unit every few months so that extra samples were sent if new staff were unsure when individual patients had come to the end of their screening period. Diagnostic samples were also often sent from patients outside the trial period if there was a clinical suspicion of CMV disease. The maximum risk for CMV disease covered by the protocol period is only the period of highest risk; the risk of CMV disease is not zero thereafter and the study reflected the normal clinical practice of trying to establish CMV disease whenever there was clinical concern.

For haematology patients, the number of screening tests (median value six tests) was significantly lower than the number specified in the protocol (10 tests); this analysis excludes diagnostic tests and patients and only considers those who survived the protocol screening period. If these are included, the number is still low (6.5 tests). This can be accounted for by well patients referred to distant hospital and primary care team follow-up from where, despite extraordinary efforts by the study scientist, submission of screening samples was somewhat unreliable. The range of tests received was also large (1–30 per patient). Haematology patients in hospital were frequently unwell owing to the rigour of their therapy. Patients tend to be pyrexial much of the time and are invariably neutropenic. This leads to a low threshold for requesting diagnostic tests (for CMV and many other viral, bacterial and fungal pathogens). This is because clinicians recognise that in these vulnerable patients the window of opportunity for treating infection successfully is very narrow and leads to an excess of 'diagnostic' tests over screening tests. The period over which haematology screening tests were carried out was also slightly shorter (median 22 weeks) than the follow-up period specified in the protocol (24 weeks), presumably owing to referral of well patients to their district general hospital.

Chapter 3

Technical evaluation of tests

Introduction

A laboratory-based technical evaluation was undertaken to establish the reliability, sensitivity/specificity and turn-around time for the four molecular tests in the diagnosis of CMV disease, versus the most commonly used non-molecular test (antigenaemia). These tests and their perceived advantages/disadvantages are described below.

pp65 antigenaemia assay

This assay measures the expression of a CMV-specific structural protein. The protein is only produced late in the viral replication cycle and, when it is present in detectable levels in peripheral blood cells, it is thought to be a good indicator of recent or current replication. The pp65 staining has a characteristic lobular appearance in polymorphonuclear leucocytes as shown in *Figure 2*.

An overview of the steps involved in the antigenaemia assay is given in Appendix 4.

A sample was regarded as being antigenaemia negative when no positive cells could be found after scanning both cell spots. The assay had been run as routine in the diagnostic laboratory before use in this study and it had been noted previously that the assay had a good positive predictive value even if only one or a few cells were found with characteristic pp65 staining. The method was found to be convenient and user-friendly and did not have the problems reported for antigenaemia assays using immunofluorescence detection in HIV-infected individuals, where low positive predictive values, were sometimes reported.[23,27] A positive result was therefore reported when one or more cells gave the characteristic staining pattern.

One problem with the pp65 assay approach is that some immunocompromised patients (particularly haematology patients) have a very low peripheral blood leucocyte count, making interpretation of results difficult. This was noted during the course of the study and in some cases it was not possible to isolate enough cells from an EDTA blood sample to validate a negative result properly.

Nested in-house PCR (semi-quantitative cell-associated CMV DNA detection by PCR)

Detection of cell-associated CMV DNA by PCR has been undertaken by a variety of laboratories using related methods. It is widely recognised that this

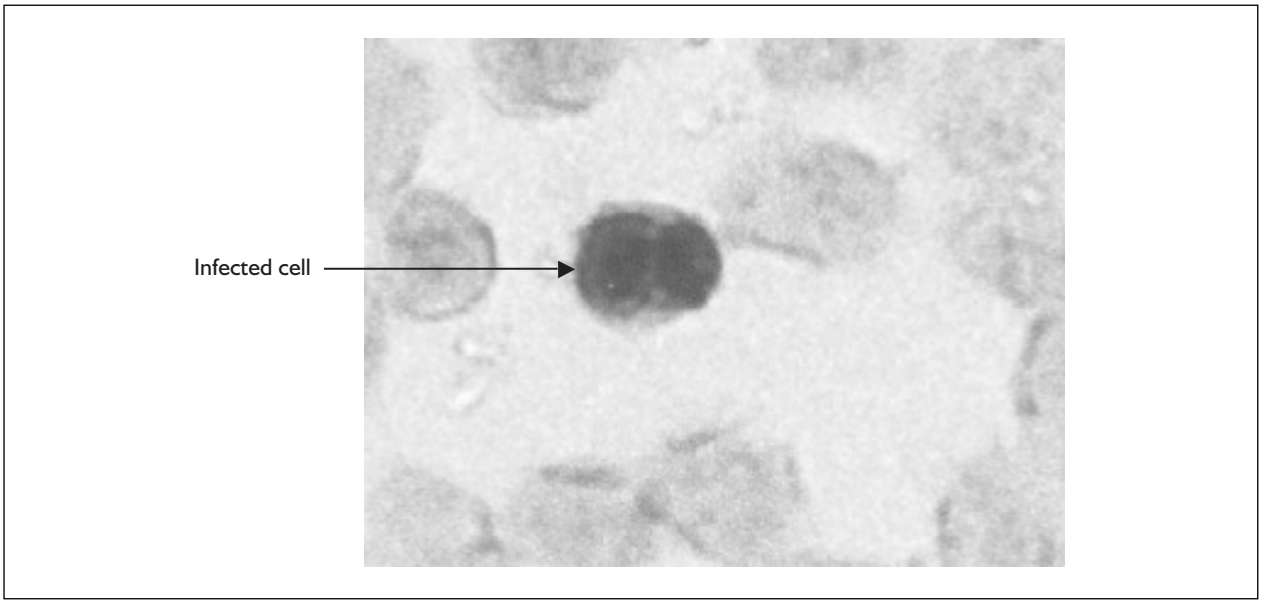

FIGURE 2 *Typical staining pattern for peripheral blood cell expressing pp65 antigen*

approach may detect latent virus DNA in the absence of replication or disease and an initial study reported positive predictive values as low as 28%.[31] Several reports claimed that rather than using qualitative assessment, quantitative assessment of cell-associated CMV DNA by PCR gives a better prediction of overt disease,[4,31–34] although many of the procedures described in these studies were very laborious and expensive for routine use.

Some feasibility work to validate a simple semi-quantitative approach to detection of the presence and level of CMV DNA was undertaken.[35] This work was later expanded and published.[36] The results demonstrated that, if CMV PCR was to be used for monitoring patients at risk from CMV disease, the level of detectable CMV DNA in whole blood extracts was critical. Using a two-step nested PCR procedure, it was possible to differentiate between 'high-level' CMV DNA (positive after a single round of PCR) and 'low-level' CMV DNA (positive only after two rounds of amplification with CMV-specific primers), and this strategy was used in the methodology adopted for this study (see Appendix 5).

Patients positive after a single round of PCR (confirmed in a nested reaction) were considered to have levels of CMV DNA which were consistent with 'active' (currently replicating) virus which was likely to be clinically significant. Nested PCR was undertaken on all samples giving a band of the appropriate size after first-round PCR. This was by way of a specificity check for the first-round amplification and to assess whether such an approach would have a high negative predictive value in transplant recipients. Feasibility data[36] had already confirmed that the use of nested PCR positive results alone would lead to an over-diagnosis of CMV in immunocompromised transplant recipients, so this was not used as a main parameter for confirmation of CMV-related disease.

The steps in the procedure for preparation and analysis of samples by semi-quantitative DNA PCR are given in Appendix 4.

The nested PCR designed for semi-quantitative detection of CMV DNA in whole blood was a straightforward assay but, as for any molecular procedure, staff undertaking the assay underwent a period of training to ensure that false-positive and false-negative results were not produced. Relatively junior staff were able to undertake the method once adequate training had been given in handling of samples for molecular amplification assays. Assessment of bands after a single round of PCR was sometimes a difficult procedure and, considering the subjective nature of this, led us to have two independent individuals interpret the results of the gel analysis before confirming a positive result.

Roche Amplicor Assay (qualitative cell-free CMV DNA detection by PCR)

This commercially available PCR-based assay (Roche Diagnostics, Lewes, UK) for the detection of CMV DNA in plasma amplifies a 365-bp portion of the CMV polymerase gene UL54.[37] Reagents and methodology provided by the manufacturer were utilised for this study, in a similar way to that described previously.[38] The main steps in the procedure are given in Appendix 4. During the course of this study others reported the use of the Roche Amplicor Assay for monitoring CMV in immunocompromised patients.[39–42]

The Roche Amplicor Assay for detection of CMV DNA in plasma was straightforward. Relatively junior staff were able to undertake the method once adequate training had been given in handling of samples for molecular amplification assays. The kit-based nature of the assay made quality control and interpretation of results uncomplicated.

Qualitative NASBA analysis of late CMV messenger RNA (NASBA Organon Teknika)

NASBA is an isothermal RNA amplification system which is available commercially (originally Organon Teknika, now bioMérieux) for detection of CMV pp67 mRNA in whole blood extracts. As pp67 is produced late in the replication cycle, one would expect detection of this mRNA to be a good marker of CMV replication. NASBA is much less laborious than reverse transcribed (RT) PCR for the detection of late CMV mRNA. The method was piloted at Cardiff.[36] During the course of this study, others reported the use of this procedure for monitoring CMV in immunocompromised patients.[41,43–48]

Use of the NASBA assay was straightforward and quality control and interpretation of results were straightforward. Relatively junior staff were able to undertake the method once adequate training had been given in handling of samples for molecular amplification assays.

At the outset of the study, the different molecular tests were perceived to have the key characteristics shown in *Table 11*.

TABLE 11 *Perceived advantages/disadvantages of molecular tests assessed*

Test method	Perceived advantages	Perceived disadvantages
Nested in-house PCR	Inexpensive Ease of use Speed Sensitivity	QC of in-house assays difficult Positive predictive value may be poor
Roche Amplicor Assay	Kit-based reagents and QC	Expensive Need to batch samples Labour intensive
NASBA, Organon Teknika	Specificity Kit-based reagents and QC	Expensive Need to batch samples

QC, quality control.

Roche COBAS Amplicor CMV Monitor Assay (quantitative cell-free CMV DNA detection by PCR)

This commercially available assay is an automated quantitative PCR system for the detection of CMV DNA in plasma (Roche Diagnostics). The assay is similar to the qualitative Roche Amplicor Assay described above. The main steps in the procedure are given in Appendix 4. A modified protocol using whole blood is available for the assay, but in this HTA study we used the extraction module of the assay which is suitable only for plasma.

The COBAS Amplicor Analyzer automates the amplification and detection steps of the PCR process on a single instrument, thus minimising hands-on time. The instrument provides high-throughput testing and is supplied by the manufacturer as part of a reagent/machine rental agreement. Relatively junior staff were able to undertake the method once adequately trained, including COBAS training (for use of the instrument). Quality control and interpretation of results were uncomplicated.

The Roche COBAS Amplicor CMV Monitor Assay has been extensively evaluated against alternative methods[39,48–55] and also compared with in-house developed CMV qualitative and quantitative assays.[47,56] In two studies, the sensitivity of the COBAS assay was found to be better than that of the original qualitative assay.[42,50]

Technical evaluation of molecular tests

The first three stages involved in the technical evaluation of these new CMV molecular tests were:

- Technical capacity: does the test perform reliably and deliver accurate (i.e. consistent and precise) information?
- Diagnostic accuracy: does it contribute to accurate diagnosis and/or prediction of CMV disease?
- Diagnostic impact: does the test replace other diagnostic tests or procedures?

These three stages are important to distinguish.[30,57] A new test may deliver consistent and precise readings, that is, deliver accurate information, but it may not contribute to accurate diagnosis and/or prediction of disease, for example because the parameters measured do not relate well enough to disease status. Similarly, even if a new test can provide an accurate diagnosis, it may still be unlikely that it will replace other existing diagnostic tests or procedures, for example because it requires special skills that are in short supply, because the time required to produce a test result is too long or because clinicians have a strong preference for another type of test.

All the molecular methods were assessed in terms of these three aspects of test performance, relative to antigenaemia.

Technical capacity

Technical capacity (see above) was assessed in the Cardiff PHLS for the non-molecular test and the four molecular test variants over the period 1998–2002. The following data were recorded for all samples processed and all antigenaemia and molecular tests undertaken as part of the study: any technical difficulties encountered in performing the test (including test not attempted

TABLE 12 *Results summary for all samples*

Test method	No. of samples	Failed tests	
		No.	%
Antigenaemia (pp65)	1741	345	19.8
Nested in-house PCR (single-round)	1341	8	0.6
Nested in-house PCR (two-round)	1341	8	0.6
Roche Amplicor Assay	1292	47	3.6
NASBA, Organon Teknika	1740	44	2.5

or failed); turn-around time in laboratory (i.e. date of receipt and date of report); and test results.

Regardless of whether the sample was a 'screening' sample from a well patient, or a 'diagnostic' sample from a patient with suspected CMV disease, the following approach was adopted during stage one of the study:

- An antigenaemia test was carried out on each sample.
- Fluid in excess of that required for antigenaemia was used to perform **all** three molecular tests.

During stage two of the study, the protocol required that, once again, antigenaemia be carried out on every sample received, but only one molecular test (NASBA chosen at the end of stage two) was to be performed on screening samples. Diagnostic samples were processed for the various PCR tests, and also for the chosen test (NASBA) according to protocol.

Molecular test results were released to clinicians in a selective manner: in stage one, quantitative single-round PCR; in stage two, qualitative NASBA. The only use made of the remaining molecular test results was within the laboratory. A total of 250 samples were stored for future testing but not tested during the study; these have been excluded from the following analyses.

Towards the end of the study, a subset of 100 samples were selected for testing using an additional (fourth) molecular test added to the protocol for technical evaluation only. Samples were selected for testing from patients for whom positive CMV results had been obtained in other assays. This was in an attempt to establish a 'threshold' for significant CMV positivity in terms of predicting CMV disease

Test failure

The study recorded all cases where the test failed to produce a result for antigenaemia and the three main molecular tests being evaluated (i.e. excluding COBAS). *Table 12* shows the number of samples tested by each method and the number (and percentage) of test failures. This table demonstrates that the level of test failure was much higher for antigenaemia than for the molecular tests. These samples were excluded from subsequent analyses.

The main technical problems associated with the antigenaemia assay that led to failure were the fact that 12% (204) of the 1741 samples submitted had insufficient cells for analysis (171 from haematology transplant patients and 33 from renal transplant recipients) and that 8% (141) of samples arrived too late in the day for analysis. For the molecular tests that exhibited >1% failure, reported technical problems were for Roche Amplicor 3.6% (47) samples insufficient for test and for NASBA 0.7% (12) samples insufficient for test and 1.8% (32) cases invalid test.

Time taken for production of test results

The time taken from receipt of specimens at the laboratory to reporting of a result was calculated for a sample of 100 specimens (undertaken once laboratory test procedures were well established) taken from (a) stage one to estimate the turn-around time for production of combined antigenaemia and nested in-house PCR (single round) test results and (b) stage two to provide a turn-around time for combined antigenaemia and NASBA test results. The median turn-around time for stage one tests was 6 days (range 1–15 days) and for stage two tests it was slightly higher at 8 days (range 2–20 days). The latter was due to the absence on maternity leave of the scientist employed on the project and the support given to the project by members of the academic department of UWCM who could not devote as much time to the project as the scientist herself. The time required for processing samples in the service setting is sensitive to the day on which

TABLE 13 *Results summary for all samples*

Test method	No. sent	No. failed	Positives/no. tested	Positive (%) (95% CI)
Antigenaemia (pp65)	1741	345	43/1396	3 (2 to 4)
Nested in-house PCR (single-round)	1341	8	84/1333	6 (5 to 8)
Nested in-house PCR (two-round)	1341	8	193/1333	14 (13 to 16)
Roche Amplicor Assay	1292	47	97/1245	8 (6 to 9)
NASBA, Organon Teknika	1740	44	9/1696	5 (4 to 6)

TABLE 14 *Positive CMV test results for all haematology specimens*

Test method	Samples (positives/tested)	% (95% CI)
Antigenaemia (pp65)	10/811	1 (1–2)
Nested in-house PCR (single-round)	27/819	3 (2 to 5)
Nested in-house PCR (two-round)	75/819	9 (7 to 11)
Roche Amplicor Assay	30/769	4 (3 to 6)
NASBA, Organon Teknika	33/1054	3 (2 to 4)

TABLE 15 *Positive CMV test results for haematology diagnostic specimens*

Test method	Samples positive/tested	% (95% CI)	Patients positive/tested	% (95% CI)
Antigenaemia (pp65)	4/119	3 (1 to 8)	3/46	7 (1 to 18)
Nested in-house PCR (single-round)	21/146	14 (9 to 21)	7/51	14 (6 to 26)
Nested in-house PCR (two-round)	37/146	25 (19 to 33)	12/51	24 (13 to 37)
Roche Amplicor Assay	20/134	15 (9 to 22)	5/48	10 (3 to 22)
NASBA, Organon Teknika	16/145	11 (3 to 21)	5/51	10 (3 to 21)

TABLE 16 *Positive CMV test results for haematology screening specimens*

Test method	Samples positive/tested	% (95% CI)	Patients positive/tested	% (95% CI)
Antigenaemia (pp65)	6/692	1 (0 to 2)	4/135	3 (1 to 7)
Nested in-house PCR (single-round)	6/673	1 (0 to 2)	5/106	5 (2 to 11)
Nested in-house PCR (two-round)	38/673	6 (4 to 8)	24/106	23(15 to 32)
Roche Amplicor Assay	10/635	2 (1 to 3)	9/98	9 (4 to 17)
NASBA, Organon Teknika	17/909	2 (1 to 3)	10/140	7 (3 to 13)

samples are received in the laboratory. It was observed that samples received early in the working week appeared to be batched for test runs later in the week, so that they were all reported on the same day.

Detectable CMV

The assays tested varied in terms of the number of samples they identified as containing detectable CMV. Results are presented for all samples (*Table 13*) and then separately for haematology (*Tables 14–16*) and renal transplant patient samples (*Table 17–19*). Results are also presented separately for the two patient groups for screening tests and tests requested for diagnostic purposes.

Haematology transplant patients

A total of 1077 samples were analysed for haematology patients. Of these samples, 930 (86%) were screening samples and 147 (14%) were diagnostic. *Table 14* shows the number (and percentage) of specimens in which CMV activation was detected by each test; two-round PCR detected most and antigenaemia least.

TABLE 17 *Results for all renal specimens*

Test method	Samples positives/tested	% (95% CI)
Antigenaemia (pp65)	33/585	6 (4 to8)
Nested in-house PCR (single-round)	57/514	11 (9 to 14)
Nested in-house PCR (two-round)	118/514	23 (19 to 27)
Roche Amplicor Assay	67/476	14 (11 to 18)
NASBA, Organon Teknika	56/642	9 (7 to 11)

TABLE 18 *Results for renal diagnostic specimens*

Test method	Samples positive/tested	% (95% CI)	Patients positive/tested	% (95% CI)
Antigenaemia (pp65)	19/80	24 (15 to 35)	10/42	24 (12 to 39)
Nested in-house PCR (single-round)	23/83	28 (18 to 37)	12/41	29 (16 to 46)
Nested in-house PCR (two-round)	39/83	47 (34 to 58)	18/41	44 (28 to 60)
Roche Amplicor Assay	23/75	31 (21 to 42)	12/41	29 (16 to 46)
NASBA, Organon Teknika	19/80	24 (15 to 35)	10/40	25 (13 to 41)

TABLE 19 *Results for renal screening specimens*

Test method	Samples positive/tested	% (95% CI)	Patients positive/tested	% (95% CI)
Antigenaemia (pp65)	14/505	3 (2 to 5)	7/98	7 (3 to 14)
Nested in-house PCR (single-round)	34/431	8 (6 to 11)	15/82	18 (11 to 28)
Nested in-house PCR (two-round)	79/431	18 (15 to 22)	31/82	38 (27 to 49)
Roche Amplicor Assay	44/401	11 (8 to 14)	18/80	23 (14 to 33)
NASBA, Organon Teknika	37/562	7 (5 to 9)	14/98	14 (8 to 23)

Table 15 shows the results for these different tests in terms of patients tested (and also samples) for diagnostic specimens only, and *Table 16* for screening specimens only. In terms of the samples tested, assays picked up CMV in 3–25% of diagnostic samples and 1–6% of screening samples. In terms of patients tested, depending on the assay used, detectable CMV was identified in 3–23% of screening cases and 7–24% of diagnostic cases.

Renal transplant patients

A total of 664 samples were analysed for renal patients. The ratio of screening to diagnostic samples was virtually identical with the pattern observed for haematology patients; 576 (87%) of renal samples were screening and 88 (13%) were diagnostic. *Table 17* presents details for all tests showing the number (and percentage) of specimens in which CMV activation was detected by each test.

Tables 18 and *19* show that in terms of the samples tested, assays picked up CMV in 24–47% of diagnostic samples and 3–18% of screening samples. When translated into patients' results, depending on the assay used, detectable CMV was identified in 7–38% of screening cases and 24–44% of diagnostic cases.

Diagnostic accuracy

The previous section presented comparable results for molecular and non-molecular tests in terms of test failure, turn-around time and levels of CMV 'detection'. The hierarchy used for the evaluation of diagnostic tests distinguishes these technical factors from the question of 'diagnostic accuracy' (i.e. does the test contribute to accurate diagnosis and/or prediction of CMV disease?).

To assess diagnostic accuracy, the results produced by a new test are usually compared with the best test currently available to detect the disease or condition, the 'gold standard'. A major problem in determining this performance characteristic of tests is often the lack of an appropriate reference (gold) standard against which to judge the new test. For most diseases, even the best available

TABLE 20 *Approaches used to identify cases with 'confirmed CMV disease'*

Scientist 1:
'Confirmed CMV disease' was defined as:

1. clinical criteria/onset of ganciclovir treatment PLUS
2. an antigenaemia (pp65) positive result OR two different molecular assay positive results (excluding nested cell-associated PCR positive)

Scientist 2:
'Confirmed CMV disease' was defined as:

1. There must be some evidence of clinical suspicion of disease. Indications of clinical suspicion included the submission of diagnostic samples (not screening samples), or starting treatment (not prophylaxis) with ganciclovir. Either of these were taken to indicate a clinical suspicion of CMV disease
2. There must be additional laboratory evidence of CMV DNA/RNA activation. Because no laboratory tests can be regarded as absolutely reliable, for a specimen to be declared positive there had to be evidence of CMV DNA/RNA detection in at least two adjacent samples
3. Second-round PCR positivity often occurs without other markers of CMV activation or disease and therefore it was not considered that a positive second round was as 'weighty' as the other less sensitive markers of DNA/RNA. A sample was only considered to be positive for CMV disease if there were at least two adjacent samples positive for CMV DNA/RNA in addition to any second-round positive nest result
4. For this definition, antigenaemia was considered equivalent to all other tests except second-round PCR

In summary:
- Clinical evidence of suspicion of CMV disease plus at least two adjacent samples giving evidence of CMV activation by at least two tests in addition to second-round positive nested PCR

diagnostic test has some associated error rate. In practice, therefore, one usually has to 'accept the best available, albeit imperfect, pseudo-reference standard'.[58] A further reference standard commonly used is the degree of concordance between the new test and results found by subsequent tissue examination. Unfortunately, case selection bias may be an important problem here, which means that the results may not be generalisable to many cases. A third approach is to use clinical follow-up as a 'proxy' reference standard. This measure may provide some relevant data, provided that outcomes are not confounded by the effects of time and intervening therapy. See Appendix 6 for further discussion.

In the present case, there was no gold standard test available to act as a reference. Furthermore, tissue examination to detect CMV disease was not undertaken in a consistent manner, so case selection bias would be high and these results could therefore not be generalisable.

Instead, a clinical reference standard was therefore constructed based on independent expert opinion as follows. Two scientists *independently* assessed all test results for each patient. This exercise was carried out blind to minimise bias. Each scientist used an independent approach (based on research evidence and personal experience) to identify cases with 'confirmed CMV disease' (see *Table 20*). Results were then compared for all samples by an independent researcher and any samples (28 in total) in which there was disagreement were fed back to the scientists concerned. At this stage, the scientists were asked to confer to see whether they could reach agreement, which they were able to do in all cases. The final agreement on 'positive' or 'negative' result was the value used as the reference standard in subsequent analyses.

Overall sensitivity/specificity and PPV/NPV values for total patient sample

Data collected in both stages of the study were first combined and used to calculate sensitivity and specificity values for each test using the whole population of patients (see Appendix 6). *Table 21* shows overall sensitivity/specificity values based on all results obtained in the first and second stages of the study: total 1396 antigenaemia results; 1333 nested in-house PCR, single-round and two-round results; 1245 Roche Amplicor assay results; and 1696 NASBA Organon Teknika results. These sensitivity and specificity values are calculated relative to the constructed reference standard described above.

The sensitivity and specificity values in *Table 21* specifically exclude cases where molecular or non-

TABLE 21 *All patients: sensitivity/specificity for all CMV tests (includes diagnostic tests)*

Test method	Failed to complete test (%)	All tests[a] (95% CI)			
		Sensitivity	Specificity	PPV	NPV
1. Antigenaemia (pp65)	19.8	0.421 (0.322 to 0.527)	0.998 (0.993 to 0.999)	0.930 (0.799 to 0.982)	0.959 (0.947 to 0.969)
2. Nested in-house PCR (single-round)	<1	0.819 (0.723 to 0.888)	0.994 (0.988 to 0.998)	0.917 (0.83 to 0.963)	0.986 (0.978 to 0.992)
3. Nested in-house PCR (two-round)	<1	0.957 (0.888 to 0.986)	0.917 (0.9 to 0.931)	0.466 (0.395 to 0.539)	0.996 (0.99 to 0.999)
4. Roche Amplicor Assay	3.6	0.837 (0.739 to 0.905)	0.978 (0.968 to 0.986)	0.742 (0.642 to 0.823)	0.988 (0.979 to 0.993)
5. NASBA, Organon Teknika	2.5	0.760 (0.66 to 0.839)	0.990 (0.983 to 0.994)	0.820 (0.721 to 0.891)	0.986 (0.978 to 0.991)

[a] Excluding all tests conducted during stages 1 and 2 where no result obtained (i.e. failed to complete). Includes all other samples, i.e. diagnostic and screening.

TABLE 22 *All patients: sensitivity/specificity of diagnostic versus screening tests*

Test method	Failed to complete test (%)	Diagnostic tests [screening tests][a]			
		Sensitivity	Specificity	PPV	NPV
1. Antigenaemia (pp65)	19.8	0.548 [0.321]	1.000 [0.997]	1.000 [0.850]	0.892 [0.969]
2. Nested in-house PCR (single-round)	<1	0.872 [0.766]	0.984 [0.996]	0.932 [0.900]	0.968 [0.990]
3. Nested in-house PCR (two-round)	<1	0.957 [0.957]	0.830 [0.932]	0.592 [0.385]	0.987 [0.998]
4. Roche Amplicor Assay	3.6	0.902 [0.778]	0.964 [0.981]	0.861 [0.648]	0.976 [0.990]
5. NASBA, Organon Teknika	2.5	0.739 [0.780]	0.994 [0.989]	0.971 [0.722]	0.937 [0.992]

[a] Calculated separately for diagnostic specimens and [screening specimens].

molecular tests or culture failed, and the level of these is listed in the first column. Positive predictive value (PPV) and negative predictive value (NPV) data were also calculated for all these tests (shown in the final two columns of the Table). Once again, these exclude cases where molecular or non-molecular tests or culture failed.

Table 22 provides sensitivity and specificity values separately for those tests ordered during the screening regime that were identified by clinicians as 'diagnostic' and those that were judged to be purely 'screening' tests (in parentheses). Diagnostic specimens included a much higher level of positive samples than did screening specimens (*Table 15* versus *Table 16*). Estimates of reliability of these sensitivity and specificity values are provided in *Table 76* in Appendix 7.

At the end of stage one of the study, the technical performance of the molecular tests was compared. Analysis of test performance demonstrated that nested in-house PCR (two-round) (Test 3) produced a large number of false positives (PPV = 0.466, see *Table 21*) and it was therefore not selected for use in stage two of the study. Detailed comparison of the remaining molecular tests [NASBA Organon Teknika (Test 5) and Roche Amplicor Assay (Test 4)], using an interim scoring system for true test positives/negatives, indicated that [after in-house PCR (single-round) which had been used in stage one], NASBA appeared to be

TABLE 23 *Haematology: sensitivity/specificity for all CMV tests (includes diagnostic tests)*

Test method	Failed to complete test (%)	All tests[a] (95% CI)			
		Sensitivity	Specificity	PPV	NPV
1. Antigenaemia (pp65)	25	0.321 (0.166 to 0.524)	0.999 (0.992 to 1)	0.900 (0.541 to 0.995)	0.976 (0.962 to 0.985)
2. Nested in-house PCR (single-round)	<1	0.846 (0.643 to 0.95)	0.994 (0.984 to 0.998)	0.815 (0.613 to 0.93)	0.995 (0.986 to 0.998)
3. Nested in-house PCR (two-round)	<1	0.923 (0.734 to 0.987)	0.936 (0.916 to 0.951)	0.320 (0.22 to 0.439)	0.997 (0.989 to 1)
4. Roche Amplicor Assay	3	0.792 (0.573 to 0.921)	0.985 (0.973 to 0.992)	0.633 (0.439 to 0.795)	0.993 (0.983 to 0.998)
5. NASBA, Organon Teknika	2	0.750 (0.562 to 0.879)	0.991 (0.983 to 0.996)	0.727 (0.542 to 0.861)	0.992 (0.984 to 0.996)

[a] Excluding all tests conducted during stages 1 and 2 where no result obtained (i.e. failed to complete). Includes all other samples, i.e. diagnostic and screening.

TABLE 24 *Haematology: sensitivity/specificity of diagnostic versus screening tests*

Test method	Failed to complete test (%)	Diagnostic tests [screening tests][a]			
		Sensitivity	Specificity	PPV	NPV
1. Antigenaemia (pp65)	25	0.235 [0.455]	1.000 [0.999]	1.000 [0.833]	0.887 [0.991]
2. Nested in-house PCR (single-round)	<1	0.905 [0.600]	0.984 [0.996]	0.905 [0.500]	0.984 [0.997]
3. Nested in-house PCR (two-round)	<1	0.952 [0.800]	0.864 [0.949]	0.541 [0.105]	0.991 [0.998]
4. Roche Amplicor Assay	3	0.895 [0.400]	0.974 [0.987]	0.850 [0.200]	0.983 [0.995]
5. NASBA, Organon Teknika	2	0.714 [0.818]	0.992 [0.991]	0.938 [0.529]	0.954 [0.998]

[a] Calculated separately for diagnostic specimens and [screening specimens].

the preferred test in terms of technical performance. This is confirmed by the PPV and NPV results in *Tables 21* and *22*.

Haematology transplant patients

Table 23 presents sensitivity/specificity values for haematology specimens alone; these are based on 811 antigenaemia results, 819 in-house PCR single-round and nested two-round results, 769 Roche Amplicor Assay results and 1054 NASBA Organon Teknika results obtained in the first and second stages of the study. PPV and NPV results are also shown for all these tests in the final two columns.

Sensitivity and specificity values are presented separately for 'diagnostic' and 'screening' tests in *Table 24*. Estimates of reliability of these values are provided in *Table 77* in Appendix 7.

Renal transplant patients

Sensitivity/specificity values and PPV/NPV results for all renal specimens are shown in *Table 25*; these are based on 585 antigenaemia results, 514 in-house PCR single-round and nested two-round results, 476 Roche Amplicor Assay results and 642 NASBA Organon Teknika results. Separate values for 'diagnostic' and 'screening' tests are presented in *Table 26*, with estimates of reliability in *Table 78* in Appendix 7.

Comparison of sensitivity/specificity and PPV/NPV results

PPVs and NPVs are not stable performance

TABLE 25 *Renal: sensitivity/specificity for all CMV tests (includes diagnostic tests)*

Test method	Failed to complete test (%)	All tests[a] (95% CI)			
		Sensitivity	Specificity	PPV	NPV
1. Antigenaemia (pp65)	12	0.463 (0.342 to 0.588)	0.996 (0.985 to 0.999)	0.939 (0.784 to 0.989)	0.935 (0.91 to 0.953)
2. Nested in-house PCR (single-round)	1	0.809 (0.692 to 0.89)	0.996 (0.982 to 0.999)	0.965 (0.868 to 0.994)	0.972 (0.951 to 0.984)
3. Nested in-house PCR (two-round)	1	0.971 (0.888 to 0.995)	0.883 (0.849 to 0.911)	0.559 (0.465 to 0.65)	0.995 (0.98 to 0.999)
4. Roche Amplicor Assay	4	0.855 (0.737 to 0.927)	0.966 (0.943 to 0.981)	0.791 (0.671 to 0.877)	0.978 (0.957 to 0.989)
5. NASBA, Organon Teknika	3	0.766 (0.64 to 0.859)	0.988 (0.974 to 0.995)	0.875 (0.753 to 0.944)	0.974 (0.957 to 0.985)

[a] Excluding all tests conducted during stages 1 and 2 where no result obtained (i.e. failed to complete). Includes all other samples, i.e. diagnostic and screening.

TABLE 26 *Renal: sensitivity/specificity of diagnostic versus screening tests*

Test method	Failed to complete test (%)	Diagnostic tests [screening tests][a]			
		Sensitivity	Specificity	PPV	NPV
1. Antigenaemia (pp65)	12	0.760 [0.286]	1.000 [0.996]	1.000 [0.857]	0.902 [0.939]
2. Nested in-house PCR (single-round)	1	0.846 [0.786]	0.983 [0.997]	0.957 [0.971]	0.933 [0.977]
3. Nested in-house PCR (two-round)	1	0.962 [0.976]	0.754 [0.902]	0.641 [0.519]	0.977 [0.997]
4. Roche Amplicor Assay	4	0.909 [0.825]	0.943 [0.970]	0.870 [0.750]	0.961 [0.980]
5. NASBA, Organon Teknika	3	0.760 [0.769]	1.000 [0.987]	1.000 [0.811]	0.902 [0.983]

[a] Calculated separately for diagnostic specimens and [screening specimens].

characteristics of a particular test; they depend on the prevalence of the condition being examined in the population tested. For example, as the disease prevalence (pretest likelihood of the condition) decreases, the proportion of individuals with a positive test result who actually have the condition falls and the proportion of non-diseased patients falsely identified as having the condition rises. In contrast, as the prevalence of a disease increases, the proportion of patients with a positive test result who do in fact have the condition rises, whereas the proportion of patients with a negative result who do not have the disease falls.

This has important implications for CMV screening tests if they are used in populations with a low prevalence of disease (for example, haematology transplant patients). In a situation where tests are used to screen for the presence of a disease which is sufficiently rare in the population, even tests with a high sensitivity and high specificity can have a low PPV, generating more false-positive than true-positive results, and therefore potentially doing as much harm as good.

In the present study, all patients who went on to develop CMV disease were identified by more than one molecular assay, but many patients who did not go on to develop CMV disease had more than one molecular assay positive result and/or consecutive positive results by the most sensitive assays.

No significant difference was seen in results obtained overall but the majority of haematology

patients with CMV disease were identified from screening samples and the majority of renal patients with CMV disease were identified from diagnostic specimens. This may reflect the more frequent screening time-points for patients receiving BMT or PBSCT.

Comparison of *Tables 23–26* demonstrates slightly different patterns for haematology and renal transplant samples.

All test results (including diagnostic samples)
Sensitivity values overall show that:

- For antigenaemia, sensitivity is low in renal patients, and particularly low in haematology patients.
- For in-house nested PCR, sensitivity is high in renal and haematology patients, but for single-round slightly higher in haematology and for two-round (nested) in renal patients.
- Roche Amplicor PCR has similar sensitivity (slightly higher in renal patients).
- NASBA has similar sensitivity in both.

Specificity values for all completed tests show that:

- Specificity is relatively high for all five tests in both renal and haematology patients.
- The lowest specificity is exhibited by nested PCR (two-round), particularly on diagnostic specimens.

Screening test results only
- Specificity is high (>0.902) in all assays.
- NPV for all assays on screening specimens is also high (>0.939), although the antigenaemia assay performs slightly worse on this test parameter.
- Sensitivity of the antigenaemia assay and Amplicor PCR is particularly poor for screening samples from haematology patients (<0.455), and antigenaemia sensitivity is very poor in renal patients (0.286).
- There is a low PPV for two-round PCR in renal patients (0.519) and very low for haematology patients (0.105), even one round PCR PPV value remains low for haematology patients (0.500).
- Roche has much lower PPV for haematology patients (0.200) than for renal patients (0.750).
- NASBA similarly has much lower PPV for haematology patients (0.529) than for renal patients (0.811).

Diagnostic test results only
- The NPV remains largely similar for all assays when used in diagnostic specimens.
- The PPV for two-round PCR in renal patients remains fairly low (0.641) and even lower for haematology patients (0.541), but the one-round PCR PPV value is now high for haematology patients (0.905) and renal patients (0.957).
- Roche PCR no longer has a much lower PPV for haematology than for renal patients (both approximately 0.85).
- NASBA similarly does not exhibit a much lower PPV for haematology than for renal patients (both >0.938).

Haematology transplant patients
From *Tables 23* and *24*, it might appear that antigenaemia would be the best test for screening haematology patients, because PPV is highest and there is not much to choose between all assays in terms of NPV. However, this does not take into account the unacceptably high failure rate for this test, particularly on haematology patients (25%).

Screening our group of haematology patients (who are mostly not high risk) by qualitative molecular assays gave good NPVs but was a poor predictor of disease (low PPV). These molecular assays performed much better on diagnostic specimens: NPV was good and PPV was better. If one examines the results for diagnostic haematology specimens in *Table 24*, then antigenaemia, NASBA and single-round in-house PCR all perform well, but one of the molecular assays would be preferable owing to the 25% failure rate for antigenaemia. However, the PPV for NASBA and single-round PCR drops away significantly, to 0.529 and 0.500, respectively, in screening specimens. However, in the screening setting all molecular tests perform significantly worse than antigenaemia. In the overall screening regime (*Table 23*), single-round PCR performs best of all molecular tests.

Renal transplant patients
For renal patients, screening tests can be more useful but the antigenaemia failure rate is still disappointing at 12%. Based on the PPV, the best qualitative molecular assay is single-round in-house PCR (0.971) or NASBA to screen renal patients.

Antigenaemia performs well on diagnostic renal specimens but, again, we must consider the 12% failure rate and the exclusion of results from what may be the 'trickiest' specimens from the antigenaemia figures. NASBA performs similarly well on these specimens.

Hence, for diagnostic specimens from both patient groups, NASBA performs the best among the

molecular tests with a relatively good balance between PPV and NPV, with single-round PCR next.

Additional molecular test

Towards the end of the evaluation, quantitative cell-free CMV DNA detection by Q-PCR (COBAS Amplicor Monitor Assay by Roche) was added as a fourth and final molecular test. This was only assessed in terms of its technical performance on selected (archived) samples owing to the limited number of assays provided.

During the study period there was an increasing recognition in several centres that quantitative assessment of CMV DNA might be of value in predicting the onset of CMV-related disease. An extension to the study was agreed by NCCHTA in December 2000 so that laboratory assessment could include quantitative assessment of CMV DNA using commercial CMV DNA (COBAS Amplicor Monitor Assay by Roche). Technical performance was assessed using selected (archived) samples owing to the limited number of assays provided.

In total, the COBAS test was run on and results produced for 68 samples. These samples came from a total of 17 patients, six of whom were haematology and 11 renal transplant patients; the 68 samples tested were predominantly renal (41/68).

The majority of the samples selected were positive (37/41 renal and 22/27 haematology), based on the reference standard result for each sample (see the previous section). The quantitative reading produced by the COBAS test was compared with the CMV result for each sample. Results are shown in Appendix 8. The nine negative specimens (four renal, five haematology) either gave an extremely low reading 'below lower level' or produced a low reading that was less than the overall median value. In contrast, the positive samples produced a broad range of quantitative values, with a number of samples producing a reading that was below the overall median value.

Because the number of CMV-negative samples was very small, it proved impossible to find a cut-off point in the quantitative CMV DNA readings to distinguish CMV-positive and CMV-negative samples and then to perform any statistical or sensitivity-specificity/analysis.

Diagnostic impact

The findings above are able to throw some light on the technical feasibility of any molecular test being able to replace antigenaemia. From the results presented, it appears that in haematology transplant patients antigenaemia is unsatisfactory as a screening or diagnostic test, principally because of its high failure rate, even though it performed well in terms of PPV and NPV. All molecular tests had a low PPV for screening samples but performed better on diagnostic samples where there was, presumably, a greater likelihood of CMV disease. Second-round nested PCR is the worst predictor of CMV disease. Single-round PCR, Roche Amplicor and NASBA each performed well on diagnostic samples, but poorly on screening samples, with NASBA performing slightly better overall.

For renal patients, antigenaemia performs better and the failure rate is only 12%. The PPV is good but the NPV is less good than for the molecular tests, that is, antigenaemia is less sensitive, and to achieve a balance between NPV and PPV single-round PCR performed better. The replacement of antigenaemia in this patient group is, however, less pressing than in the haematology group.

Based on the technical assessment, it does not appear that molecular tests should be used as an 'add-on test' in either patient group. However, for renal patients other practical factors, such as the time of arrival of the sample in the laboratory, might make an 'either/or' option the most favoured by clinicians and laboratory staff.

Indeed, the manner in which these molecular tests are likely to be used in practice will be dependent on several other factors, **apart from technical performance**. These include current provision of CMV screening for immunocompromised patients and clinicians' preferences and perceptions about the test (or tests) that should be used. Similarly, the test preferences of laboratory scientists and any laboratory constraints on the introduction of specific CMV tests might influence 'diagnostic impact' or patterns of use and the likely replacement of existing tests by molecular methods.

The following chapter explores laboratory stakeholders' views on different CMV tests and CMV screening regimes.

Chapter 4

Current CMV laboratory testing practice (UK)

Introduction

In addition to assessing the technical performance of molecular tests, part way through the study the pattern of use of CMV tests nationally was explored through a UK survey of virology laboratories. The survey also aimed to identify any factors that might influence the adoption of particular molecular tests. Factors explored included the preferences of laboratory staff and clinicians, relative costs, skills of existing laboratory staff, availability of specialist equipment and organisation of laboratory services.

At the outset of the study, it was known that a number of virology laboratories in the UK were already using molecular tests for CMV detection and possibly for screening. It was assumed that this was generally as an adjunct to traditional antigenaemia tests. Prior to the start of the study, there had been no reports of large-scale studies to evaluate different molecular methods as screening tests for detection of CMV disease.

UK survey of NHS virology laboratories

A postal survey of UK virology laboratories was conducted in 2002. The questionnaire was developed in conjunction with Cardiff PHLS laboratory staff and piloted before use.

Thirty-two virology laboratories were identified and sent a copy of the questionnaire. Three laboratories reported that they did not currently process samples to diagnose CMV disease in immunocompromised patients **and** had no plans to introduce such tests; for four laboratories joint responses were provided on another questionnaire, there were three non-responders and we received 22 completed questionnaires. Hence out of 25 centres from which we would have liked a response, we obtained responses from 22/25 (88%). Appendix 9 provides details of the laboratories that responded.

Respondents

The survey questionnaire was addressed to Laboratory Directors. A covering letter asked that, where this individual was not responsible for CMV testing or for the possible introduction of such tests, he or she should pass the questionnaire on to the relevant person.

Individuals who replied mainly described themselves as Consultants/Consultant Virologists, although in one case the respondent was a Laboratory Manager, in one case a Clinical Head of Microbiology and in a further case a Virologist.

Existing and planned use of CMV screening tests in NHS virology laboratories

Existing CMV screening tests for immunocompromised patients

Survey responses indicated that 21/22 virology laboratories currently process samples to diagnose CMV disease in immunocompromised patients, and the remaining laboratory was planning to introduce these tests; 21 laboratories also reported that they conduct some CMV screening tests, and the final laboratory was planning to introduce such screening tests.

Laboratories were asked about the total number of CMV tests undertaken in 2001 and the proportion of these that were screening tests. *Table 27* shows that numbers of CMV tests ranged from 18 to 6776 per annum; on average, the 21 laboratories undertaking CMV testing received 1797 CMV test requests in 2001 and 1385 (77%) of these were screening tests. The estimated percentage of CMV test workload linked to screening requests ranges from 30 to 100% (in two laboratories). The average picture nationally is similar to that recorded in the study laboratory where for renal patients 87% were screening samples and 13% diagnostic (haematology 86% screening and 14% diagnostic (see the section 'Detectable CMV', p. 23).

CMV test workload for different patient groups

Laboratories were asked to break down their 2001 CMV test workload into the three patient groups being considered. Results are shown in Appendix 10. Their responses are summarised below.

TABLE 27 *Laboratory CMV testing patterns (2001)*

Centre no.	No. of CMV tests per year in 2001	Estimated percentage of CMV tests for screening for 2001	Estimated total number of tests for screening
1	220	50	110
2	178	80	142
3	2,630	90	2,367
4	4,200	50	2,100
5	1,500	50	750
6	41	100	41
7	550	70	385
8	1,700	75	1,275
9	1,414	90	1,273
10	650	99	644
11[a]	0	0	0.0
12	18	30	5
13	475	95	451
14	306	36	110
15	4,250	75	3,188
16	698	50	349
17	1,100	90	990
18	2,500	65	1,625
19	6,776	100	6,776
20	1,500	95	1,425
21	2,600	50	1,300
22	4,435	85	3,770
Total	37,741		29,076
Average no. of tests per annum (centres undertaking CMV testing)	1,797	Average % screening tests: 77%	1,385

[a]Excluded from averages; planning to introduce CMV testing and screening.

Haematology transplant patients

- The average number of CMV tests (diagnostic and screening) was 23 tests per week (based on 17 laboratory responses); range <1–80 tests per week.
- On average, 78% of CMV tests were screening tests; range <1–100% (based on 17 respondents).

Renal transplant patients

- The average number of CMV tests was 6.5 tests per week (based on 16 responses); range <1–25 tests per week.
- On average, 71% of CMV tests were screening tests; range 0–100% [based on 12 respondents (these respondents were those providing both estimates of the average number of CMV tests at their laboratory and estimates of the percentage of these which were screening tests for CMV)].

HIV patients

- The average number of CMV tests was three per week (based on 13 responses); range <1–16 tests per week.
- On average, 54% of CMV tests were screening tests; range 0–100% [based on nine respondents (see comment above for renal transplant patients)].

These responses indicate that laboratories are performing nearly four times as many CMV tests for haematology transplant patients as for renal patients. Approximately three-quarters of these are screening tests. Fewer tests are being performed for HIV patients (in fewer laboratories) and only half are screening tests.

CMV tests used by laboratories for different patient groups

Laboratories were asked for details of the type(s) of CMV diagnosis and screening in the three patient groups.

Haematology transplant patients

All 21 laboratories provided information on the CMV tests currently used; *Table 28* shows the main tests used by laboratories. One laboratory used three types of test, listing two as the main test used.

Three laboratories indicated that specimens are processed off-site and gave no details of the test

TABLE 28 *Main type of CMV test used on-site for haematology transplant patients*

Type of test	No. of laboratories	Comments
Not applicable	3	3 labs: samples processed off-site
Real-time quantitative PCR	8	6 labs: LightCycler PCR 2 labs: TAQMAN
Other quantitative PCR	2	1 lab.: quantitative plasma viraemia 1 lab.: in-house nested PCR
Qualitative PCR	3	2 labs: in-house qualitative PCR 1 lab.: plasma PCR
Antigenaemia (pp65)	3	1 lab.: only for patients with full blood count $>1 \times 10^9$/l.
Antigenaemia (pp65) and DEAFF	1	1 lab.: listed both these as main tests; uses 3 tests in total
DEAFF	1	

used. Of the remaining 18 laboratory sites, 13 (72%) use some form of PCR test for CMV detection in haematology transplant patients. Of these, the majority (8/13 or 62%) indicated that they use real time quantitative PCR (LightCycler or TAQMAN). A further 3/13 (23%) use qualitative PCR. Only 4/18 respondents (22%) report that their main test is antigenaemia; two laboratories reported that they use the DEAFF test (one also using antigenaemia).

Thirteen laboratories provided information on secondary tests used. Two laboratories referred samples elsewhere for further tests. Of the remainder, seven laboratory sites used some form of quantitative PCR as a secondary test, including one laboratory that reported the use of real-time quantitative PCR in cases where there is a lack of cells (i.e. the patient is neutropenic). A further two laboratories reported the use of non-molecular techniques such as antigenaemia and two laboratories used DEAFF tests as a back-up.

Renal transplant patients

All 21 laboratories undertaking CMV testing provided information on the tests used for renal transplant patients. A similar picture emerges to that reported for haematology transplant patients in *Table 28*. First-choice tests were identical, except that one laboratory reported the use of antigenaemia in renal patients, rather than in-house qualitative PCR as for haematology transplant patients. The Renal Transplant Laboratory carried out this test.

Slightly fewer (11/21) laboratories also provided information on secondary CMV tests used for renal transplant patients. Of these, six respondents indicated that they use some form of quantitative PCR. A further two laboratories use antigenaemia and two use DEAFF tests. One laboratory refers samples elsewhere for secondary testing.

HIV patients

Similar information was provided on CMV testing in HIV patients from all 21 laboratories (see *Table 29*).

Compared with the haematology and renal transplant patients (*Table 28*), there appeared to be slightly more first choice use of PCR tests for this patient group. Overall, 14 out of the 18 (78%) laboratories that test specimens on-site indicated that they use some form of PCR, compared with 13/18 (72%) for renal and haematology transplants. More laboratories reported the use of quantitative PCR for HIV patients (three rather than two), although the numbers recording use of real-time quantitative PCR (LightCycler or TAQMAN) and qualitative PCR were the same as for renal and haematology patients.

One laboratory reported the use of quantitative PCR instead of antigenaemia in HIV patients. Thus, fewer laboratories that test on-site (4/18 versus 5/18 haematology transplants and 6/18 renal transplants) reported that the main test they use is a non-molecular method (antigenaemia or DEAFF).

Only 9/21 respondents indicated that a second test was used to test HIV patients for CMV. Of these, five laboratories indicated the use of on-site PCR-based tests, and three reported that antigenaemia or the DEAFF test is used.

Summary

1. Most respondents use a nucleic acid amplification (molecular) test for CMV detection in immunocompromised patients.

TABLE 29 *Main type of CMV test used on-site for HIV patients*

Type of test	No. of laboratories	Comments
Not applicable	3	3 labs: samples processed off-site
Real-time quantitative PCR	8	6 labs: LightCycler PCR 2 labs: TAQMAN
Quantitative PCR	3	1 lab.: in-house quantitative PCR on CSF 1 lab.: quantitative PCR mainly 1 lab.: in-house nested PCR
Qualitative PCR	3	2 labs: qualitative PCR 1 lab.: plasma PCR
Antigenaemia (pp65)	2	
Antigenaemia (pp65) and DEAFF	1	1 lab.: listed both these as main tests; uses 3 tests in total
DEAFF	1	

2. A small minority of laboratories use antigenaemia and DEAFF tests. It is recognised that antigenaemia may be suboptimal in neutropenic patients and is more likely to be fit for purpose for renal transplant patients than for stem cell transplant patients or those with advanced HIV infection.
3. No laboratory specifically reported use of the commercial NASBA Organon Teknika for pp67 detection, or the commercial Roche COBAS Amplicor Monitor Quantitative Assay or Roche Amplicor qualitative CMV PCR assay.
4. Laboratories using nucleic acid amplification tests had selected one of three technologies
   (a) 'in-house' nested PCR which may be quantified
   (b) real-time quantitative PCR using the LightCycler platform
   (c) real-time quantitative PCR using the TAQMAN platform.

Reasons given for use of more than one type of CMV test

Laboratories were asked to explain (if applicable) why a second type of test might be used in patients. From open text comments, it would appear that there is a general move towards using quantitative nucleic acid amplification assays for the assessment of CMV DNA, with many laboratories developing or using real-time quantitative PCR. Laboratories have largely abandoned the DEAFF test as a first-line test; however, as they move towards using molecular assays, laboratories may keep more traditional assays for patients in whom they work well (e.g. antigenaemia for renal transplant patients and others with a good peripheral white blood cell count).

Laboratories also sometimes use two tests if one gives a qualitative result and one a quantitative result. The qualitative test will be used to examine all samples and the quantitative assay reserved for those positive by the qualitative test. If the quantitative test is the more expensive, this strategy saves money.

Samples for PCR can be stored whereas those for antigenaemia and DEAFF cannot, so some laboratories may wish to keep the option of a PCR available for samples which need storage (e.g. arrive too late in the day to be processed on that day). Another practical consideration is the use of 'one-off' or 'stat' tests versus tests which can only be offered as a batch. The use of 'stat' testing for urgent (non-screening) samples is a well-established option for service providers. 'Stat' testing tends to be costly and reserved for particularly urgent diagnostic samples. Other laboratories may routinely use different tests for diagnosis as opposed to screening for CMV.

In addition, some laboratories may wish to use a second test to confirm the result of a first test. Finally, some laboratories reported they were using more than one test for validation purposes, and would cease to do this once validation of quantitative methods is complete.

Reporting times for CMV test results

Laboratories were asked for the average turn-around time for CMV tests (i.e. time from receipt of sample to despatch of report), whether carried out on-site or not. The range of reporting times for the 20/21 centres currently undertaking CMV testing is shown in *Table 30*; some laboratories identified an exact number of days, whereas others

TABLE 30 *Laboratory turn-around time for CMV tests*

Turn-around time (days)	Number of centres (%)
1	6 (30%)
1–2	3 (15%)
2	5 (25%)
1–3	1 (5%)
3	2 (10%)
10	2 (10%)
5 or 14	1 (5%)

specified a range. Overall, 70% of centres stated that they have a turn-around time of between 1 and 2 days on average. A smaller proportion (15%) indicated that their average turn-around time is up to 3 days. The remaining three laboratories stated that turn-around is up to 2 weeks; in these cases, samples were processed off-site.

Possible impact of technological advances on type of CMV test used

An open question asked respondents to comment on whether they considered that further technological advances are likely to impact on their laboratory's current form of CMV testing. Only four laboratories (18%) said 'No', 15 laboratories (73%) said 'Yes' and three laboratories did not offer a valid reply.

From laboratory responses it would appear that those laboratories not anticipating a change are mostly using real-time PCR. Real-time PCR was not available when the study protocol was drafted in 1996. By 1998 when the study was begun, real-time PCR was just being developed and a few laboratories had the necessary equipment for this technology.

This technology has a number of advantages over all previous methods of detecting CMV DNA and its rapid diffusion into practice since around 2000 has accelerated with the development of two commercial platforms for real-time PCR, the Roche LightCycler and the Roche TAQMAN.

It is therefore not surprising that real-time PCR had been adopted as the molecular method of choice by 8/21 laboratories surveyed in 2002. A further nine laboratories were planning a switch to quantitative forms of PCR testing (usually real-time PCR) once quantitative techniques had been validated in routine use.

For the future, the promise of DNA microarray technology was viewed with some enthusiasm. Thus far there are no microarray systems developed for detection of infectious disease markers in the routine diagnostic laboratory. Developments are being actively pursued and this may be the technology to supersede real-time PCR in tubes. Two respondents explicitly mentioned advances in DNA microarray technology.

CMV screening for different patient groups

Introduction of CMV screening

Laboratories were asked when CMV screening was first introduced for different types of patients. Although not all laboratories were able to provide this information, responses show that CMV screening was first introduced for renal transplant patients in 1992, and that this had continued to diffuse steadily since that time. For haematology and HIV patients, CMV screening was first introduced 2 years later in 1994. Its use had spread most rapidly in haematology transplant patients and least rapidly in patients with HIV.

The responses also indicate that when the HTA study started (end of 1998), fewer than half of the laboratories that reported CMV screening in 2002 had introduced this. There had therefore been a significant diffusion of CMV screening for immunocompromised patients during the period of the study.

CMV screening protocols

Laboratories were asked about any CMV screening protocols used in their hospitals. They were asked to record the duration of CMV screening (i.e. number of weeks in total during which screening takes place) for the three patient groups.

Haematology transplant patients

Sixteen laboratories provided information on CMV screening protocols for haematology transplant patients; eight centres specified absolute figures and eight specified a range. There was relatively little variation in the reported interval between screening tests but more variability in the duration of screening.

The overall pattern of responses is summarised in *Table 31*. This indicates that (where stated) weekly screening is most common and that the period of screening is not less than 3 months (12 weeks), with an almost equal number of laboratories screening up to 24 weeks (the study screening period) as for 16 weeks, and a very few reporting screening for longer than 24 weeks.

TABLE 31 *Haematology transplant patients: CMV screening period and test frequency*

Frequency of CMV screening tests	Period over which CMV screening undertaken				Total
	Not stated	Up to 12–14 weeks	Up to 16–24 weeks	Up to 24–52 weeks	
Every 3–5 days	1	2[a]			3
Every 7 days		4[b]	6[c]	2	12
Not stated		1		1[d]	2
Total	1	7	6	3	17

[a] Weekly, but maybe twice per week.
[b] 1 laboratory every 7–14 days.
[c] Every 2 weeks after first 6 weeks or 3 months.
[d] Day to day.

TABLE 32 *Renal transplant patients: CMV screening period and test frequency*

Frequency of CMV screening tests	Period over which CMV screening undertaken				Total
	Not stated	Up to 12–14 weeks	Up to 16–24 weeks	Up to 24–52 weeks	
Every 3–5 days	1[a]				1
Every 7 days		5[b,c]	2		7
Not stated	3[c]	1[d]			4
Total	4	6	2		12

[a] Continuous while in Trust.
[b] 1 laboratory every 7–14 days.
[c] *Ad hoc*/not routine/when ill.
[d] Week 4 and week 12.

In summary:

- Approximately half of laboratories (7/15 or 47%) reported screening for CMV in haematology patients until 12–14 weeks post-transplant, and (where stated) this screening is weekly or more frequently.
- Six centres (40%) undertake screening for longer (between 16 and 24 weeks post-transplant), all testing weekly (but one only for the first 6/24 weeks and another for the first 12/24 weeks, and then every 2 weeks).
- Only two centres (13%) provide CMV screening for longer than 6 months, both testing once per week.
- Three laboratories did not state either the screening period or the interval between screening tests.

Renal transplant patients

Thirteen centres provided information on their renal transplant CMV screening protocols; six centres specified absolute figures and seven specified a range.

The overall pattern of responses for renal patients is summarised in *Table 32*. This shows that, once again, weekly screening is most common and that the period of screening is not less than 3 months (12 weeks). Unlike the protocols reported for haematology patents, fewer laboratories screen up to 24 weeks and none reported a screening period longer than 24 weeks post-transplant (16 weeks was the study screening period).

Compared with the protocols reported for haematology patients, analysis of renal responses shows a clearer pattern in terms of duration and frequency of CMV screening:

- Nearly three-quarters of laboratories (5/7 or 71%) report that screening for CMV in renal transplant patients is undertaken until 12–14 weeks post-transplant. Where the screening interval is stated for these laboratories, this is weekly.
- Only two centres (29%) undertake screening until between 16 and 24 weeks post-transplant, both testing weekly.

TABLE 33 *Constraints on introduction of CMV screening tests*

Constraint	No. of laboratories (%)
Total respondents	22
Staff shortages: own staff not able to undertake extra tests	7 (32%)
Skill shortage: difficulty recruiting staff capable of undertaking test	5 (23%)
Cost of additional staff time	10 (45%)
Cost of consumables and kits	11 (50%)
Equipment costs (if applicable)	9 (41%)
Accommodation/working space	6 (27%)

- No centre undertakes CMV screening of renal transplant patients for longer than 6 months post-transplant.
- However, a further five centres did not state the period over which screening tests are carried out and/or the interval between screening tests.

HIV patients

The information provided on CMV screening protocols for HIV patients was limited. Only five laboratories provided a response stating that they undertook screening described as *'ad hoc'*; 'only if symptomatic'; 'not routine'; 'pro-active'; and 'continuous'. The use of HAART has changed the pattern of clinical disease seen in patients with longstanding HIV infection. Therapy has delayed progression to AIDS and the incidence of CMV disease has fallen. This has substantially reduced the pressure for CMV screening. Screening is therefore likely to adopt an '*ad hoc*' pattern as described in these responses.

Test preferences for CMV screening of immunocompromised patients

All respondents were asked for information on their first choice of test for CMV screening of immunocompromised patients. There were 19/22 respondents to this question. Most (16/19 or 84%) indicated a preference for a PCR-based test. Of these, five laboratories expressed a preference for some form of real-time quantitative PCR [LightCycler (three laboratories); TAQMAN (one laboratory); real-time quantitative PCR unspecified (one laboratory)]. A further five laboratories indicated a preference for other forms of quantitative PCR, and only six laboratories specified qualitative PCR tests. Of the remaining three laboratories, one specified DEAFF for HIV/renal transplants; one laboratory replied 'quantitative assay' (unspecified); and one laboratory expressed a preference for antigenaemia 'if the whole blood count is adequate'.

A 'second-choice' screening test was identified by 8/22 laboratories. Once again, the majority (5/8) indicated a preference for some form of PCR (one laboratory specifically mentioning quantitative PCR).

Barriers to introduction of CMV screening in UK laboratories

Laboratories were asked what constraints if any they had faced (or anticipated facing) when introducing CMV screening tests. Responses are summarised in *Table 33*.

One in three laboratories reported staff pressures within the laboratory as a constraint on the introduction of CMV screening tests and one in four report difficulties in recruiting skilled staff to conduct these tests as a constraint. Financial constraints were the most important factors likely to limit the introduction of CMV screening. In approximately half of laboratories, the costs of consumables/kits and of additional staff time were perceived to be an important obstacle to the introduction of CMV screening tests, as was equipment costs (although in slightly fewer laboratories). The accommodation/working space required was viewed as a constraint in only one in four laboratories.

Reported cost of CMV testing in UK laboratories

Finally, respondents were asked about the average cost of a CMV test conducted in their laboratory. They were asked to provide 2001 cost figures, if available, and to indicate whether these figures included the following five cost elements: staff salary costs; costs of consumables and kits; equipment costs; cost of accommodation; and hospital overhead costs.

Table 34 presents the cost figures provided by UK laboratories for different types of test. The care with which individual laboratories estimate true test costs, as opposed to a 'price' to be charged to purchasers, will vary. However, two-thirds of the test costs listed (13/21 or 62%) are reported to include all five cost elements listed above; the

TABLE 34 *Reported cost of CMV testing in UK laboratories*

Type of test	Laboratory cost (£)	Costs included				
		Salary	Consumables/ kits	Equipment	Accommodation	Overheads
Real-time quantitative PCR						
LightCycler PCR	40	✓	✓	✓	✓	✓
LightCycler PCR	20	✓	✓	✓	✓	✓
TAQMAN real-time PCR	32.25	✓	✓	✓	×	×
Quantitative PCR						
Quantitative PCR (Roche)	65	✓	✓	✓	✓	✓
Quantitative PCR (manual not real-time)	53	✓	✓	✓	×	✓
Quantitative PCR	48	✓	✓	✓	×	×
Quantitative PCR	25	✓	✓	✓	✓	✓
Qualitative PCR						
Plasma PCR	40	✓	✓	✓	✓	✓
Qualitative PCR	30	✓	✓	✓	✓	✓
Qualitative PCR	23	✓	✓	✓	✓	✓
Qualitative PCR	22	✓	✓	✓	✓	✓
Qualitative PCR	14–73	✓	✓	×	×	×
Qualitative PCR	9	✓	✓	×	✓	×
Antigenaemia (pp65)						
Antigenaemia	64	✓	✓	✓	×	✓
Antigenaemia	38	✓	✓	✓	×	×
Antigenaemia	22	✓	✓	✓	✓	✓
Antigenaemia	15	✓	✓	✓	✓	✓
Antigenaemia	10	✓	✓	×	×	×
DEAFF						
DEAFF	22	✓	✓	✓	✓	✓
DEAFF	17	✓	✓	✓	✓	✓
DEAFF	15	✓	✓	✓	✓	✓

✓, Included in cost estimate; ×, not included in cost estimate.

remaining one-third (eight cases) do not include accommodation and/or hospital overhead costs, and in three cases equipment costs are also not included.

Of the eight laboratories reporting the use of real-time PCR for CMV detection in haematology/renal transplant patients, only three provided an estimate of costs, two for LightCycler and one for TAQMAN. The cost per sample for the former is reported to be £20–£40 and for TAQMAN £32.25 (the latter figure does not include accommodation and hospital overhead costs).

Overall, four laboratories reported costs for other quantitative PCR tests, although only two laboratories used these for CMV detection in immunocompromised patients. The costs reported were generally higher than those for real-time PCR. One laboratory estimated a cost of £65 based on the use of a commercial quantitative PCR test (Roche Diagnostics). Two laboratories estimated the cost of their quantitative PCR tests at £48 and £53 (although neither of these figures included all cost elements) and one laboratory estimated the full cost of a quantitative PCR as £25 per sample tested.

Once again, although only three laboratories reported use of qualitative PCR tests for CMV detection (including one using plasma PCR), six laboratories were able to provide estimates for the cost of such tests. The plasma PCR test cost was estimated at £40. Three further laboratories provided full cost estimates for qualitative PCR tests, ranging from £22 to £30; two other laboratories provided estimates of £9 and £14–£73 (although these did not include all cost elements).

A wide range of antigenaemia costs was reported by the five laboratories using these tests, from £10 to £64 per test. The two laboratories whose costs included all five elements provided less disparate estimates (£15 and £22 per sample tested).

However, these findings do not allow discrimination of molecular tests on cost grounds. The costs of different molecular tests (2001 prices) reported by laboratories in the UK survey overlap: antigenaemia unit test costs are reported to range from £10 to £64 and those for qualitative PCR from £14 to £73 and other laboratories report the cost of tests such as qualitative PCR Roche as £65 per test and another non-specified quantitative PCR test as £25–£53.

The range of costs reported above is not unexpected. The actual cost of a particular test would be expected to vary depending on the grade of staff used, type and age of equipment, size of accommodation and type/location of hospital (and therefore central overhead costs). In addition, laboratory throughput will influence the amount of any fixed cost element finally attributed to an individual test. Test costs are considered in more detail in the next chapter.

By the time the study was completed, it was clear that real-time PCR offers key advantages and the study laboratory had chosen to use it in preference to any of the molecular tests which were available in 1996 or in 1998.

Chapter 5

Costs of antigenaemia and molecular tests

Introduction

In order to assess the relative cost-effectiveness of introducing alternative molecular test CMV screening regimes, the study had first to estimate the costs of performing these various tests, and then compare the resources used for a number of different scenarios. A combination of a primary costing approach (i.e. obtaining cost information relating to costs faced by the UHW in Cardiff, where the evaluation occurred), together with some cost modelling (i.e. for the purposes of sensitivity analysis), was used. Sensitivity analysis made some allowance for potential variations in costs. Differences contingent upon differential staff gradings and potential differences in the amount of time expended undertaking the tests were taken into account. Thus separate sensitivity analyses allowed for differences in costs associated with either having an MLSO grade one or grade two conduct the test and also allowed for a ±10% difference in staff time expended.

A detailed cost analysis was carried out, first for the four main diagnostic tests studied:

1. CMV pp65 antigenaemia assay.
2. Semi-quantitative cell-associated CMV DNA detection by PCR (nested in-house PCR). Costs were assessed assuming both a manual extraction process and an automated extraction process. Costs were calculated for single round and two round nested PCR.
3. Qualitative NASBA late messenger RNA (pp67). Costs were assessed assuming both a manual extraction process and an automated extraction process.
4. Qualitative cell-free CMV DNA detection by PCR (Roche Amplicor Assay).

In addition at a later date, because the COBAS Amplicor Monitor Assay (Roche), became available, we also made some assessment of the costs involved with its use.

Methodological approach to the cost analysis

During stage one of the study, a detailed study of variable costs (including consumables) and semi-variable costs (including labour costs) was conducted for the four main tests. Costing was then subsequently carried out using the most recently available resource costs to ensure costings were as up to date as possible. The costing was conducted from the perspective of the NHS and the principal focus was on the direct cost of performing each diagnostic test. **Capital costs** were not separately calculated; these were instead assumed to be subsumed within other costs. For commercial 'kit-based' assays the required capital equipment tended to be provided by those supplying the test kits and capital costs were therefore built into suppliers 'kit' prices. For 'in-house' assays, locally purchased capital equipment was used. This equipment was not purchased solely or specially for CMV assays but was shared by many users, both for research and diagnostic purposes. General-use equipment was made available to the study by the department at UHW and does not appear as a funding stream for the study. Therefore, to avoid the danger of 'double counting', it was not considered appropriate to cost and build in capital costs separately in the way we had originally envisaged since these were largely subsumed in other costs.

Overhead costs associated with the laboratory space used (which varies by sample batch size) were allowed for by making an apportionment of capital charges levied, associated with the laboratory's occupation of space for the purposes of undertaking a CMV test. Cleaning costs were also apportioned in a similar way, using the cost of cleaning rather than capital charges as the basis of apportionment. Ideally, we would have liked to also look at the costs associated with heating, lighting and power consumption. Unfortunately, UHW Trust personnel informed us that it would be impractical to obtain reliable costings for these. However, the costs of these items are likely to be negligible relative to other costs of testing.

The cost modelling involved assessing costs based on actual amounts of time expended by laboratory staff who were based at UHW. In order to calculate labour costs, a health economist from Warwick University visited the laboratory at UHW to observe working patterns for each of the tests (the

TABLE 35 *Baseline analysis – test costs for batch size of five samples*

	Cost per sample (batch of 5) (£)	
	MLSO 1	**MLSO 2**
pp65 antigenaemia	22.36	25.80
Quantitative CMV PCR – manual extraction (nested in-house PCR) – 1 round	10.56	11.84
Quantitative CMV PCR – automated extraction (nested in-house PCR) – 1 round	12.49	13.33
Quantitative CMV PCR – manual extraction (nested in-house PCR) – 2nd round	13.46	15.28
Quantitative CMV PCR – automated extraction (nested in-house PCR) – 2nd round	15.17	16.16
Qualitative NASBA – manual extraction (Biomerieux)	30.27	33.04
Qualitative NASBA – automated extraction (Biomerieux)	31.20	33.58
Qualitative CMV PCR (Roche Amplicor)	27.47	28.65
COBAS CMV PCR (Roche COBAS Assay)[a]	Not available	Not available

[a] COBAS had to be costed using list prices supplied by Roche, because the actual kits were donated rather than charged for.

only exception to this approach related to costing of COBAS, where a laboratory technician monitored time expended, rather than a health economist). In the interests of trying to ensure the generalisability of findings to a routine service setting, working patterns were observed only after the personnel involved had become familiar with a test, rather than during the initial learning period. Particular care was taken to observe the actual working time spent on testing (it should be noted however that a small allowance was nevertheless made for morning coffee breaks, because these are thought to be common practice in many laboratories in the UK). This means that we did not simply use start and finish times for testing. Given the staged nature of some of the tests, very often staff may well be involved in other activities in the laboratory during times when they are not actually working on the test in question. Therefore, particular care was taken to ensure that only actual time expended undertaking a test (plus unavoidable deadtime) was reflected in estimates of labour time expended. An allowance (25 minutes per break) was also made for morning coffee breaks, common practice in many laboratories throughout the UK. Information on **consumable costs** used in the testing process was also obtained from the PHLS based at UHW (on rare occasions this was not possible, i.e. Roche COBAS). These reflect the cost of such items as test kits, reagents and disposable items. Consumable costs were quantified and valued at market rates, based wherever possible on actual resource costs at the UHW. However, in the event that kits were provided free of charge (so the test could be evaluated, i.e. Roche COBAS), list prices from suppliers were used.

Average costs were calculated and the effect of batch size on cost per test was also considered. Because some tests could only be conducted economically in given defined batch sizes (which sometimes varied between tests), this undermines strict comparability of relative costs between all CMV tests and batch sizes. Nonetheless, we attempted to make comparisons whenever practicable.

Costs not associated with diagnostic test performance (such as those arising from sample transportation, laboratory reception costs and the costs of despatching test results) were excluded. It is possible that some of these costs might be affected by the choice of CMV test selected. For example, some tests which are only feasible in large batch sizes may be associated with economies of scale when it comes to transportation costs or the dispatching of results. However, it was considered that such costs are inevitably very much related to the individual circumstances faced by laboratories and the nature and location of hospitals that they serve. Therefore, it would be virtually impossible to cost them meaningfully based on experiences at only one site, in a way that would be generalisable elsewhere.

Baseline analysis of comparative costs

A baseline analysis of comparative costs was undertaken to include overhead, labour and consumable costs for various batch sizes. Full details are presented in Appendix 11. In the analyses below, costs assuming the use of an MLSO scale one are presented in roman font, followed by the equivalent costs for an MLSO scale two in italics.

Batch sizes of five samples

Assuming a batch size of five, *Table 35* shows that the least expensive test was single-round nested in-

TABLE 36 *Baseline analysis – test costs for batch size of one sample*

	Cost per sample (batch of 1) (£)	
	MLSO 1	**MLSO 2**
Quantitative CMV PCR – manual extraction (nested in-house PCR) – 1 round	23.36	27.22
Quantitative CMV PCR – automated extraction (nested in-house PCR) – 1 round	23.18	25.60
Quantitative CMV PCR – manual extraction (nested in-house PCR) – 2nd round	32.32	37.80
Quantitative CMV PCR – automated extraction (nested in-house PCR) – 2nd round	31.22	35.37
Qualitative NASBA – manual extraction (Biomerieux)	Not available	Not available
Qualitative NASBA – automated extraction (Biomerieux)	Not available	Not available
Qualitative CMV PCR (Roche Amplicor)	Not available	Not available
COBAS CMV PCR (Roche COBAS Assay)[a]	Not available	Not available

[a] COBAS had to be costed using list prices supplied by Roche, because the actual kits were donated rather than charged for.

house PCR (manual extraction) at £10.56 per sample (*£11.84 for MLSO 2*), then automated extraction single-round nested PCR. Two-round nested PCR (manual extraction) was the next least expensive test at £13.46 per sample (*£15.28 for MLSO 2*) followed by the automated variant of the two-round test. This is followed by the pp65 antigenaemia test at £22.36 per test (*£25.80 for MLSO 2*) and Roche Amplicor Assay at £27.47 per sample (*£28.65 for MLSO 2*). The most expensive test was NASBA at £30.27 per test (*£33.04 for MLSO Two*) for manual extraction and £31.20 (*£33.58 for MLSO 2*) for automated extraction. A costing for COBAS for a batch of five samples was not available owing to the impracticalities of conducting this test for such small volumes of tests. However, given that at higher volumes (i.e. a batch size of 12 samples) this test was estimated to cost £49.86 per sample (*£50.45 for MLSO 2*), it would have been the most expensive of the tests in *Table 35*, had it been run for a batch size of five samples.

Batch size of one sample

Analyses were also undertaken to calculate costs for a single sample batch size, that is, 'one-off' or 'stat' testing. Because the clinical relevance of a CMV result is highest if it is available within a few hours, optimum patient management may require more expensive one-off tests to be performed. Unfortunately, it was not possible to estimate one-off costs in relation to some of the tests. However, a comparison could be made between the two tests that worked out least expensive for a batch size of five (i.e. pp65 antigenaemia and nested in-house PCR). This indicates that the semi-quantitative nested PCR test remains the least expensive test of the two. *Table 36* shows that the automated extraction of single-round PCR is least expensive, followed by the manual version. The two-round version, automated and then manual extraction, is the next most expensive. However, all variants of the in-house nested PCR test proved much less expensive than the pp65 antigenaemia test [at £73.60 per test (*£87.99 for MLSO 2*)]. Moreover, given that the cost of two-round nested in-house PCR for a one-off test is similar to the cost of NASBA for a batch size of five samples, it would appear unlikely that either variant of the NASBA tests will be the least expensive at low volumes (this is because the cost per test is generally higher at lower volumes). Figures for Roche Amplicor and COBAS were not available at such low volumes.

Larger batch sizes

Of the molecular tests, it would appear that in-house nested PCR is the least expensive at higher volumes of batch size 30 (see Appendix 11). Using the single-round variant, it is estimated to cost £8.14 (*£8.94 for MLSO 2*) using manual extraction and £10.50 using automated extraction (*£11.05 for MLSO 2*). Nested two-round PCR costs £9.89 per sample (*£11.03 for MLSO 2*) using manual extraction and £12.19 per sample (*£13.13 for MLSO 2*) using automated extraction. This is less expensive than both versions of qualitative NASBA, with the manual extraction variant costing £22.96 per sample (*£24.16 for MLSO 2*) and the automated extraction variant costing £25.50 per sample (*£26.67 for MLSO 2*).

The only costing we were able to obtain for qualitative CMV PCR (Roche Amplicor Assay) was for a batch size of 32 at £23.36 per sample (*£23.66 for MLSO 2*), which is roughly comparable to manual extraction NASBA at these volumes. COBAS is likely to be by far the most expensive test. Indeed it has been costed at £48.68 per sample for a batch size of 24 (*£49.01 for MLSO 2*).

It was not possible to calculate costs for pp65 antigenaemia tests using a batch size of 30, since this batch size was not possible in the laboratory. For a batch of 20 antigenaemia test samples however, the cost was calculated to be £13.03 per sample (*£14.47 for MLSO 2*). Assuming the same rate of decline in costs between a batch size of five and one of 20 samples, and between a batch size of 20 and 30, then the cost of antigenaemia can be projected to fall to around £6.22 per test. However, that is a fairly strong assumption, and it could well be that the rate of decline reduces at higher volumes, owing to more limited scope for further economies of scale.

Sensitivity analysis

In order to consider the likely effect, on the test costs calculated, of varying the assumptions made in the costing study, a sensitivity analysis was conducted. This examined the impact upon cost estimates of the use of MLSO grade one or MLSO grade two staff; it also looked at the impact of a ±10% change in staff time expended.

Sensitivity analysis for a batch size of 5 tests

For a sample batch size of five tests, allowing for possible changes in both the grade of staff employed, and a ±10% change in staff time expended, the cost of the tests is within the following ranges. Results are detailed in Appendix 12. Overall, the sensitivity analysis demonstrates that

- The cost of a pp65 antigenaemia test ranges from £21.07 to £27.44.
- In relation to single-round nested in-house PCR, the unit test cost varies from £10.07–£12.45 (manual extraction) to £12.17–£13.72 (automated extraction).
- For two-round nested in-house PCR, unit test cost varies from £12.77–£16.15 (manual extraction) to £14.63–£17.29 (automated extraction).
- For NASBA, the cost varies from £29.23–£34.35 (manual extraction) to £30.30–£34.71 (automated extraction).
- For the qualitative Roche Amplicor Assay, the cost varies between £27.02 and £29.21.

The implications of the above are that the most inexpensive test appears to be nested in-house PCR (manual extraction) with the single-round variant inevitably cheaper and the automated single-round version and the two-round versions marginally more expensive.

Another clear finding that emerges from the sensitivity analysis is that even comparing the lowest range costs for pp65 antigenaemia, (£21.07) with the highest range costs for two-round nested in-house PCRs (£16.15 with manual extraction and £17.29 with automated extraction), it is evident that nested in-house PCR is always a less expensive option than pp65 antigenaemia for a batch size of five.

Interestingly also, it appears that the NASBA test – both manual and automated extraction – is always more expensive than pp65 antigenaemia. Comparing the highest estimate of cost for pp65 antigenaemia (£27.44) with the lowest estimates of cost for qualitative NASBA (£29.23 with manual extraction and £30.30 with automated extraction), it is clear that antigenaemia enjoys a clear cost advantage for a batch size of five samples.

The sensitivity analysis does indicate, however, that the cost of the qualitative Roche Amplicor Assay, although not quite in the same price range as NASBA (the highest estimate of its cost is £29.21), is very close to being in the same price range (the lowest estimate of price for manually extracted NASBA is £29.23). Moreover, it would appear that this test is actually less expensive than both variants of NASBA, although not by a particularly large margin.

Sensitivity analysis for larger batch sizes

It did not always prove possible to observe identical batch sizes to estimate costs at higher volumes. This was partly constrained by the fact that batch size had to reflect actual numbers of specimens coming through the laboratory. It was also constrained by the fact that some tests need to be conducted in defined units (i.e. Roche Amplicor Assay for 32, 64 or 96 samples or COBAS for 12 or 24 samples). The implications of these assumptions for changes in relative costs at higher batch size are detailed in Appendix 12. Overall, the sensitivity analysis indicated that

- The cost of a pp65 antigenaemia test ranges from £12.49 to £15.16 (batch size 20).
- For single-round nested in-house PCR, the unit test cost varies from £7.84–£9.33 (manual extraction) to £10.29–£11.32 (automated extraction) (batch size 30).
- For two-round nested in-house PCR, the unit test cost varies from £9.46–11.57 (manual extraction) to £11.83–13.59 (automated extraction) (batch size 30).

- For NASBA, the cost varies from £22.51–24.74 (manual extraction) to £25.06–27.22 (automated extraction) (batch size 30).
- For the qualitative Roche Amplicor Assay, the cost varies between £23.25 and £23.80.

The implications of the above are that with larger batch sizes it is less clear that nested in-house PCR is necessarily the least inexpensive test. Our estimate of cost for pp65 antigenaemia for a batch size of 20 (£12.49–15.16) is likely to overestimate the cost for a batch size of 30, because of economies of scale. However, just how much costs would further decline if the batch size rose from 20 to 30 is unclear. What is clear, however, is that of the molecular methods, the single-round nested in-house PCR (manual extraction) variant is cheapest, with a price in the range £7.84–9.33 for a batch size of 30. Moreover, even using optimistic assumptions about the rate of decline in the cost of pp65 antigenaemia as the batch size increases from 20 to 30, we would not anticipate that antigenaemia would be much lower in cost than a single-round (manually extracted) nested in-house PCR, and it may in reality not enjoy a cost advantage over this test. However, in relation to some of the more costly variants of this nested PCR test, antigenaemia may enjoy a cost advantage.

Once again it would appear that the NASBA test (manual extraction) is always more expensive than pp65 antigenaemia (as for a batch size of five). Moreover, as before (for a batch of five samples), NASBA is always more expensive than any of the variants of the nested in-house PCR test.

Further, the small price advantage of the Roche Amplicor Assay relative to NASBA, which applies for a batch size of five samples, seems to be maintained at higher batch sizes. It would appear that the Roche COBAS test is simply much more expensive than any of the other molecular tests.

In conclusion, the order of relative costs of the molecular based tests is not affected very much by differences in the number of samples being processed in a batch. However, as one might expect, the cost of processing samples does tend to fall as the number of samples increases, owing to economies of scale. What is less clear (because of a paucity of data available on the costs of antigenaemia testing using larger batch sizes) is the extent to which the clear cost advantage of each of the versions of nested in-house PCR for a batch size of five samples may be eroded as the batch size increases to around 30 samples. The fact remains, however, that even using these larger batches the relative costs of the molecular-based tests are little affected by an increase in the batch size from five to 30. This is a finding that we would expect to be replicated at the average throughput of 9.4 samples per week, observed at UHW, if samples were processed together. At this batch size, we would not expect antigenaemia to have a cost advantage over any of the variants of nested in-house PCR tests.

Chapter 6

Clinical impact of CMV screening tests

Introduction

Evaluation of CMV screening tests should include assessment of patient outcomes and clinical and cost-effectiveness, adapted Fineberg hierarchy levels four and five, respectively.[30,57] During the study, molecular CMV screening tests were trialled in a service setting so that these final levels in the assessment hierarchy could be addressed. This chapter and the next cover the first of these, and the final level (cost-effectiveness) is considered in Chapter 8.

In the study, clinicians were dependent on a combination of clinical symptoms, laboratory test results and other diagnostic investigations when making decisions about the management of their patients, and any clinical impact of CMV screening tests must be viewed in this context.

Clinical signs and symptoms

Clinical diagnosis of CMV disease relies upon presentation of a range of signs and symptoms, as shown in *Table 37*. However, as pointed out earlier, many of the presenting symptoms are non-specific and may be due to a wide variety of causes.

Hence a purely clinical diagnosis can lead to an incorrect conclusion and inappropriate use of antivirals. This, in its turn, may lead to possible loss of the transplant, prolonged hospital stay, delay in engraftment and systemic toxicity affecting particularly the bone marrow and liver. Clearly, in such a situation other sources of information are necessary to support any diagnosis and to inform patient management.

Other diagnostic investigations

In addition to laboratory CMV tests, other investigations may also be used by clinicians in order to identify clinically significant CMV infection and its location (*Table 37*). These include various imaging modalities [e.g. X-ray, magnetic resonance imaging (MRI), computed tomography (CT), ultrasound scans] and bronchoscopy. However, definitive diagnosis of CMV infection will usually involve histological findings, possibly including post-mortem examination. This information is, of course, not available for all cases.

Measurement of impact on diagnosis and patient management

Assessing diagnostic tests (includes screening, monitoring and primary diagnosis) is notoriously difficult for various reasons. This may be because of local forms of disease manifestation or clinical practice, the interplay between the different tests/investigations used, a lack of standardisation or the fact that diagnostics is further removed from the clinical outcome than therapeutics.[59] The optimal design for assessing the accuracy (sensitivity and specificity) of a diagnostic test is considered to be a prospective blind comparison of the test, and a reference test in a consecutive series of patients should be taken from a relevant clinical population. This was the approach adopted in the current study. A recent systematic review of the literature has demonstrated that strict application of such methodological criteria would invalidate the application of most study results.[60]

Not only are there difficulties in the technical evaluation of diagnostic tests, there are even more difficulties in assessing clinical impact. This is principally because information from one test may be combined with that from various other sources, e.g. clinical signs and symptoms, other laboratory tests, imaging and other investigations. Furthermore, the information provided by a particular test may have a number of subtle effects, including simply changing the level of diagnostic certainty or confirming patient management that is already planned (reassurance). These subtle effects are almost impossible to capture retrospectively, and this may result in the benefits of a particular test being undervalued. This is particularly likely to be the case for screening tests such as those considered here.

Hence one of the strongest study designs for evaluating the clinical impact of a diagnostic test is a prospective study in a consecutive series of patients.[58] This should capture data under conditions of uncertainty, with recording of planned patient management *ex ante* as the test is ordered, and then actual management *ex post* on receipt of test results. This was the approach

TABLE 37 *Criteria for CMV syndromes*[a]

CMV disease	Presumptive criteria	Definitive criteria
CMV Pneumonitis	None	Symptoms: dyspnoea, cough or fever, with hypoxaemia and infiltrates on chest X-ray, *plus* histology showing typical lesions or detection of antigen from affected tissue, *and* absence of other pathogens or persistence of symptoms after appropriate treatment for other pathogens (because CMV is uncommon as the sole aetiology of pneumonia, exclusion of other pathogens requires negative routine bacterial, fungal and mycobacterial cultures of uncontaminated sputum or bronchoalveolar lavage and negative stains for *Pneumocystis carinii* pneumonia and toxoplasmosis)
CMV retinitis	*Symptomatic or asymptomatic plus signs:* typical lesions include fluffy white areas with or without haemorrhages and/or grey–white areas or retinal necrosis with or without haemorrhages. **Must** be confirmed by an experienced ophthalmologist using dilated fundoscopy	None
CMV gastrointestinal disease	None	Symptoms: retrosternal pain, pain on swallowing, or dysphagia, abdominal pain, diarrhoea, rectal pain; *plus* histology showing typical lesions or detection of antigen from affected tissue, *plus* endoscopy showing mucosal erythema, oedema, friability, erosion or *ulceration*.
CMV meningoencephalitis	*Symptoms:* rapid (days to 4 weeks) syndrome with progressive delirium, cognitive impairment ± seizures and fever (often with other SMV end-organ disease), fatal outcome unless treated, *plus*: CT/MRI may show periventricular abnormalities with or without contrast enhancement, CSF may be normal, or show evidence of CMV by PCR, or rarely by culture, typical lesions on cytology	Symptoms plus histology or autopsy
Other CMV end-organ disease	None	*Symptoms:* compatible clinical presentation, *plus* histology showing typical lesions or detection of antigen from affected tissue

[a] Based on the 1993 CDCP (US) definition (MMWR 1992; 41 [No RR-17]): 1–19 and modified for this trial. Criteria for CMV syndromes based on ACTG 204 trial.

adopted in the present study. It had the added benefit that *ex ante* responses could be used to model the likely impact of tests that were performed in the laboratory but not fed back to clinicians. In order to gather the relevant information on CMV test impact and perceived benefits, a series of structured proformas were developed for completion by the clinician at the point when a CMV test was requested for a patient and on receipt of each test result. These proformas recorded the patient's clinical status and the clinician's level of diagnostic certainty at the time of test request, and any subsequent impact on diagnostic certainty and patient management of the test result. The clinician's retrospective judgement on whether an individual test result had been of benefit to the patient was also recorded to allow for the existence of a 'latent' benefit that is not reflected in changes to patient management such as reassurance.[57] Slightly

TABLE 38 *Sequence of administration and content of clinical questionnaires*

Questionnaire	Time completed	Questionnaire content
Baseline Questionnaire A	At recruitment into study	• Age/gender • Type/date of transplant • Donor/recipient CMV status
Request Questionnaire B	At the point of requesting each CMV test	• Clinical status • Current drug therapy • *Ex ante* likelihood of CMV disease • *Ex ante* likely impact of positive test result on therapy and further investigations • *Ex ante* likely impact of negative test result on therapy and further investigations
Result Questionnaire C	On receipt of each screening test result	• Impact of actual result on diagnostic certainty • Changes planned to patient management • Perceived benefit

different questionnaires were developed for the two main patient groups (renal and haematology transplants). These were administered at specific points during the CMV screening process. *Table 38* shows the sequence of administration and content of each questionnaire.

Questionnaire A was completed at entry into the study for each patient recruited. Following this, a structured request form B had to be completed and attached to all CMV samples sent to the laboratory for that patient, and a structured questionnaire C was sent out with each CMV test result, for completion and return to the laboratory. Once a patient had been recruited to the study, the virology laboratory adopted a policy of not accepting CMV samples for that individual without a structured request form; all missing forms were diligently chased, as were any questionnaires attached to test results that were not returned to the laboratory. All questionnaires were administered by staff in the laboratory and coded with the patient's study number before being sent for data analysis.

Questionnaires were developed in conjunction with clinicians in the Renal and Haematology Departments at the UHW and piloted prior to use. The format was adapted slightly at the end of stage one, following feedback from clinicians, in order to improve comprehension and layout and to simplify data collection.

Completed questionnaires

For the 98 renal and 140 haematology transplant patients recruited to the prospective study, a total of 1381 **request** forms were received in the laboratory during the study period. Of these, 104 were subsequently excluded from the analysis for various reasons, including 18 samples that were for storage only, six forms without a sample, 39 that were requested **before** transplantation and therefore fell outside the post-transplant screening protocol and 41 where there was no response from the clinician on receipt of the test result.

The number of **results** forms returned to the laboratory totalled 1416. Of these, 139 were excluded from the final analysis, including three forms without a matching sample, 39 that were for tests performed before transplantation, six samples that were for storage only and 91 with no completed test request form.

Table 39 shows the pattern of returned questionnaires separately for each stage of the study and *Table 40* for the two patient groups studied. During both stages of the prospective study, clinicians received two types of CMV test result routinely (a molecular test and antigenaemia). In stage one, the molecular test was first-round, nested in-house PCR and in stage two it was NASBA. There were no significant differences between the two stages, or between the renal and haematology groups, in terms of return of questionnaires. The numbers of questionnaires available for analysis is shown in *Table 41* separately for each type of transplant patient and for each stage of the study.

The forms finally used to analyse impact on patient management therefore included 1277

TABLE 39 *Numbers of returned questionnaires for each stage*

Questionnaire	Stage 1 (130 patients)	Stage 2 (108 patients)	Total (238 patients)
Baseline Questionnaire A	126	105	231
Request Questionnaire B	623	759	1382
Result Questionnaire C	613	803	1416

TABLE 40 *Numbers of returned questionnaires for each patient group*

Questionnaire	Renal transplants (98 patients)	Haematology transplants (140 patients)	Total (238 patients)
Baseline Questionnaire A	98	133	231
Request Questionnaire B	591	791	1382
Result Questionnaire C	618	798	1416

TABLE 41 *Numbers of analysed questionnaires (request + result forms)*

Patient group	Stage 1 (130 patients)	Stage 2 (108 patients)	Total (238 patients)
Renal (98 patients)	269	304	573
Haematology (140 patients)	418	286	704
Total	687	590	1277

request forms and an equal number of results forms, or 2554 questionnaires in total.

Completion rates for returned questionnaires were generally high, with 75–90% of clinical and other key data being provided.

Clinical signs and symptoms

Table 42 presents the incidence of clinical signs and symptoms recorded at the point when tests were requested. The frequency of symptoms (relative to the number of samples sent for testing) is shown separately for diagnostic and screening tests, for the two patient groups studied and for samples judged to be true positives versus those which proved to be negative. Significant differences are evident between diagnostic and screening tests with a higher likelihood of fever, chest symptoms or graft versus host disease being present ($p < 0.001$) when a diagnostic test was requested. This is entirely to be expected. Significant differences were also recorded between renal and haematology patients, with a greater likelihood of fever ($p < 0.001$) or chest symptoms ($p < 0.01$) being reported for haematology patients.

The value of clinical signs and symptoms in discriminating CMV infection was, however, minimal. The final columns show that the only significant difference between true positive and negative samples was limited to the presence of fever ($p < 0.05$). In cases where CMV disease was suspected, clinicians were also asked to indicate where the site of infection was thought to be. There was no pattern in the predicted site of any CMV infection; most clinicians said 'unknown' or 'systemic'. Clinicians were also asked about other (bacterial) infections. Clinicians recorded a documented bacterial infection on 39 request forms (28 patients); these infections were predominantly recorded for haematology patients (24 cases) rather than renal patients (four cases).

Patient CMV infection patterns and outcomes at end of screening period

The clinical impact of a CMV screening strategy will be largely, although not exclusively, dependent on the number of cases of CMV disease identified. CMV disease patterns (observed and predicted) were analysed and compared with reported patient

TABLE 42 *Pattern of clinical signs and symptoms at test request*

	Diagnostic no. (% of tests)	Screening	Renal	Haematology	True positive	True negative
Fever	47*** (27%)	126*** (11%)	17*** (3%)	156*** (22%)	17* (21%)	156* (13%)
Chest symptoms	20*** (12%)	16*** (1%)	7** (1%)	29** (4%)	5 (6%)	31 (3%)
GVHD	19*** (21%)	30*** (5%)		49 (7%)	3 (4%)	46 (4%)
Raised[a] WBC	5 (9%)	66 (16%)	71 (15%)		8 (16%)	63 (13%)
High[b] platelets	6 (11%)	41 (10%)	47 (10%)		3 (6%)	44 (10%)
Raised[c] creatinine	18 (90%)	147 (71%)	165 (73%)		3 (75%)	162 (71%)

[a] Normal white blood cell (WBC) count = 4.0–10.5 x 10^9/l.
[b] Normal platelet count = 150–400 x 10^9/l.
[c] Normal creatinine = 60–120 µmo/l.
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

outcomes (successful transplant, relapse/failure, death) at the end of the screening period.

Table 43 presents information for the renal transplant group on the predicted likelihood of CMV disease (based on published risk factors) and actual CMV cases, the final screening outcome recorded for these patients at 16 weeks (i.e. at the end of the screening period) and, in the last three columns, information on longer term outcomes for these same patients.

Table 43 demonstrates that the number of CMV cases in the renal cohort was lower than predicted (13% versus 21%). In terms of the final 'screening outcome' at 16 weeks, one patient had died, two suffered a transplant failure and the remainder were judged 'successful'. Transplant failures and death rates were concentrated in the two groups with the greatest likelihood of CMV disease. The longer term outcome information shows that the one further patient who died was in a high CMV risk group; there were no additional transplant failures recorded. CMV was not judged to be a contributory cause of death in either case.

Table 44 presents a similar analysis for haematology transplant patients. It illustrates that the incidence of CMV cases in the haematology group was again slightly lower than predicted; in the 140 patients included in the prospective study one might predict 8.6 cases of CMV, whereas only six study patients had at least one (true) positive CMV sample.

Examination of final 'screening outcome' at 24 weeks shows that 30 patients had died, six patients relapsed and 104 were reported as successful transplants at this stage. Death rates were highest in the allogeneic transplant group, although this is not related to predicted likelihood of CMV disease. In terms of relapse, the highest rates were recorded in autologous transplant patients, none of whom had a high likelihood of CMV disease. Longer term outcomes available at the end of the study period show that 43 patients had died, nine patients relapsed and 88 were reported as 'successful' transplants. Of the patients who died, only 3/30 who died within the screening period had a positive CMV sample and only 4/43 whose longer term outcome was recorded as death had a positive test result. However, CMV was recorded as a contributory cause of death in one patient within the screening period (of the three patients with a positive sample within this period) and for no further cases in the longer term.

Comparison of the two patient groups shows that, in terms of CMV disease, the level identified was lower than expected for renal patients at 13% (versus 21% expected), whereas for haematology transplant patients it was largely as expected (4% versus 6%). The lower level of CMV disease observed in renal transplants appears to be linked to a lower than expected incidence in the R+D+ group (3% versus 25%). The likelihood of death or transplant failure was low (<5%) among renal transplant patients but much higher for

TABLE 43 *Renal transplants: serostatus of recipients and donors, number of CMV cases, final 'screening outcome' at 4 months and longer term outcome*

CMV status	No. of patients	Probability of CMV[a] (95% CI)	No. of CMV cases in study sample		Final 'screening outcome' at 16 weeks[d]			Longer term recorded outcome[d]		
			Predicted[b]	Actual[c]	D No. (%)	F No. (%)	S No. (%)	D No. (%)	F No. (%)	S No. (%)
R–D–	22	0% (0 to 15)	0	0	0	0	22 (100%)	0	0	22 (100%)
R–D+	28	44%[e] (29 to 61)	12.3	12 (43%)	0	1 (3.5%)	27 (96%)	1 (3.5%)	1 (3.5%)	26 (93%)
R+D–	18	6% (2 to 16)	1.1	0	0	0	18 (100%)	0	0	18 (100%)
R+D+	30	25%[e] (17 to 37)	7.5	1 (3%)	1 (3%)	1 (3%)	28 (94%)	1 (3%)	1 (3%)	28 (94%)
Total	98	21%	20.9 (21%)	13 (13%)	1 (1%)	2 (2%)	94 (96%)	2 (2%)	2 (2%)	94 (96%)

D, donor; R, recipient.
[a] Likelihood of CMV infection in patient group.
[b] Predicted no. of cases based on 1.
[c] Patients who had at least one true positive CMV test.
[d] D, died; F, transplant failure; S, successful.
[e] Significantly different from R–D–.
Data from Fox *et al*. (1995)[4] and Emery *et al*. (2000).[5]

haematology transplants, with 25% dead or relapsed at the end of the 6-month screening period and 37% at longer term follow-up. Hence the potential for improvement is higher in this patient group.

Overview of impact of CMV screening test results

If one examines all test results sent to clinicians (of which only 80/1277 or 6.3% were positive), the number of reported effects is low. Clinicians recorded that a test result (positive or negative) led to a change in patient management in fewer than 5% (61 test results). Changes reported included the start of CMV therapy linked to 4% (50/1277) of test results, and ordering of further investigations (X-rays, CT/MRI or bronchoscopy) in 3% (38/1277) of cases. These figures exclude any reassurance effects.

Stage 1 versus Stage 2 responses (all patients)

Because the molecular test provided differed between the two stages of the study, responses were analysed separately for stage one and stage two patients, as shown in *Table 45*. Analysis of the 687 results questionnaires for stage one patients shows that one in six test results (17%) were reported to have any impact on diagnostic certainty, with 1% leading to a reduction in certainty.

In terms of impact on patient management, 5% (37 tests) resulted in CMV treatment being initiated and only one test led to existing CMV treatment being stopped. There was also a limited effect on prescribing of other drugs; it was reported that one patient was treated with antibiotics and one patient with intravenous immunoglobulins (IVIg) as a consequence of the CMV test result. In addition, 5% (32/655) of the test results were reported to have resulted in one or more further investigations being ordered (e.g. X-rays). If only screening test requests are considered (see *Table 46*), the observed pattern of clinical impact remains very similar.

In retrospect, clinicians identified just over half (53%) of the stage one CMV test results as being of benefit, with 44% identified as of no benefit. *Table 46* shows that the figures are only slightly less favourable if screening tests alone are considered, with 48% being reported as of benefit.

TABLE 44 *Haematology transplants: serostatus of recipients and donors, number of CMV cases, final 'screening outcome' at 6 months and longer term outcome*

CMV status	No. of patients (% sample)	Probability of CMV[a] (95% CI)	No. of CMV cases in study sample		Final 'screening outcome' at 16 weeks[d]			Longer term recorded outcome[d]		
			Predicted[b]	Actual[c]	D No. (%)	R No. (%)	S No. (%)	D No. (%)	R No. (%)	S No. (%)
Allogeneic transplant										
R–D–	15 (10.7%)	3% (1 to 11)	0.45	0	6 (40%)	0	9 (60%)	8 (53%)	0	7 (47%)
R–D+	4 (2.9%)	4% (1 to 21)	0.16	1 (25%)	2 (50%)	0	2 (50%)	3 (75%)	0	1 (25%)
R+D–	8 (5.7%)	44%[e] (27 to 63)	3.52	2 (25%)	5 (63%)	0	3 (38%)	5 (63%)	0	3 (38%)
R+D+	14 (10%)	12% (7 to 28)	1.68	2 (14%)	7 (50%)	0	7 (50%)	7 (50%)	0	7 (50%)
Autologous transplant										
R–	42 (30%)	~0%	~0	0	5 (12%)	3 (7%)	34 (81%)	8 (19%)	5 (12%)	29 (69%)
R+	57 (41%)	5%	2.85	1 (2%)	5 (9%)	3 (5%)	49 (86%)	12 (21%)	4 (7%)	41 (72%)
Total	140 (100%)		8.66 (6.2%)	6 (4%)	30 (21%)	6 (4%)	104 (74%)	43 (31%)	9 (6%)	88 (63%)

D, donor; R, recipient.
[a] Likelihood of CMV infection in patient group.
[b] Predicted no. of cases based on 1.
[c] Patients who had at least one true positive CMV test.
[d] D, died; R, relapsed; S, successful.
[e] Significantly different from R–D–.
Data from Fox *et al*. (1995)[4] and Emery *et al*. (2000).[5]

Comparison of parameters for stage one and stage two of the study shows that there was a significant fall ($p < 0.01$) in the reported likelihood of a positive test result leading to other investigations being ordered (e.g. X-rays, CT) on moving from stage one to stage two. Also, in terms of reported benefit there was a significant increase in the proportion of CMV test results that were reported *ex post* to have been of benefit (83% in stage two versus 48% in stage two, $p < 0.01$). Both of these effects are consistent with a lower confidence in screening test results in stage one. This may be related to the different molecular tests used (in stage one, first-round, semi-quantitative, in-house PCR; in stage two, NASBA) or a simple learning curve effect. However, there were no differences between stage one and stage two in reported impact on prescribing or diagnostic certainty.

Renal versus haematology transplant patient responses

Proformas were also analysed separately for renal and haematology transplant patients (see *Table 47*). Results questionnaires demonstrated a number of differences between renal and haematology patients. Analysis of the 573 renal questionnaires (10.3% test results positive) shows that only 5% of test results were reported to have had an impact on diagnostic certainty, with one in five of these leading to a reduction in certainty.

In terms of impact on patient management, only 3% (18/573) of test results were reported to lead to CMV treatment being initiated, and in three cases existing CMV treatment was stopped. There was also a very limited effect on prescribing of other drugs, with only one patient being prescribed antibiotics and one antifungals. Similarly, very low levels (0.7%) of test results were reported to have led to further investigations being ordered. However, 72% of the CMV test results were identified retrospectively by renal clinicians as having been of benefit and only 22% were identified as of no benefit. The pattern is very similar if only screening test requests are considered (see *Table 48*).

TABLE 45 *Stage 1 and 2 responses to clinical impact questionnaires*

Measure	Total (1277 tests)	Stage 1 (687 tests)	Stage 2 (590 tests)
Ex post *responses: test result led to*			
Increased diagnostic certainty	112 (9%)	105 (16%)**	7 (1%)**
Decreased diagnostic certainty	20 (2%)	9 (1%)	11 (2%)
No change in diagnostic certainty	1078 (89%)	544 (83%)	534 (97%)
Start CMV therapy	50 (4%)	37 (5%)	13 (2%)
Stop CMV therapy	5 (0.4%)	1 (<0.2%)	4 (0.5%)
Prescribe other therapy	4 (0.3%)	2 (<0.3%)	2/ 590 (<0.4%)
Order X-ray	34 (2.7%)	29 (4.2%)**	5 (0.9%)**
Order CT/MRI	26 (2%)	26 (3.8%)**	0 (0%)**
Order bronchoscopy	15 (1.2%)	11 (1.6%)	4 (0.7%)
Ex post *responses: test result*			
Of benefit	852 (67%)	362 (53%)**	490 (83%)**
Of no benefit	425 (33%)	325 (47%)**	100 (17%)**

** Significant, $p < 0.01$.

TABLE 46 *(Screening tests only): stage 1 and 2 responses to clinical impact questionnaires*

Measure	Total (1106 tests)	Stage 1 (580 tests)	Stage 2 (526 tests)
Ex post *responses: test result led to*			
Increased diagnostic certainty	83 (8%)	76 (14%)**	7 (1%)**
Decreased diagnostic certainty	16 (1.5%)	5 (1%)	11 (2%)
No change in diagnostic certainty	961 (91%)	478 (86%)	483 (96%)
Start CMV therapy	35 (3%)	27 (5%)	8 (1.5%)
Stop CMV therapy	2 (<0.2%)	0 (0%)	2 (0.4%)
Prescribe other therapy	4 (0.4%)	2 (<0.3%)	2 (<0.4%)
Order X-ray	21 (2%)	19 (3%)**	2 (0.5%)**
Order CT/MRI	18 (2%)	18 (3%)**	0 (0%)**
Order bronchoscopy	7 (0.6%)	5 (1%)	2 (0.4%)
Ex post *responses: test result*			
Of benefit	714 (65%)	278 (48%)**	436 (83%)**
Of no benefit	351 (32%)	282 (48%)**	69 (13%)**

** Significant, $p < 0.01$.

A separate analysis of the 704 haematology results questionnaires (3% test results positive) shows that a higher proportion of haematology than renal test results (15% versus 5%) were reported to have had an impact on diagnostic certainty, with one in seven of these (2% of test results) leading to a reduction in certainty.

In terms of impact on patient management, only 5% were reported to lead to CMV treatment being initiated, and two tests led to existing CMV treatment being stopped. Once again, only a very limited effect was reported on prescribing of other drugs, with one patient being prescribed antibiotics and one IVIg. In contrast, 5% of results were reported to have led to further investigations being ordered (e.g. CT, bronchoscopy, X-rays). Once again, if screening test requests only are considered, the pattern is very similar.

However, in retrospect, haematology clinicians identified fewer test results as being of benefit than did renal consultants (59% versus 72%) and 39% were identified as of no benefit. This pattern remains if only screening test requests are considered.

Comparison of renal and haematology patient questionnaires demonstrates that the likelihood

TABLE 47 *Renal and haematology responses to clinical impact questionnaires*

Measure	Renal (573 tests)	Haematology (704 tests)
Ex post *responses: test result led to*		
Increased diagnostic certainty	24 (4%)**	88 (13%)**
Decreased diagnostic certainty	5 (1%)	15 (2%)
No change in diagnostic certainty	506 (95%)	572 (85%)
Start CMV therapy	18 (3%)	32 (5%)
Stop CMV therapy	3 (0.6%)	2 (0.3%)
Prescribe other therapy	2 (<0.4%)	2 (<0.3%)
Order X-ray	3 (0.5%)**	31 (4.4%)**
Order CT/MRI	1 (0.2%)**	25 (3.6%)**
Order bronchoscopy	3 (0.5%)	12 (1.7%)
Ex post *responses: test result*		
Of benefit	412 (72%)**	440 (63%)**
Of no benefit	128 (23%)**	252 (36%)**

** Significant, $p < 0.01$.

TABLE 48 *(Screening tests only): renal and haematology responses to clinical impact questionnaires*

Measure	Renal (506 tests)	Haematology (600 tests)
Ex post *responses: test result led to*		
Increased diagnostic certainty	17 (4%)**	66 (11%)**
Decreased diagnostic certainty	5 (1%)	11 (2%)
No change in diagnostic certainty	451 (95%)	510 (87%)
Start CMV therapy	12 (2%)	23 (4%)
Stop CMV therapy	2 (0.4%)	0 (0%)
Prescribe other therapy	2 (<0.4%)	2 (<0.3%)
Order X-ray	1 (0.2%)**	20 (3%)**
Order CT/MRI	1 (0.2%)**	17 (3%)**
Order bronchoscopy	5 (1%)	2 (0.4%)
Ex post *responses: test result*		
Of benefit	358 (71%)**	356 (59%)**
Of no benefit	117 (23%)**	234 (39%)**

** Significant, $p < 0.01$.

that a positive test result would lead to prescribing of CMV therapy was significantly higher ($p < 0.01$) for haematology tests, although the level was low in absolute terms (5%). In terms of reported benefit, renal physicians were significantly ($p < 0.01$) more likely to report *ex post* that the CMV test result had been of benefit than were the haematologists (72% versus 63%). This analysis of CMV test result questionnaires indicates that the screening tests appear to have had only a limited effect in terms of changes to diagnostic certainty or to patient management.

CMV test results and patient outcomes

The CMV screening regimes introduced in the study site did not exclude diagnostic tests. Hence, the number of tests requested for a patient might be expected to differ from protocol if infection was suspected.

Renal transplant patients

Table 49 shows the intensity of CMV testing (median number of tests per patient) and the

TABLE 49 *Renal transplant patients: CMV testing and patient outcome*

Recorded outcome	No. of patients	CMV testing pattern (all[a] tests)			
		Median no. of CMV tests per patient	Median period[b]: (days) (weeks)	Median no. of true positives	Mean no. (SD) of true positives
Within 4 months					
Died	1	3	56 (8 weeks)	0	0
Transplant failure	2	4.5	70.5 (10 weeks)	0	0
Successful	95	4	77 (11 weeks)	0	0.6 (1.7)
Longer term					
Died	2	7.5	117 days (17 weeks)	4.5	4.5 (6.4)
Transplant failure	2	7	151 days (22 weeks)	0	0
Successful	94	6	153 days (22 weeks)	0	0.6 (1.8)
Total	98	6	153 days (22 weeks)	0	0.7 (2.0)

SD, standard deviation.
[a] Tests before transplant are excluded.
[b] Period over which samples were taken from patients.

period over which these were administered. The diagnostic yield (number of true positive test results per patient) is also shown for renal patients together with outcomes (death, transplant failure, successful transplant) at the end of the 4-month screening period. *Table 49* demonstrates that the median number of CMV tests was similar in the three groups, but that positive CMV samples (mean number of true positives = 4.5) were clearly concentrated in the two renal transplant patients whose longer term outcome was death. As pointed out above, CMV was not judged to be a contributory cause of death in either case. In other patients, the mean number of true CMV positives over the longer term was very low. Furthermore, neither of the patients whose transplant failed had a positive CMV sample, and CMV was also not judged to be a contributory cause of failure for either.

Instead of longer term outcomes, if the figures based on final 'screening outcome' (i.e. at the end of 4 months) are examined, the observed pattern changes. CMV tests and positive CMV test results during the screening period are now no longer concentrated in the patients who died or whose transplant failed, and instead positive results are observed (mean 0.6) in those whose transplant was judged successful at this stage. This suggests that positive CMV samples in those who died were obtained beyond the screening period, rather than as part of screening. However, it should be borne in mind that none of the mean test numbers is significantly different from zero.

Haematology transplant patients

The intensity of CMV testing and diagnostic yield is compared for haematology patients with different outcomes in *Table 50*. This table demonstrates that, unlike renal patients, there is no evidence over the longer term that CMV tests were concentrated in the patients who died (median five tests), although they do appear to be slightly concentrated in haematology patients who relapsed (median seven tests). This is probably an artefact because the patients who died survived for a shorter period and therefore had fewer opportunities for screening tests to be undertaken. There is some slight indication that true positive results were concentrated in the patients who died, both in the longer term and during the 6-month screening period. Furthermore, the mean number of true CMV positive tests in the whole patient group was low (0.5 in the patients who died).

CMV prescribing and test results

In order to explore these patterns further, the association of positive test results with prescribing was also examined. It would be anticipated that the provision of a positive CMV test result should influence whether or not a study patient is prescribed CMV therapy, and that conversely a negative result would prevent inappropriate prescribing of ganciclovir.

Renal transplant patients

There were 22 (22.5%) renal transplant patients

TABLE 50 *Haematology transplant patients: CMV testing and patient outcome*

Recorded outcome	No. of patients	CMV testing pattern (all[a] tests)			
		Median no. of CMV tests per patient	Median period[b]: (days) (weeks)	Median no. of true positives	Mean no. (SD) of true positives
Longer term					
Died	43	5	49 (7 weeks)	0	0.5 (2.0)
Relapsed	9	7	191 (27 weeks)	0	0 (0)
Successful	88	6	153 (22 weeks)	0	0.1 (0.8)
Within 6 months					
Died	30	3.5	21 (3 weeks)	0	0.7 (2.3)
Relapsed	6	7	137 (20 weeks)	0	0 (0)
Successful	104	6	130 (19 weeks)	0	0.2 (0.7)
Total	140	6	127 days (18 weeks)	0	0.2 (1.3)

SD, standard deviation
[a] Tests ordered before transplant are excluded.
[b] Period over which samples were taken from patients.

TABLE 51 *Renal transplant patients: CMV test results and CMV prescribing*

CMV therapy	CMV disease indicated (at least one sample true positive)		Total
	No	Yes	
Yes	9[a] (9.2%)	13[b] (13.3%)	22 (22.5%)
No	72 (73.5%)	4[c] (4.1%)	76 (77.6%)
Total	81 (82.7%)	17 (17.4%)	98 (100.0%)

[a] Clinician received positive test result in 1/9 cases.
[b] Clinician received positive result in 13/13 cases.
[c] Clinician received positive result in 4/4 cases.

who received CMV therapy during the trial. *Table 51* shows that of the 17 patients who had generated one or more true positive CMV screening test results, only 13 of these individuals received CMV treatment (76.5% of those testing positive or 13.3% of all patients).

Hence four patients who were judged to be true positives did not receive ganciclovir. This may be partly linked to perceived clinical risk; three of these transplants were low risk for CMV (R+D+) but one was high risk (R–D+).

It may also partly be linked to poor technical performance (low sensitivity) of the molecular and other tests reported. Overall, in approximately one-quarter of total cases (27% or 16/59), although a true positive sample was identified **retrospectively** based on the clinical reference standard used in the study, the clinicians received a negative test result. However, in the four patients under question this was not the case: all had a positive test result reported. The decision not to prescribe based on a positive result appears to be due to a combination of clinical symptoms and the toxicity of ganciclovir. None of these patients suffered a transplant failure or death. Renal clinicians are less likely to treat well individuals with a laboratory diagnosis of CMV since they know that ganciclovir is toxic to the transplanted kidney and they may prefer to wait until there are definite signs of disease before instituting treatment. This is reasonable for renal patients who respond well to therapy. For haematology patients, pre-emptive therapy, before the patient becomes unwell, is more often considered.

Table 51 also indicates that nine individuals (40.9% of those receiving therapy or 9.2% of all patients) did not have a single sample that tested (true) positive but still received ganciclovir. The two study patients whose transplant failed were both in this group, although the remainder were successful transplants. For these nine patients, four patients

TABLE 52 *Haematology transplant patients: CMV test results and CMV prescribing*

CMV therapy	CMV disease indicated (at least one sample true positive)		Total
	No	Yes	
Yes	7[a] (5.0%)	6[b] (4.3%)	13 (9.3%)
No	127 (90.7%)	0 (0.0%)	127 (90.7%)
Total	134 (95.7%)	6 (4.3%)	140 (100.0%)

[a] Clinician received positive test result in 2/7 cases.
[b] Clinician received positive result in 6/6 cases.

received both diagnostic and screening tests (three R–D+ and one R–D–) so CMV was suspected by clinicians. For the remaining five patients, clinicians ordered only screening tests; one R+D+, two R–D+ and two R–D– (one of whom was suffering severe rejection). In all cases, therefore, the clinician instituted pre-emptive CMV therapy although screening tests were negative. The nine cases included two patients (one R–D+ and one R–D–) in whom the clinician *ex ante* reported the likelihood of CMV infection to be high. In one case the probability was judged to be 100%, but this was at a point well beyond the end of the 16-week screening period; therapy started 44 weeks post-transplant. In the second case, the probability was judged to be 70% and the patient also had clinical signs (fever); therapy started at 21 weeks post-transplant (equivalent to the median screening follow-up period observed; see *Table 8*). In one case a positive antigenaemia result (but negative molecular test result) was reported, although this was judged not to be a true positive in retrospect (the patient was high-risk R–D+).

Haematology transplant patients

As was the case with renal transplants, the relationship between CMV prescribing and CMV test results was complex. *Table 52* shows that there were 13 (9.3%) haematology study patients who received ganciclovir, compared with 22.5% of renal patients. *Table 52* also shows that only six of these individuals (46% of those receiving therapy or 4.3% of all patients) had at least one (true) positive CMV sample; 4/6 of these patients died and the remaining transplants were successful. It further shows that seven individuals (53.8% of those receiving CMV therapy or 5% of all patients) did not have a single sample that tested (true) positive; 4/7 of these patients died and the remaining transplants were successful. Unlike renal patients, there were no haematology patients who were judged (retrospectively) to have had a true positive CMV test who did not receive CMV therapy (compared with 4/17 renal patients).

For three of the seven patients prescribed ganciclovir without a positive CMV test result, both diagnostic and screening tests had been ordered so clinicians suspected CMV disease (one R+D+ and two R–D–); for the remaining four patients, clinicians ordered screening tests only (two R+D+, one R–D+, one R–D–). The seven cases prescribed ganciclovir included four patients (two D+R+ and two D–R–) in whom the existence of CMV infection was judged as highly likely (60–95% probability, fever in all cases, lung infection suspected in three cases) when the test was requested. The date on which therapy started ranged from one to 57 days (eight weeks) post-transplant.

Comparison of the two patient groups shows that for renal patients there were 17 individuals with true positive CMV test result and 4/17 of these were not prescribed ganciclovir. Furthermore, 9/81 true negatives were prescribed ganciclovir (9/98 or 9% of patients). For haematology transplant patients, the six true positives were all prescribed ganciclovir, but 7/134 of the patients judged retrospectively to have had no positive samples were also prescribed CMV therapy (7/140 or 5% of patients). From the analysis above, it appears that there is limited evidence of CMV therapy being influenced exclusively by CMV test results.

Clinicians' interpretation of CMV test results

It may be that clinicians found it difficult to interpret CMV screening results because two test results were provided and the antigenaemia and molecular tests might produce different results. *Table 53* shows the frequency of different test result patterns.

TABLE 53 *Frequency of different combinations of test results*

Results combinations	All tests	Stage 1 (PCR1)	Stage 2 NASBA	Renal tests	Haematology tests
Both positive Antigenaemia positive Molecular test positive	29 (2.3%)	20 (2.9%)	9 (1.5%)	23 (4.0%)	6 (0.9%)
Positive/negative Antigenaemia positive Molecular test negative	5 (0.4%)	5 (0.7%)	0 (0%)	4 (0.7%)	1 (0.1%)
Both negative Antigenaemia negative Molecular test negative	979 (76.7%)	516 (75.1%)	463 (78.5%)	455 (79.4%)	524 (74.4%)
Negative/positive Antigenaemia negative Molecular test positive	32 (2.5%)	17 (2.5%)	15 (2.5%)	22 (3.8%)	10 (1.4%)

Table 53 demonstrates that instances where a clinician received a positive antigenaemia result and a negative molecular test result occurred extremely infrequently (0.4% of test results). This combination was more likely to occur for stage one molecular tests (i.e. first-round nested in-house PCR) and also for renal transplant patients, although neither of these effects was statistically significant. *Table 53* also shows that although the reverse combination (negative antigenaemia result and positive molecular test result) occurred slightly more often, this was still an infrequent occurrence (2.5% results).

As a further indication of any difficulties in **interpreting** CMV test information, clinicians were also asked to record any cases where they contacted the laboratory to discuss test results. This happened extremely infrequently (11/1277 results). Also, the times when it happened were confined exclusively to stage one, and the results were mostly (10/11) negative. These 11 tests related to seven patients, both haematology (four) and renal (three).

Historical controls

In order to explore further any impact linked to the introduction of CMV screening, a group of historical control patients for whom only diagnostic CMV tests had been available was identified. This consisted of a series of consecutive patients who had undergone transplants immediately prior to the commencement of the study. In addition to identifying transplant outcomes, a number of 'proxy' outcome measures were to be extracted from patients' notes and compared with values for the prospective study patients, such as length of inpatient stay, levels of confirmed CMV infection (positive CMV tests), and levels of CMV therapy. Any statistically significant differences in these measures might indicate a marginal benefit for patients in the study who had access to CMV screening and also diagnostic tests.

The initial objective was to identify a minimum of 150 historical patients matched for type of procedure/condition and type of CMV donor/recipient status. This sample size was based on a preliminary estimate from the literature of an average 40% of study patients developing CMV infection; mean reported rate of 70% for renal patients and up to 40% for haematology patients.[4] In the event, the percentage of patients in the prospective study with a positive CMV test result was much lower than these figures; 13% of renal patients and only 3.6% of haematology transplant patients had one or more positive samples. This made it unlikely that significant differences in CMV infection rates could be observed with the sample size initially planned. Effort was therefore expended in increasing the number of historical controls, although extending the period over which data were collected would inevitably increase the likelihood of secular trends having a significant confounding effect.

Data collection: renal and haematology transplant patients

Initial attempts to collect data on historical controls through examination of a sample of 53 haematology patient records demonstrated that the information required could not be extracted consistently. In particular, key data items were often missing and in several instances the notes were not available because a patient's care had

been transferred. The latter effect was important because it meant notes for a **consecutive** series of historical patients could not be examined, risking the introduction of spectrum bias.[61] It was therefore decided that data for historical patients (both renal and haematology) should instead be identified through a combination of routine hospital data sources.

For the renal transplant patients, most of the required data could be downloaded from the Renal Directorate's patient database. However, laboratory test data were not available from this source, so these were provided by the laboratory and matched to historical controls on patient hospital number. CMV prescribing data were similarly not available from the renal patient database, and this information could also not be extracted at an individual patient level from either pharmacy or ward records at UHW. Prescribing data therefore could not be included in the comparison of historical and study patients.

For the haematology patients, historical data was provided via the Haematology Department's own database, plus the laboratory database. However, the haematology database was not as well established as the renal database and the data on historical cases proved to be of too poor quality to be used. Of 165 patients identified, only 94 (57%) had age, sex and CMV status of recipient recorded (basic demographics) and only 24 patients (14%) had outcome data. It was decided, therefore, that the data from the Haematology Department's historical database could not be used for comparison purposes, although information on CMV testing patterns was obtained from the laboratory and matched to the haematology historical controls on patient hospital number.

Comparison of historical and prospective study cases

Renal transplant patients

Information was collected on 199 consecutive renal patients who underwent transplants over a period of 29 months before the prospective study started (1 April 1996–25 September 1998).

The main clinical benefit of CMV screening was expected to be a therapeutic one, with more accurate detection of disease leading to more appropriate use of drug therapy. However, since it had proved impossible to identify CMV drug therapy for the historical controls, analysis was necessarily limited to comparison of the remaining parameters, that is, length of inpatient stay, number of CMV tests and their results and 'final' outcome (i.e. survival/transplant success) at the end of a defined period. The time point selected for recording this outcome measure for the two groups was 9 months; this was a pragmatic choice, since it was equivalent to the follow-up period available for the final renal patient recruited to the prospective study. A 28-week follow-up period was also selected as best representing the 'screening period' as implemented; this was the upper quartile value of the observed CMV screening period for renal transplant patients in the study sample.

Historical and study patient groups were compared in terms of basic descriptors. There were no significant differences between the 199 historical controls and 98 prospective study patients in terms of gender (Fisher's exact test, two-sided $p = 0.37$); age ($p = 0.66$); patient's CMV status ($p = 1.00$); donor's CMV status ($p = 0.61$); or cadaveric versus live transplant ($p = 0.13$).

Table 54 presents comparative data on **patient testing profiles** showing the proportion of patients in the two periods who had a CMV test, and (separately once screening was introduced) the proportion of patients who continued to have **diagnostic** CMV tests (i.e. tests to confirm the presence of suspected disease). As would be expected, the data demonstrate a significant increase in the percentage of patients receiving a CMV test once screening was introduced (30–100%). However, there is also a significant increase ($p = 0.037$) in the percentage of patients who received a **diagnostic** test (30–43%), although this effect ceases to be significant if a 28-week cut-off is applied. The latter effect is suggestive of carryover of CMV testing behaviour beyond the screening period. Furthermore, when diagnostic tests were ordered, the percentage of patients who tested positive was lower for study patients (24%) than for the historical group (43%), suggesting a general lowering of clinicians' thresholds for ordering diagnostic tests, although this difference was not significant at the 5% level either at 28 weeks or longer term.

In terms of the **intensity of testing**, *Table 54* also shows that not only were more patients in the prospective study receiving diagnostic tests, they were also receiving nearly twice the intensity of diagnostic tests (0.82 versus 0.44 per patient); study patients were generating only a slightly higher number of positive diagnostic test results (0.19 versus 0.14 per patient). Neither of these effects was statistically significant. Finally, the percentage of **diagnostic** tests that proved to be

TABLE 54 *Key renal patient parameters: historical controls versus prospective study*

Parameter	Longer term period		28 weeks post transplant	
	Study group (98 patients)	Historical controls (199 patients)	Study group (98 patients)	Historical controls (199 patients)
No. (%) of patients with ≥ 1 positive CMV test[a] result/total no. of patients	13/98 (13%)	26/199 (13%)	12/98 (12%)	25/199 (13%)
Unsuccessful[c]/successful outcome at 9 months	4/94**	35/164**		
Inpatient stay (mean days)	15.37*	18.95*		
Patient testing profile				
No. (%) of patients who had ≥ 1 CMV test[a]/total no. of patients	98/98*** (100%)	60/199*** (30%)	98/98*** (100%)	60/199*** (30%)
No. (%) of patients who had ≥ 1 CMV diagnostic[b] test/total no. of patients	42/98 (43%)*		39/98 (40%)	
No. (%) of patients with ≥ 1 positive CMV diagnostic[b] test/total no. of patients who received a diagnostic test	10/42 (24%)	26/60 (43%)	10/39 (26%)	25/60 (42%)
Intensity of testing				
No. of CMV tests[a]/total no. of patients	579/98 5.91	88/199 0.44	527/98 5.38	64/199 0.32
No. of CMV diagnostic tests[b]/total no. of patients	80/98 0.82		65/98 0.66	
No. of positive CMV tests[a]/total no. of patients	33/98 0.34	28/199 0.14	32/98 0.33	27/199 0.14
No. of positive CMV diagnostic tests[b]/total no. of patients	19/98 0.19		19/98 0.19	
No. (%) of positive CMV tests[a]/total no. of tests	33/579*** (5.7%)	28/88*** (32%)	32/527*** (6.1%)	27/64*** (42%)
No. (%) of positive CMV diagnostic tests[b]/ total no. of diagnostic tests	19/80 (24%)		19/65 (29%)	
Percentage at risk transplants based on: R–D+ risk 44%; R+D+ risk 25%; R+D– risk 6%; R–D– risk 0%	21%	21%[d]		

[a] Screening and diagnostic tests (antigenaemia pp65 tests only).
[b] Tests (antigenaemia pp65) ordered for diagnostic purposes, i.e. excluding screening tests.
[c] Transplant failure or death.
[d] Figure based on only 75% of historical cases where donor/recipient CMV status available.
Compared to historical group: * significant, $p < 0.05$; ** significant, $p < 0.01$; *** significant, $p < 0.001$.

positive was lower in the study patients than in the historical group (24% versus 32%), again suggesting a lowering of clinicians' thresholds for ordering diagnostic tests, but this effect was non-significant.

Table 54 also shows that there was no indication of a significant difference in the reported levels of CMV disease, with 13% of historical and study patients testing positive on antigenaemia, although a slightly lower proportion (12%) of study patients tested positive during the 28-week screening period. The antigenaemia test results were used for comparison because these were the only results available for **both** groups.

However, there was a significant difference between historical and study patients in terms of outcomes at 9 months and in terms of the mean length of inpatient stay. *Figure 3* shows that there was no clear trend in length of stay (LOS) for the majority of patients (study start date month 37), although there was a fall in the number of long-stay 'outliers' after this date. The introduction of

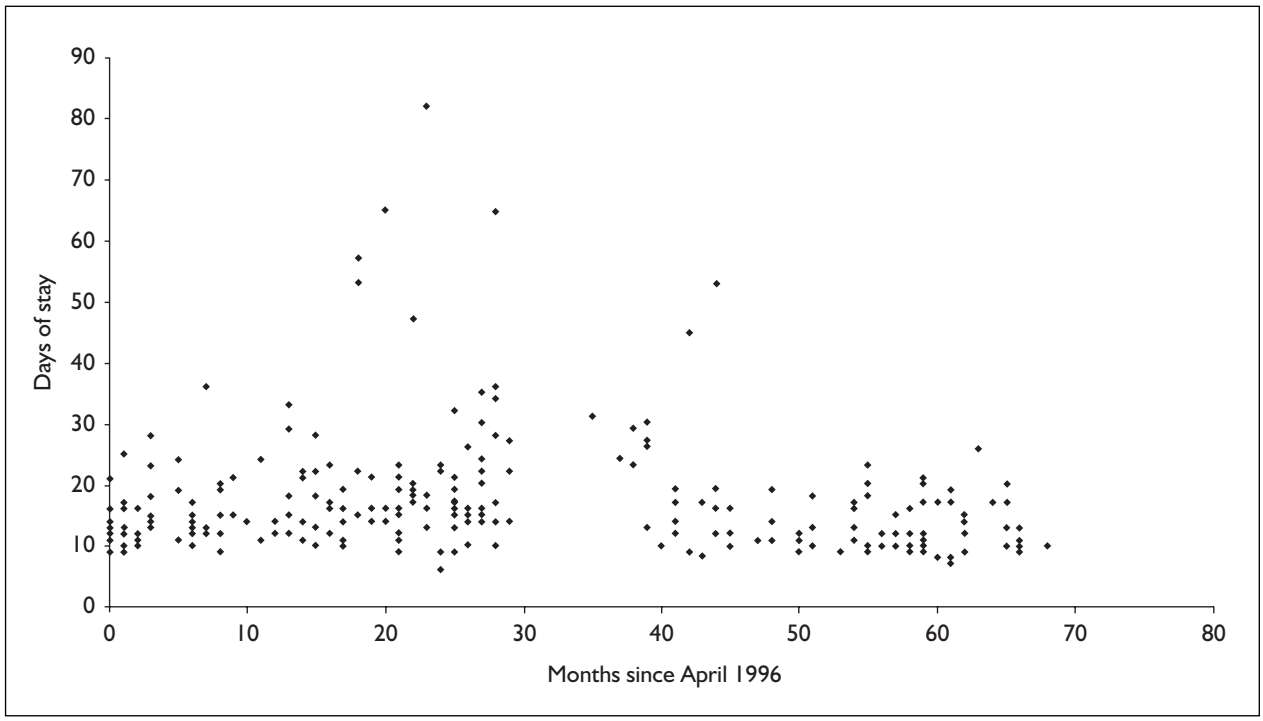

FIGURE 3 *Reported length of inpatient stay for historical and study patients (excluding patients who died). Data points are given in Appendix 19.*

screening was closely followed by organisational changes in the renal department which may have contributed to this observed effect.

Haematology transplant patients

More limited data were available on the 136 haematology transplant patients who underwent transplants over a period of 27 months before the prospective study started (26 June 1996–20 September 1998). It was therefore not possible to confirm that the 136 historical controls and 140 prospective study patients were comparable in terms of gender, age, patient's and donor's CMV status or type of transplant.

Table 55 is therefore only able to compare study patients and historical controls on laboratory data. Unlike renal transplants, this table demonstrates a difference in the reported CMV disease level, with 2.2% of patients in the historical control group testing positive with antigenaemia compared with 3.6% in the prospective study group, although the difference is not significant.

The **patient testing profile** also shows, as for renal transplant patients, a significant increase ($p < 0.005$) in the percentage of study patients who received a **diagnostic** test within 28 weeks (32% versus 21%), although this effect is not significant for longer term data. Among patients receiving a diagnostic test, there was no significant difference in the percentage of study patients who tested positive compared with historical controls at 28 weeks (6.7% versus 7.1%), providing no evidence of a lowering of clinicians' thresholds for ordering diagnostic tests (unlike renal physicians).

Once again, as with the renal transplant patients, *Table 55* also shows that in terms of the **intensity of testing**, not only were patients in the screening period undergoing a higher average number of CMV tests per patient, they were also undergoing nearly twice the intensity of diagnostic tests (0.84 versus 0.54 tests per patient). Neither of these effects was statistically significant at the $p = 0.05$ level. Haematology study patients also generated the same low number of positive diagnostic tests (0.03 per patient) as historical patients. Finally, the percentage of **diagnostic** tests that proved to be positive was lower in the screening trial period than in the historical group (3% versus 5.5%), although this difference was once again not significant.

In summary, the comparison of a consecutive series of historical patients with the prospective study patients demonstrates a highly significant difference in outcomes for renal patients (deaths, transplant failures) at 9 months; there was no similar data available for haematology transplant

TABLE 55 *Haematology patient parameters: historical controls versus prospective study*

Parameter	Longer term period		28 weeks post transplant	
	Study group (140 patients)	**Historical controls (136 patients)**	**Study group (140 patients)**	**Historical controls (136 patients)**
No. (%) of patients with ≥ 1 positive CMV test[a] result/total no. of patients	5/140 (3.6%)	3/136 (2.2%)	5/140 (3.6%)	2/136 (2.2%)
Patient testing profile				
No. (%) of patients who had ≥ 1 CMV test[a]/ total no. of patients	129/140*** (92%)	30/136*** (22%)	129/140*** (92%)	28/136*** (21%)*
No. (%) of patients who had ≥ 1 CMV diagnostic[b] test/total no. of patients	46/140 (33%)		45/140 (32%)*	
No. (%) of patients with ≥ 1 positive CMV diagnostic[b] test/total no. of patients who received a diagnostic test	3/46 (6.5%)	3/30 (10%)	3/45 (6.7%)	2/28 (7.1%)
Intensity of testing				
No. of CMV tests[a]/total no. of patients	717/140 5.12	73/136 0.54	660/140 4.71	55/136 0.40
No. of CMV diagnostic tests[b]/total no. of patients	118/140 0.84		110/140 0.79	
No. of positive CMV tests[a]/total no. of patients	9/140 0.06	4/136 0.03	9/140 0.06	4/136 0.03
No. of positive CMV diagnostic tests[b]/total no. of patients	4/140 0.03		4/140 0.03	
No. (%) of positive CMV tests[a]/total no. of tests	9/717* (1.3%)	4/73* (5.5%)	9/660*** (1.4%)	4/55*** (7.3%)
No. (%) of positive CMV diagnostic tests[b]/ total no. of diagnostic tests	4/118 (3%)		4/110 (4%)	
Allogeneic/autologous transplants	41/99	N/A[d]		
Percentage at risk transplants based on: Allogeneic R–D+ risk 4%; R+D+ risk 12%; R+D– risk 44%; R–D– risk 3% Autologous R– ~0%; R+ ~5%	8.7%	N/A[d]		

[a] Screening and diagnostic tests (antigenaemia pp65 tests only).
[b] Tests (antigenaemia pp65) ordered for diagnostic purposes, i.e. excluding screening tests
[c] Relapse or death.
[d] Allogeneic/autologous breakdown and donor/recipient CMV status not available for historical cases.
Compared to historical group: *significant, $p < 0.05$; *** significant, $p < 0.001$.

patients. This effect does not appear to be linked to CMV disease. However, the introduction of CMV screening tests does appear to have produced a significant increase in the number of CMV diagnostic tests requested for patients (both renal and haematology), and also the number of tests overall. There was also some indication that a lower percentage of diagnostic tests were positive once screening was introduced, possibly linked to a lowering of clinicians' thresholds for ordering diagnostic tests. There was no evidence that significantly more cases of CMV infection were detected among study patients than among historical cases.

Predicted impact of alternative CMV screening test options

When measuring the impact of a diagnostic test, evaluation conventionally focuses on the effect of positive test results, that is, estimating the benefit of test information for patients in whom disease is detected. However, only 6.3% of the CMV tests in

TABLE 56 *Stage 1 and 2 responses to clinical impact questionnaires*

Measure	Total (1277 tests)	Stage 1 (687 tests)	Stage 2 (590 tests)
Ex ante *responses: positive test will lead to*			
Repeat CMV test request	337 (26%)	128 (19%)**	209 (35%)**
Start CMV therapy	523 (41%)	298 (43%)	225 (38%)
Prescribe other therapy	53 (4%)	34 (5%)	19 (3%)
Order X-ray	128 (10%)	93 (14%)**	35 (6%)**
Order CT/MRI	39 (3%)	36 (5%)**	3 (0.5%)**
Order bronchoscopy	101 (8%)	76 (11%)**	25 (4%)**
Ex ante *responses: negative test will lead to*			
Start CMV prophylaxis	7 (0.5%)	7 (1%)	0
Stop CMV prophylaxis	2 (<0.2%)	2 (<0.3%)	0
Start CMV therapy	0	0	0
Prescribe other therapy	1 (<0.1%)	1 (<0.2%)	0
Order X-ray	3 (<0.3%)	1 (<0.2%)	2 (<0.4%)
Order CT/MRI	0 (0%)	0 (0%)	0 (0%)
Order bronchoscopy	3 (<0.3%)	2 (0.3%)	1 (<0.2%)

** Significant, $p < 0.01$.

the present study (80/1277) produced a truly positive result. At the same time, more than twice this number (14.1% or 180/1277) tested positive on one or more of the three molecular tests being evaluated. Therefore, in such a situation it is clearly important to consider the likely impact of these alternative molecular test results, **were they to be reported**. At the time when a screening sample was sent to the laboratory, a patient who would test positive on a particular CMV test could not be distinguished. The likely effect of different molecular tests could therefore only be estimated by asking clinicians to record, at the time of test request, the *ex ante* likely impact of a positive or negative test result on their clinical management and then linking this to subsequent results for different types of test.

Analysis of the test request questionnaires demonstrated that *ex ante* clinicians were reporting that, even if a **positive result** were received, this would **not necessarily** lead to the start of therapy in all patients; only in 41% of patients. In addition, it would be likely to lead to a repeat test request in 26% of cases and ordering of other investigations in several instances, namely X-rays (10% of cases), CT/MRI (3% of cases) and bronchoscopy (8% of cases). In contrast, clinicians reported that a **negative result** would have virtually no impact on planned patient management; the only effects predicted were further investigations in <0.3% cases. Clinicians did not anticipate that there would be any changes to prescribing. However, this does not rule out effects such as reassurance about planned management.

Clinicians were also asked *ex ante* to record the likelihood that a particular patient would have a positive CMV test result. This was judged to be very likely in only 26 cases (3%), likely in 95 cases (8%), unlikely in 816 instances (71%) and very unlikely in 208 cases (18%).

Stage one versus stage two responses (all patients)

Table 56 presents the predicted impact for all CMV tests, and separately for stage one and stage two patients; these differ from the actual clinical impact of test results recorded which was dependent on the pattern of results, which in its turn was dependent on the tests used. Clinicians recorded the *ex ante* likelihood that a particular patient would have a positive CMV test result to be very similar in stages one and two, judged 'very likely' or 'likely' (11% versus 12% in stage two), or thought to be 'unlikely' or 'very unlikely' (89% versus 88% in stage two).

Analysis of the 687 request questionnaires from stage one indicates that *ex ante* clinicians reported a *positive* CMV test result would lead to the start of CMV therapy in 43% of cases, ordering of a repeat test request in 19% and ordering of other tests in a similar proportion of cases, namely X-rays (14%), bronchoscopy (11%) and CT/MRI (5%). At the same time, clinicians reported very few anticipated effects following a *negative* test result. Clinicians

TABLE 57 *Renal and haematology responses to clinical impact questionnaires*

Measure	Renal (573 tests)	Haematology (704 tests)
Ex-ante *responses: positive test will lead to*		
Repeat CMV test request	1 (<0.2%)**	336 (48%)**
Start CMV therapy	184 (32%)**	339 (48%)**
Prescribe other therapy	1 (<0.2%)**	52 (7%)**
Order X-ray	2 (<0.4%)**	126 (18%)**
Order CT/MRI	0 (0%)**	39 (6%)**
Order bronchoscopy	2 (<0.4%)**	99 (14%)**
Ex ante *responses: negative test will lead to*		
Start CMV prophylaxis	5 (<1%)	2 (<0.3%)
Stop CMV prophylaxis	1 (<0.2%)	1 (<0.2%)
Start CMV therapy	0 (0%)	0 (0%)
Prescribe other therapy	0 (0%)	1 (<0.2%)
Order X-ray	1 (<0.2%)	2 (<0.3%)
Order CT/MRI	0	0
Order bronchoscopy	0	3 (<0.5%)

** Significant, $p < 0.01$.

were very unlikely to report that they would order further investigations; they anticipated ordering only one X-ray and bronchoscopy for two patients. There were also relatively few effects predicted on prescribing: in 1% of tests (seven cases) the start of CMV prophylaxis, cessation of prophylaxis in two cases, and start of antifungal therapy for one test. There were no anticipated effects of a negative test result on prescribing of antibiotics, growth factor or IVIg.

A similar analysis of the 590 requests for patients recruited in stage two shows that a positive CMV test result was predicted to lead to the start of medication in an equivalent proportion (38%) of cases; a repeat test request in significantly more cases than for stage one (35% vs 19%, $p < 0.01$); and ordering of other tests less frequently namely X-ray (6% versus 14%), bronchoscopy (4% versus 11%) and CT/MRI (0.5% versus 5%), all significant ($p < 0.01$). These predicted patterns mirror those identified through analysis of results questionnaires. Clinicians reported that a *negative* test result would have no effect on CMV prophylaxis, and no effects were reported on antibiotic therapy, antifungal therapy or growth factor. There were similarly very few anticipated effects in terms of further investigations following a negative test result (<0.4%). Once again, this does not rule out effects such as reassurance.

In summary, comparison of responses in stages one and two of the study shows that *ex ante* clinicians in stage two were significantly more likely to report that a positive test result would lead to a repeat test request (35% versus 19%, $p < 0.01$) and less likely ($p < 0.01$) to report that it would lead to ordering of other investigations (X-rays, CT/MRI, bronchoscopy). This may be linked to an emerging requirement for a confirmatory positive CMV screening test result, as reported nationally (see survey of UK clinicians, Chapter 7). Otherwise, there were no significant differences.

Renal versus haematology transplant patient responses

The predicted effect of positive and negative test results was analysed separately for renal and haematology transplant patients (see *Table 57*). *Ex ante* renal clinicians judged the likelihood that their patients would have a positive CMV test result to be higher than did haematology clinicians; likelihood was recorded as 'very likely' or 'likely' in 18% of renal requests versus 6% of haematology requests; this mirrors the differences in actual CMV disease levels.

Analysis of the 573 renal request questionnaires indicates that *ex ante* clinicians reported that a **positive** CMV test result would lead to the start of medication in 32% of cases, a repeat test request in only one case (<0.2%) and ordering of other tests in extremely few cases, namely X-rays (<0.4% of cases), CT/MRI (0%) and bronchoscopy (<0.4%).

Clinicians also reported that a **negative** test result would have very few effects (<1%): the start of prophylaxis in five cases, but there was no

anticipated effect on CMV therapy antibiotic treatment, antifungal therapy or prescribing of growth factor. Predicted effects on further investigations were limited to ordering of X-rays in one case.

For haematology patients, analysis of the 704 request questionnaires indicates that *ex ante* clinicians reported that a *positive* CMV test result would lead to the start of medication in a higher number (48% versus 32%, $p < 0.001$) of cases, a repeat test request in significantly more cases than for renal patients (48% versus <0.2%, $p < 0.01$) and ordering of other tests also in far more cases, namely X-rays (18% versus <0.4% of cases), CT/MRI (6% versus 0% of cases) and bronchoscopy (14% versus <0.4% of renal cases).

There were, once again, very low levels of anticipated effects reported by clinicians for **negative** test results. Negative results were identified as likely to lead to the start of prophylaxis in two cases and cessation in one case, start of CMV therapy in two cases and antifungal therapy in one case. There were no anticipated effects on antibiotic therapy or prescribing of growth factor. Negative results were also expected to have very few effects on ordering of further investigations, limited to two cases in which a clinician reported they would order an X-ray and in three cases a bronchoscopy.

In summary, comparison of renal and haematology patients demonstrates that *ex ante* haematology clinicians were significantly ($p < 0.01$) more likely to report that a positive test result would lead to a repeat test request and to other investigations being ordered (e.g. X-rays, CT/MRI, bronchoscopy). The likelihood that a positive test result would lead to prescribing of CMV therapy was also significantly higher in haematology patients ($p < 0.01$), but in neither patient group was it 100% certain. As discussed earlier, clinicians will consider the result of a screening test in the light of other factors. During the course of the study, practice also moved towards clinicians requiring a second positive CMV result in some cases.

The next chapter considers the results of a national survey of renal and haematology transplant centres exploring CMV screening and the response of clinicians to positive CMV tests more widely.

Chapter 7

CMV screening: views of UK clinicians

Introduction

In order to assess the views of a wider group of clinicians' views about CMV screening and prescribing, all renal transplant centres and bone marrow transplant units in the UK were surveyed. These surveys had three main objectives:

- to estimate the degree to which formal protocols are in use for CMV screening of immunocompromised patients
- to explore the preferences of clinicians for tests to be used in CMV screening
- to ascertain their views on the use of CMV treatment and prophylaxis for transplant patients and its relationship to CMV testing.

Questionnaires were developed in conjunction with clinicians in the Renal and Haematology Departments at the UHW and piloted prior to use.

Survey of UK renal transplant centres

Questionnaires were posted in the second half of 2002 to 115 consultant clinicians in 31 renal transplantation centres in the UK (England, Scotland, Wales and Northern Ireland). In most cases individual centres had two or more named consultants who were included in the survey sample. In terms of follow-up of non-responders, we took the view that although it was useful to obtain more than one response per centre (because individual clinicians may have slightly different views), the main requirement was to obtain at least one response from each centre surveyed. Hence reminders were only sent to clinicians in non-responder sites. After one postal reminder had been sent, telephone follow-up was used for all centres that had not provided at least one response.

Overall a total of 37 clinician responses were received, representing at least one response from 24 centres (77%). Fifteen centres provided just one response, seven centres provided two responses, one centre provided three responses, and one centre provided five responses. In the analysis that follows, certain questions are addressed at the centre level and for others the analysis is presented at the individual clinician level.

Renal screening and diagnostic testing for CMV

Over two-thirds of renal transplant centres (13/19 or 68%) reported that they undertake CMV screening in addition to diagnostic tests. In six centres, clinical staff stated that they use only diagnostic tests (i.e. testing when there are clinical indications of CMV). Five centres did not answer this question. In the 11 centres providing more than one response, just under half (five) reported differences between physicians in their responses. In some centres, these differences were linked to screening being undertaken by a specific type of clinician (e.g. paediatric nephrologists). In other centres, it appeared to be indicative of variations in practice amongst renal consultants.

In the 13 centres where clinicians reported CMV screening, they were asked how frequently and over what period of time renal patients are screened post-transplant. Details were provided by 12 of these centres and the results demonstrate a similar pattern to that reported previously by laboratories (see Chapter 4, *Table 32*). The most common screening period reported was 12–14 weeks. Only two centres reported screening for longer, one to 16 weeks and one for 24 weeks post-transplant. No centre undertook screening for longer than 6 months (24 weeks). In terms of testing frequency, the most popular interval between screening tests was 7 days, with slightly more frequent testing (about twice per week) being the next most likely. The screening period used in the present study is similar but the testing frequency was slightly lower; our renal transplant patients were screened for CMV during the first 4 months (16 weeks) following transplant, and testing was at a 4-weekly interval.

Stratification of renal transplant patients for CMV testing

Individual clinicians were asked whether, when testing for CMV post-transplant, they stratify patients according to risk (e.g. type of transplant and/or patient/donor CMV status). Overall 35/37 respondents answered this question;

approximately one-third (12/35 or 34%) indicated that they use a predefined protocol and a further 3/35 (9%) stratify on a case-by-case basis using clinical signs and symptoms. However, the majority (20/35 or 57%) indicated that they do *not* stratify patients when testing for CMV. Hence the national picture is similar to the screening protocol implemented in the Cardiff study site, where all patients were screened.

The 15 respondents who do stratify patients for CMV screening were asked to provide further information; 13 provided details, covering 12 different centres. These centres all stratified based on CMV risk assessment, with the R–D+ group being mentioned specifically in all cases; seven respondents (from seven centres) also all indicated that these patients would usually receive CMV prophylaxis and be carefully observed.

Renal clinicians' views on frequency and duration of CMV testing

Renal consultants were asked whether they are satisfied with the CMV screening protocol used in their centre. All 37 respondents answered this question. Only six (16%) indicated that they were not satisfied. Five of these identified two main areas for improvement:

- The fact that regular follow-up of patients is poorly done, including sending samples when scheduled or follow-up post-discharge (two respondents).
- That a programme of screening using simple reliable tests is required (three respondents).

Renal clinicians' views on types of CMV tests used

Clinicians were asked what type of CMV test is available for their patients. Overall 36/37 clinicians completed this question, with 24 centres providing a response.

The majority of centres (18/24 or 75%) and clinicians (25/36 or 69%) indicated that a PCR-based technique is used. Antigenaemia was reported to be used in only 5/24 (21%) centres and by 10/36 (28%) of clinicians. In addition, five centres (21%) and six clinicians (17%) reported the use of the DEAFF test. This pattern largely mirrors that reported by laboratories (see Chapter 4). One clinician did not know the type of test used.

Clinicians were also asked whether they consider that the types of CMV tests currently available to them are satisfactory. Overall 30/37 respondents thought their current test was satisfactory, although one also pointed out that it 'could be improved' and a further respondent said that they were 'still learning what it means'.

Only a small minority (7/37 or 19%) considered that the test they currently use is unsatisfactory. The tests used were approximately equally divided between PCR, antigenaemia and DEAFF. When asked to expand on their reasons, one respondent commented that there 'is a need for quantitative PCR testing'. A second respondent pointed out that the PCR 'quantitative test does not appear to have been validated' by the laboratory. A third focused on antigenaemia and commented that they have had 'patients with a negative test in whom tissue biopsies have subsequently demonstrated active CMV disease'; at the same time, this respondent also pointed out that PCR is 'possibly too sensitive' rendering quantification unreliable. Three further responses were all made by clinicians from the same centre (not the study site). They pointed out that their current regimen had been set up as a research project and that they should 'have CMV antigenaemia assay and PCR routinely available'. A final respondent commented that the CMV test available (PCR) 'is satisfactory for diagnostic purposes'; this centre had completed a study of screening high risk patients for the first 3 months post-transplant by weekly testing and 'in only half of the patients who developed CMV disease was PCR positive before onset of disease'.

Protocols for CMV test results

Renal clinicians were asked whether they have formal protocols in place to act upon the results of CMV tests. Just over half (21/37 or 57%) indicated that a protocol is in place, and 14/21 of these centres (67%) appear to have a formal protocol. Hence over half of renal centres overall had no formal screening protocol.

Respondents who reported **no formal protocol** were asked for further details, and nine clinicians provided responses. In essence, these fell into two very similar groups as follows:

- That CMV test results are interpreted alongside all other data, such as fever or falling full blood count, and the clinical status of the patient (six respondents). One respondent also stated that they would use aciclovir prophylaxis if the donor is CMV positive and the recipient CMV negative.
- That ganciclovir is prescribed in the event of clinically proven infection (three respondents).

TABLE 58 *Renal clinicians: groups given CMV therapy following single positive test*

Type of transplant	Patient group given CMV therapy (37 clinicians)			
	Yes No. (%)	Maybe No. (%)	No No. (%)	Total
R–D+ (cadaver donor transplant)	21[a] (62)	1 (3)	12 (35)	34[c]
R+D– (cadaver donor transplant)	13[a] (38)	1 (3)	20 (59)	34[c]
R+D+ (cadaver donor transplant)	14[a] (41)	1[b] (3)	19 (56)	34[c]
R–D– (cadaver donor transplant)	12[a] (35)	1[b] (3)	21 (62)	34[c]
R–D+ (living donor transplant)	20[a] (59)	1[b] (3)	13 (38)	34[c]
R+D– (living donor transplant)	13[a] (38)	1[b] (3)	20 (59)	34[c]
R+D+ (living donor transplant)	14[a] (41)	1[b] (3)	19 (56)	34[c]
R–D– (living donor transplant)	12[a] (35)	1[b] (3)	21 (62)	34[c]
Patients on antibody induction therapy	12[a] (35)	1[b] (3)	21 (62)	34[c]

[a] One clinician commented Yes 'if CMV illness'.
[b] One clinician 'not clear'.
[c] No response from 3 centres.

Of the clinicians who reported a **protocol** in place, 16/21 (76%) provided further details. Certain of these protocols were essentially formalised versions of the informal behaviour described above, that is intervene when CMV disease is clinically suspected (six respondents). Others indicated that a particular trigger will mean they treat pre-emptively with ganciclovir, such as when the viral load rises above a set level or is rising steeply and approaching this level, patients convert to CMV PCR positive, or in the event of two consecutive CMV positive PCR results (two respondents). Other responses were more complex, several including a link to prophylactic treatment, for example:

> "Post-transplantation both donor and recipient are given antibody screening. There is also CMV monitoring if possible to avoid having a CMV-positive donor being matched with a CMV-negative recipient. There is PCR screening of CMV-positive/CMV-negative patients for 3 months. Finally, there is prophylaxis for all CMV positive/CMV negative patients."

> "All CMV-negative recipients receive sero-negative blood products. If they are CMV positive on PCR, they receive ganciclovir for 14 days intravenously. If they are still CMV positive on PCR after 2 weeks, a further 14-day course of ganciclovir is given."

Renal clinicians' response to newly positive CMV test

Clinicians were also asked in what situations they would prescribe anti-CMV therapy if a renal transplant patient tested positive after previously testing negative for CMV. Their responses to this question are given in *Table 58*.

It is evident that renal clinicians are most likely to prescribe therapy for R–D+ renal transplants, regardless of the type of transplant (cadaver or living donor), with approximately two-thirds prescribing. For all other types of transplant (R+D–; R+D+; R–D–) and for patients on antibody induction therapy, a similar majority of clinicians report that they would **not** prescribe anti-CMV therapy based on a single positive result. Three clinicians did not reply to this question.

Clinicians were also asked to provide details of the therapy typically used if they had answered 'Yes'. The responses recorded show that the main drug therapy used is ganciclovir, but that prescribing will usually only take place if the patient also exhibits clinical symptoms.

In a follow-on question, respondents were asked under what circumstances a positive result would not result in the prescribing of anti-CMV therapy. Twenty-one responded to this question. The majority (15/21) specifically mentioned the presence of clinical symptoms as a requirement for prescribing, and the remainder either mentioned the need for two positive test results or for a certain level (in quantitative tests) to be exceeded. This appears to mirror the pattern observed in the study site, where renal clinicians did not necessarily prescribe following a positive screening test result and would often order a further CMV test.

TABLE 59 *Renal transplant centres: use of CMV prophylaxis*

Form of prophylaxis	Number of centres	Method of delivery	Provided on inpatient or outpatient basis	Estimated transplants given this type of prophylaxis (%)
Aciclovir	2	Oral	IP/OP	20–60
Aciclovir (and ganciclovir)	3	Oral	IP/OP	10–80 (1–10)
Aciclovir (and ganciclovir and valaciclovir)	1	Oral	IP/OP	40 (5% and <5%)
Valaciclovir	2	Oral	IP/OP	12–60
Valaciclovir (and valaganciclovir)	1	Oral	IP/OP	1–10
Ganciclovir	4	Oral	IP/OP	10–30
Ganciclovir (i.v. and oral)	3	i.v. and Oral	IP/OP	10–25
Ganciclovir (and valaganciclovir)	2	Oral	IP/OP	10–20 (2–10)

IP, inpatient; OP, outpatient.

TABLE 60 *Renal transplants: patient groups given CMV prophylaxis*

Type of transplant	Patient group given CMV prophylaxis		Total
	Yes No. (%)	No No. (%)	
R–D+ (unrelated donor transplant)	27 (90)	3 (10)	30
R+D– (unrelated donor transplant)	6 (20)	24 (80)	30
R+D+ (unrelated donor transplant)	7 (23)	23 (77)	30
R–D– (unrelated donor transplant)	0 (0)	30 (100)	30
R–D+ (related donor transplant)	25 (86)	4 (14)	29[a]
R+D– (related donor transplant)	6 (21)	23 (79)	29[a]
R+D+ (related donor transplant)	7 (24)	22 (76)	29[a]
R–D– (related donor transplant)	0 (0)	29 (100)	29[a]
Patients on antibody induction therapy	17 (57)	13 (43)	30

[a] One centre no information because these types of transplant done elsewhere.

CMV antiviral prophylaxis

Renal clinicians were also asked whether their department currently uses CMV prophylaxis and, if so, what type(s) of drugs are used and in what percentage of transplant patients. *Table 59* demonstrates that ganciclovir is most widely used (13/18 centres), with aciclovir next (6/18); these are prescribed as oral drugs on both an outpatient and inpatient basis. Where ganciclovir is used, clinicians estimate that only 1–30% of patients receive this type of prophylaxis; a much higher proportion of patients (up to 80%) are reported to receive aciclovir prophylaxis; figures for valaciclovir are intermediate.

Clinicians were then presented with a range of transplant patient types and asked to indicate which of these **might** be given anti-CMV prophylaxis in their transplant centre (*Table 60*).

The responses in *Table 60* indicate that no centres report that they would give prophylaxis to R–D– transplants and over-three quarters would not for R+D+ or R+D–. Most report, however, that they would give prophylaxis to R–D+ transplants and over half to patients on antibody induction therapy.

Renal consultants were also asked about any impact of CMV prophylaxis on the management of a patient with a new positive CMV test result, namely whether a patient would be managed differently when faced with a newly positive CMV test depending on whether the patient is on prophylaxis or not.

One-third of respondents (10/31 or 32%) answered 'Yes' to this question, and three respondents (10%) recorded 'Don't know'. Respondents who answered 'Yes' offered explanations, including the following:

- That the patient would be switched from prophylaxis to ganciclovir (6/31 or 19% of respondents).
- That a patient on prophylaxis may get disease which is asymptomatic and short-lived and it would not be necessary to treat this unless it is a high-risk transplant (one response).
- That if a patient is on prophylaxis one might be happier to observe a patient who is otherwise well, but who has a positive CMV PCR result (one response).
- That this would depend on clinical status (one response).

Suggested improvements to CMV testing of renal transplant patients

A final question asked whether CMV testing could be improved in any ways that had not already been indicated. Most of the 37 respondents expressed satisfaction with their CMV testing. The main issues raised related to the speed of reporting (seven respondents) and the evolving nature of testing for CMV (two respondents). One renal clinician described how CMV screening plus targeted treatment had been tried, but the centre had reverted to prophylaxis as a less burdensome strategy:

> "When post-operative ganciclovir became available we used a prophylactic approach (with audit). We then for about 12 months trialed 'pre-emptive monitoring' and treated with intravenous ganciclovir only those patients who showed increased titre. The administrative burden and need for inpatient care persuaded us to re-adopt 'blanket prophylaxis' (postoperative valganciclovir may mean we re-evaluate the situation)."

Survey of UK haematology transplant centres

Haematology transplant centres were similarly surveyed in order to assess their existing CMV screening practices and the views of clinicians on these. Questionnaires were sent in the second half of 2002 to lead clinicians (Directors of Bone Marrow Transplant Units, Professors of Haematology and Haematology Consultants) in 21 transplant centres in the UK [the mailing list was supplied by Dr David Marks (Chairman of the Clinical Trials Committee, British Society of Bone Marrow Transplantation)]. Non-responders were sent a further questionnaire and then telephone follow-up was used for any centre which had not provided a response.

A total of 11 responses were received from the 21 centres (this represents a 52% response rate, compared with a 77% response rate for renal transplant centres).

Haematology screening and diagnostic testing for CMV

All 11 respondents provided information on CMV testing in their transplant unit. Most (9/11 or 82%) indicated that they undertake both screening and diagnostic testing, but two centres indicated that they use screening tests only. Clinicians were asked how frequently and over what period patients are screened post-transplant. Responses once again demonstrate a similar pattern to that reported by laboratories (see Chapter 4, *Table 31*). The most common screening period reported was 24–26 weeks, with 12–17 weeks post-transplant being the second most likely. Only two centres screened for longer, namely 36–52 weeks post-transplant (and possibly longer if the patient is still on immunosuppressive therapy). In terms of testing frequency, the most common interval between tests was 7 days, with slightly more frequent testing (about twice per week) being the next most likely. Hence the screening period used in the present study is similar but our testing interval was slightly higher; in the study, bone marrow/stem cell transplant patients were screened during the first 6 months (24 weeks) following transplant, and testing was at 2-weekly intervals for the first 3 months and then every 4 weeks.

Stratification of haematology transplant patients for CMV testing

Respondents were next asked whether, when testing for CMV post-transplant, they stratify haematology transplant patients according to risk. Most (10/11 or 91%) reported that some form of stratification **is** undertaken; this contrasts with only 41% of renal centres. Furthermore, in 8/10 cases (80%) this is according to a protocol, and in the remaining 2/10 cases stratification is on a case-by-case basis using clinical indicators. Only one centre did not use some form of stratification when screening for CMV.

Staff in the 10 centres who stratify patients were asked to provide further information. Five centres provided comments and three enclosed a copy of their centre's protocol. All the protocols excluded autologous transplants from screening, but they adopted slightly different screening patterns for the

remaining high-risk and medium-risk groups (see Appendix 13). In other cases it was reported that autologous transplants are not screened, and there was a level of consistency in the type of allogeneic transplants screened. Of the five centres that described their stratification process (rather than sending a copy of their protocol), one commented that, for allogeneic transplants, if either donor or recipient was CMV positive pretransplant then ideally CMV screening would last for at least 6 months; if donor or recipient was CMV negative then there would be no screening. Two centres indicated that there would be screening of allogeneic BMT if either patient or donor is CMV positive, with one reporting weekly screening. Two more centres reported routine screening only for allografts, although there was no difference between different types, with one indicating that all allografts are screened weekly until week 26 (longer if they remain positive at this point).

Hence for this patient group national practice appears to differ from the screening protocol implemented in the study site, where all haematology transplant patients were screened (both autologous and allogeneic transplants).

Haematology clinicians' views on frequency and duration of CMV testing

Haematologists were asked about their satisfaction with the frequency and duration of CMV testing in their centre. All except one respondent (10/11) indicated that they were satisfied. No comments were made about follow-up post-discharge, unlike the renal consultants, possibly because patients return to the transplant centre for follow-up, rather than their local hospital. The one respondent who identified a need for improvements commented that:

> "because of personnel issues antigenaemia tests can only be done on three days per week ... the centre has no arrangements for rapid CMV tests to be done after routine hours or at the weekend, e.g. if a patient needs an emergency bronchoscopy/endoscopy ... a more rapid test with faster turn-around is needed, and a better on-call service is needed."

In addition, this respondent questioned the type of test currently used, pointing out that a PCR-based test is required rather than antigenaemia, owing to the number of neutropenic patients.

Haematology clinicians' views on types of CMV tests used

Haematologists were asked what type of CMV test is available for their patients. All clinicians completed this question. Most centres (10/11 or 91%) indicated that a PCR-based technique is used. Nearly half of these (four centres) made explicit reference to the use of quantitative PCR as their main test; one centre used commercial (Roche) quantitative PCR; one used quantitative PCR (LightCycler technology); one did not specify more than quantitative PCR; and one used both qualitative and quantitative PCR. Clinicians in the remaining centres had access to in-house PCR tests (two centres); plasma PCR (two centres); and PCR (unspecified) in two centres. The one centre that did not have access to a PCR test listed antigenaemia and DEAFF as the tests used. Once again, this pattern largely mirrors that reported by laboratories (see Chapter 4, *Table 28*).

When asked whether they consider that the types of CMV tests available are satisfactory, 7/10 clinicians thought they were. However, three respondents indicated that current tests were unsatisfactory. One of these respondents was based at a centre where antigenaemia and DEAFF are used, and had already commented that a PCR-based test was needed rather than antigenaemia because of the number of neutropenic patients. A second respondent at a centre where PCR was used made the comment that "access to pp65 antigenaemia would provide additional valuable information". The third respondent, from a centre where qualitative PCR is used, mentioned resource constraints pointing out that "(we) need quantitative PCR. We have no funds to employ staff to run the LightCycler that they have already purchased."

Protocols for CMV results

Haematologists were similarly asked whether their centre has formal protocols in place to act upon the results from CMV tests. All except one centre (10/11 or 91%) stated that they have. The centre with no formal protocol commented that if quantitative PCR is positive and a patient has symptoms they would treat, but in asymptomatic patients they would observe and consider antigenaemia testing. The other 10 centres with formal protocols were asked to provide further details, and seven did so.

Most respondents reported that they would require two positive results before initiating treatment. One indicated that if CMV PCR transcripts are >400 they would simply repeat the PCR the following day for confirmation. A second respondent indicated that they would order an immediate repeat test and would treat (pre-emptively) based on a second set of positive results; or if the patient is in a high-risk group

TABLE 61 *Haematology clinicians: groups given CMV therapy following positive test*

Type of transplant	Patient group given CMV therapy			
	Yes No. (%)	Maybe No. (%)	No No. (%)	Total
Unrelated donor transplant (CMV positive donor)	8[a] (80)	2[b] (20)	0 (0)	10
Unrelated donor transplant (CMV negative donor)	8[a] (80)	2[b] (20)	0 (0)	10
Other (i.e. related) allografts (CMV positive donor)	8[a] (80)	1[b] (10)	1 (10)	10
Other (i.e. related) allografts (CMV negative donor)	8[a] (80)	1[b] (10)	1 (10)	10
Autografts (CMV positive donor)	2[a] (20)	5[b] (50)	3[c] (30)	10
Autografts (CMV negative donor)	2[a] (22)	4[b] (44)	3[c] (33)	9[d]

[a] One case commented Yes 'but would re-test'.
[b] One case commented: only if patient had symptoms compatible with CMV, e.g. enteritis/liver function tests.
[c] One case commented: these patients not screened routinely.
[d] No response from 1 centre.

they would give immediate pre-emptive therapy, and then repeat the test. A further respondent stated that they would commence ganciclovir if CMV PCR was positive on two occasions at 0–80 days post-transplant. A fourth respondent indicated that if a patient was positive twice on qualitative PCR they would treat the patient with ganciclovir and then monitor the patient's response with quantitative PCR. Two respondents simply stated that they would use pre-emptive ganciclovir with follow-up testing. A final respondent indicated that they would use antiviral therapy until PCR negative (or CMV specific T cell immunotherapy as part of a clinical trial). Therefore, the majority of protocols involve a repeat CMV test, usually PCR, which is positive before treatment (4/7 respondents; one of these would also treat pre-emptively if a high-risk case). The remaining 3/7 respondents have formal protocols that involve pre-emptive treatment with follow-up testing.

Haematology clinicians' response to newly positive CMV test

All clinicians were asked in what cases they would prescribe anti-CMV therapy if a haematology transplant patient tested positive after previously testing negative for CMV. Ten of the 11 centres responded and their responses to this question are given in *Table 61*.

It would appear that in unrelated donor transplants and allografts (irrespective of the CMV status of the donor), most clinicians would respond to a newly positive result by initiating anti-CMV therapy; a few would use an element of discretion (contingent upon clinical context); and one physician would not prescribe therapy in the case of related allografts. In relation to autografts, *Table 61* indicates that there appears to be less consensus about the use of anti-CMV therapy in a patient with a positive CMV test result.

Clinicians were also asked to provide details of the therapy typically used. The responses recorded show that the main drug therapy used is ganciclovir.

In a follow-on question, respondents were asked under what sort of circumstances a positive result would not result in prescribing of anti-CMV therapy. The majority of respondents (7/11) specifically mentioned the need for two positive test results. Only one respondent mentioned the need for clinical symptoms as a requirement for prescribing (unlike renal transplants, where this was the major reason) and the remainder mentioned that it would be contingent upon the type of transplant and donor source.

CMV antiviral prophylaxis

Haematologists were asked whether their department currently uses CMV prophylaxis and, if so, what type(s) of drugs are used and in what percentage of transplant patients. *Table 62* shows that, unlike renal responses, aciclovir is the drug most often reported (2/4 centres), with ganciclovir or valaciclovir used for prophylaxis in the other two sites; drugs are mainly administered orally and prescribed on both an outpatient and inpatient basis.

Clinicians were asked to identify which patient groups might be given anti-CMV prophylaxis in their transplant centre. *Table 63* shows their answers.

No centre would give CMV prophylaxis to R–D– transplants. Three centres reported that they

TABLE 62 *Haematology transplant centres: use of CMV prophylaxis*

Form of prophylaxis	Method of delivery	Provided on inpatient or outpatient basis	Estimated transplants given prophylaxis (%)
Aciclovir	i.v. then after discharge 800 mg q.d.s. p.o.	Not indicated	50% of allogeneic transplants
Aciclovir	i.v.	IP	60
Valaciclovir	Oral	IP/OP	50
Ganciclovir	Defined by protocol	IP/OP	75% of unrelated transplants

IP, inpatient; OP, outpatient.

TABLE 63 *Haematology transplants: patient groups given CMV prophylaxis*

Type of transplant	Patient group given CMV prophylaxis		Total[a]
	Yes No. (%)	No No. (%)	
Unrelated donor transplants (R–D+; R+D–; R+D+)	3 (30)	7 (70)	10
Unrelated donor transplants (R–D–)	0 (0)	10 (100)	10
Other allografts (R–D+; R+D–; R+D+)	2 (20)	8 (80)	10
Other allografts (R–D–)	0 (0)	10 (100)	10
Autografts (R/D+; R/D–)	0 (0)	9 (100)	9[b]
Non-myeloablative allograft with Campath (alemtuzumab) conditioning (R–D+; R+D–; R+D+)	2 (22)	7 (78)	9[b]
Non-myeloablative allograft with Campath (alemtuzumab) conditioning (R–D–)	0 (0)	9 (100)	9[b]
Non-myeloablative allograft without Campath (alemtuzumab) conditioning (R–D+; R+D–; R+D+)	2 (20)	8 (80)	10
Non-myeloablative allograft without Campath (alemtuzumab) conditioning (R–D–)	0 (0)	10 (100)	10

[a] One centre response missing throughout (specific questions not addressed).
[b] One centre respondent said 'not applicable'.

would give prophylaxis to all other unrelated transplants; two centres would also provide prophylaxis to other (i.e. related) allografts; and two centres would provide it for non-myeloablative allografts with or without Campath (alemtuzumab) conditioning.

Haematologists were asked about any impact of CMV prophylaxis on the management of a patient with a new positive CMV test result. Only two respondents (2/11 or 18%) answered 'Yes' to this question, compared with one-third of renal clinicians, and two respondents did not record an answer to this question, both reporting no use of prophylaxis. One of the two respondents who answered 'Yes' indicated that treatment doses would be changed; the other indicated that an alternative antiviral agent would be considered for treatment.

Suggested improvements to CMV testing of haematology patients

When asked whether CMV testing could be improved in any way, the main issues raised related to the need for ready availability of testing, the speed of reporting and the need for appropriate CMV tests (i.e. those which were considered to be suitable by the clinician). Specific comments included the desire to have a daily rather than twice-weekly service (something considered not to be practical by the respondent, and the comment was made that urgent PCRs can

still be accommodated if they insist). Two clinicians commented upon speed of reporting; one stated that there was a 3-day wait for results, another commented that in relation to speed of reporting "the faster the better", although they were "happy with current reporting times". Another person commented upon the need for antigenaemia tests and upon difficulties sending samples and obtaining results promptly. Finally, a further respondent indicated that quantitative PCR was required, but otherwise provision was O.K.; and one clinician stated that there was a need for CMV quantitative tests with rapid turnaround time plus clinical advice.

Chapter 8

Cost-effectiveness of CMV screening regimes

Introduction

It is evident that the introduction of CMV screening has the potential to lead to a substantial increase in laboratory CMV testing costs (77% of CMV tests nationally are reported to be screening tests; see *Table 27*). Also, the analysis of clinical impact reported in the previous chapter indicates that, depending on the CMV testing strategy adopted, there may in addition be various 'associated' or concomitant costs linked to treatments and investigations ordered as a result of screening test information, some of which may be incorrect. A full economic evaluation of the different molecular CMV screening strategies therefore requires that the relative resource costs (both direct test cost and other associated or concomitant costs) measured in the study be compared with any quantifiable benefits (or disbenefits) that can be identified.

In the case of diagnostic technologies, a number of elements enter into a chain of inquiry, representing different measures that may be indicators of benefit (see Appendix 14). A recent overview of diagnostic studies has identified that most measure benefits primarily in terms of the accuracy of the test results produced.[60] Occasionally, they are also measured in terms of the impact of results on diagnostic certainty or patient management and, less rarely, in terms of impact on patient outcome. Diagnostic certainty and patient management effects are both process measures. The impact of normal or negative results is normally largely ignored, although this is not necessarily always the case.[62] In the present study, it may be argued that an important benefit of CMV screening tests is the reassurance provided to clinicians by negative results, since positive CMV results represented a very small minority of samples (see *Table 13*). However, even though most screening results may only provide reassurance, the level of **real** benefit whether for primary diagnosis or for monitoring of a patient's condition will be dependent on provision of accurate test results.

In addition to benefits, CMV screening may also result in certain disbenefits for patients, particularly if there is an inability to detect CMV disease in some cases (false negatives) coupled with incorrect identification of disease in other cases (false positives). Ideally, a comprehensive economic evaluation should measure and value the impact of these, and also the benefits associated with any correct, early diagnosis which allows pre-emptive treatment. From the results in Chapter 3, it is evident that the number and types of incorrect result will differ depending on the test used. This in turn will also influence the level of other NHS costs associated with the test, and therefore the balance of costs and benefits will vary depending on the type of testing regime adopted.

During both stages of the prospective study, molecular CMV screening tests were trialled in a service setting so that the final level in the Fineberg assessment hierarchy could be addressed. The study measured impact of each test on diagnostic certainty, any effects on patient management, clinicians' perception of benefits to the patient and also the accuracy of test results and longer term patient outcomes.

Ideally, in addition to comparing the costs and benefits of alternative CMV screening regimes, these regimes should also be compared against a diagnostic testing only regime using pp65 antigenaemia, that is, the *status quo* in UHW prior to the study. However, the CMV screening regimes trialled allowed for diagnostic tests to be requested between screening tests, if the clinician considered this necessary, that is, the strategies on which prospective data were collected are inevitably 'mixed' strategies.

A number of drivers would be expected to influence the relative cost-effectiveness of these different CMV screening regimes. Among others, these will include the level of:

- reduction in CMV disease/death
- reduction in transplant failure due to CMV disease
- increase in diagnostic certainty
- reduction in inappropriate CMV therapy (i.e. ganciclovir)
- increase in CMV testing costs
- increases in inappropriate therapy/further investigations due to false positives/negatives.

These drivers are now considered further.

Reduction in CMV disease/death (study patients versus historical controls)

The first of these (reductions in CMV-related deaths) could not be assessed in the study because numbers were far too low to detect a statistically significant difference between historical and study patients (for comparison of CMV screening with diagnostic test only strategies) or in stage one and stage two (for comparison of different screening tests). For example, in the group with the largest number of deaths (haematology transplant group) there was only one CMV-related death in the study period and none in the renal transplant patient group [see the section 'Patient CMV infection patterns and outcomes at end of screening period' (p. 50)].

Although differences in CMV-related deaths could not be measured, reductions in the **level of CMV disease** might provide a surrogate indicator of this. For historical controls, the number of patients with a positive antigenaemia test result (the only common test) was used as a measure of this. There was no significant difference observable for renal patients; the 199 historical controls and 98 prospective study patients both included exactly 13% of patients who tested positive on antigenaemia alone (see *Table 54*). In the case of haematology transplant patients, there was no statistically significant difference recorded, with 2.2% of the 136 patients in the historical control group testing positive on antigenaemia compared with 3.6% in the 140 prospective study group (see *Table 55*). There was therefore no evidence of a change in levels of CMV detection as a result of the introduction of CMV screening.

Reduction in transplant failure (study patients versus historical controls)

A second factor that might influence cost-effectiveness would be a reduction in the level of transplant failures or rejections associated with CMV screening, if any. Once again, analysis provided no indication of a positive effect. Although there was a difference observed for renal transplants between historical and study transplant failure rates at 9 months ($p < 0.01$), this could not be attributed to lower levels of CMV disease (see *Table 54* and *Figure 3*). There were no similar data available for haematology transplant patients.

Impact on diagnostic certainty/patient management (study patients)

There were no historical data on the impact of diagnostic tests on diagnostic certainty or patient management. However, in the prospective study the impact of test results was recorded prospectively. Screening results were reported to have a very limited impact on diagnostic certainty, although a significantly greater ($p < 0.01$) effect was reported for haematology than for renal results (see *Tables 47* and *48*). Overall, an increase in diagnostic certainty was associated with only 4% of all renal screening regime results (24/573) and 13% of all haematology results (88/704). If screening test results alone are considered (excluding tests ordered for diagnostic purposes), this figure is the same for renal cases (4%) and only slightly lower for haematology results (11%). The reporting of an increase in diagnostic certainty was significantly linked to the presence of a positive test result for haematology patients ($p = 0.026$) but not for renal transplant patients ($p = 0.0585$). Hence approximately one in 10 purely screening results increased diagnostic certainty in haematology patients and fewer than one in 20 in renal patients; positive CMV test results were also more likely to increase diagnostic certainty in haematology cases than in renal transplant cases. It should be borne in mind that diagnostic and screening tests cannot be separated in the screening regime as easily as this might imply, since the introduction of screening has been shown to lead to an increase in the number of CMV diagnostic tests requested (see *Tables 54* and *55*). In addition, a small number of respondents reported a **decrease** in diagnostic certainty; 1% of renal and 2% of haematology test results.

In summary, only a limited impact was observable on diagnostic certainty, and in most instances screening appeared simply to provide reassurance and did not affect patient management. However, there may be a latent benefit from screening test results in terms of providing general reassurance **without** influencing diagnostic certainty.[62] It appears that an effect of this type may be in operation since overall 63% of haematology test results and 72% of renal test results were identified as of benefit by clinicians (see *Tables 47* and *48*).

Even where there is a reported impact on diagnostic certainty, this will not necessarily be linked to measurable changes in planned patient management. Of the 24 renal test results that were reported to increase diagnostic certainty, five were positive tests, with three resulting in a change in patient management; the remaining 19 negative tests mostly had no influence and only four led to a change in management. Thus, of the 24 renal CMV tests reported to increase diagnostic certainty, only seven led to a change in patient management and the remainder (71%) presumably provided reassurance for management that was already

under way or planned. Similarly, of the 88 haematology tests that led to an increase in diagnostic certainty, five were positive tests, with three resulting in a change in patient management, and of the remaining 83 negative tests, only 20 led to a change in management. Hence 74% of the haematology tests that were reported to increase diagnostic certainty presumably also provided reassurance. For screening only tests, these figures were very similar, 76% and 74% for renal and haematology results, respectively. Overall, 7/573 renal test results (1.2%) and 23/704 (3.3%) of haematology results led to a change in diagnostic certainty and patient management.

Reduction in inappropriate CMV therapy (ganciclovir)

Analysis of the clinical impact recorded in the prospective study indicates that only 2/506 renal screening test results (<0.5%) led to planned CMV therapy being avoided (see *Table 48*). A similar analysis of the haematology questionnaires shows that 0/600 screening test results (0%) led to CMV treatment being stopped. This is not unexpected since only a very limited effect on diagnostic certainty was reported. There is therefore no evidence available from the prospective study that CMV screening is likely to have a significant effect in terms of reducing inappropriate CMV therapy. The relationship between prescribing and test results is also not straightforward, as evidenced in *Tables 51* and *52*. Reductions in inappropriate CMV therapy could not be assessed by direct comparison with the historical control groups because no data were available on ganciclovir prescribing in the prestudy transplant patients.

Cost-effectiveness analysis

Although there was no evidence of a significant impact of screening on CMV disease levels or on inappropriate ganciclovir prescribing, there were significant differences in the number of cases detected by the various CMV screening regimes (i.e. due to differences in test sensitivity and specificity). These differences are likely to have an impact on various other measures, including CMV prescribing, initiation of other therapies and ordering of further investigations. CMV molecular screening tests were therefore compared in terms of their cost-effectiveness based on the following:

1. the number of samples identified as positive by a particular test during the screening period versus the direct cost of laboratory testing in order to enable a cost-effectiveness ratio (calculated as the test cost per positive sample identified) to be calculated
2. the number of samples identified as true positive by the test over the same period of time versus the direct cost of laboratory testing in order to enable a second cost-effectiveness ratio (calculated as the test cost per **true** positive sample detected) to be calculated
3. the number of samples identified as true positive by the test over the same period of time versus direct and associated/concomitant cost consequences (i.e. cost of testing plus any further treatment/investigation costs associated with false-negative and false-positive test results) in order to enable a third cost-effectiveness ratio (calculated as the direct and **associated cost** per true positive sample detected) to be calculated.

The next section explores these ratios for the different CMV screening regimes introduced in the study setting, and compares these with diagnostic tests alone (antigenaemia pp65). The subsequent section models the cost-effectiveness of alternative CMV screening regimes that might be implemented; these include regimes that stratify patients according to their inherent risk of CMV disease.

Cost-effectiveness ratios for UHW CMV screening strategies

The findings presented in Chapters 5 and 6 can be used to estimate cost-effectiveness ratios for different CMV screening test regimes as implemented in UHW versus estimates for a regime consisting of diagnostic antigenaemia tests only (using data on diagnostic tests requested in association with screening tests). The analysis was undertaken from the perspective of the NHS and excluded any patient costs.[63] As explained above, the primary outcome measures used were the number of CMV positives detected and the number of true positives detected. These were compared with the cost of CMV testing using baseline costs (see Chapter 5), taking into account differences in test failure rates (Chapter 3). A batch size of five samples was assumed initially. In Cardiff, the CMV screening protocols used resulted in the laboratory processing an average 4.2 samples each week for renal transplant patients; for haematology patients this figure was 5.2 samples per week [see the sections 'CMV testing patterns for renal transplant patients' (p. 13) and 'CMV testing patterns for bone marrow/stem cell transplant patients' (p. 14)] Since

TABLE 64 *Cost-effectiveness of CMV screening regimes[a] for renal patients (direct costs)*

CMV screening/diagnostic test regime	Total direct cost per 100 test results[b] (£)	Average cost per positive sample (£)	Average cost per true positive sample detected (£)	Total no. of positives undetected (false negatives)[c]	Total no. of positives wrongly detected (false positives)[c]
Antigenaemia (pp65): diagnostic tests only	3217	130	130	9.9	0
Antigenaemia (pp65): screening regime	3217	607	643	6.5 (6.2)	0.4 (0.3)
NASBA, Organon Teknika: Screening regime	3389	404	458	2.3 (2.3)	1.1 (1.1)
Roche Amplicor Assay PCR: Screening regime)	2972	209	256	2.0 (1.9)	2.7 (2.9)
Nested in-house PCR (single-round): screening regime	1191	113	116	3.0 (2.5)	0.2 (0.4)
Nested in-house PCR (two-round): screening regime	1537	67	120	0.4 (0.4)	10.1 (10.1)

[a] Screening regimes include diagnostic tests ordered as part of regime.
[b] Based on batch size of 5 samples and allowing for observed level of test failure.
[c] Figures in parentheses based on all completed questionnaires. Main figures based only on complete sets (i.e. request and results questionnaire).

the laboratory undertook CMV screening for both types of patient, as do most laboratories nationally (see Chapter 4), this was equivalent to 9.4 samples per week. Assuming that tests are run twice per week (2–3 days), it would be possible to run test batch sizes of about five samples.

Average UHW direct cost per positive detected and per true positive detected

The direct costs of testing per CMV positive detected and per **true** positive detected for the five different CMV screening regimes are presented in *Tables 64* and *65* for renal transplant and haematology transplant patients, respectively. Estimates for a diagnostic testing only regime (using antigenaemia pp65) are shown in the first row of the tables. Below this, the CMV screening regimes are arranged in the third column in terms of decreasing cost per positive detected (i.e. increasing cost-effectiveness).

The cost-effectiveness ratios presented in these tables are based on all CMV tests requested as part of the screening protocol. The final two columns in both tables provide estimates of the number of CMV-positive samples that will not be identified (false negatives) and the number of samples that will be identified incorrectly as CMV (false positives) for each type of test. Data are included only if a complete set of questionnaires was completed, that is, request questionnaire and result questionnaire. For comparison purposes, figures are also provided (in italics) based on all questionnaire responses, regardless of whether both questionnaires were completed; these demonstrate no significant differences.

In terms of the basic cost-effectiveness ratio (cost per positive detected), two-round nested PCR would appear to be the most cost-effective screening test for both renal and haematology transplant patients. However, as the final column shows, this test also has by far the highest number of false positives.

If **CMV diagnostic tests** [see the top row in *Tables 64* and *65*, bearing in mind the caveat in the section 'Impact on diagnostic certainty/patient management out (study patient)' (p. 78)] are compared with the various screening test regimes in terms of **true positives**:

- For renal transplant patients, it appears that on the indicator of cost per true positive detected the in-house PCR (single-round and two-round) screening regimes perform better (£116–120 per true positive detected) than the antigenaemia diagnostic testing regime (£130);

TABLE 65 *Cost-effectiveness of CMV screening regimes[a] for haematology patients (direct costs)*

CMV screening/diagnostic test regime	Total direct cost per 100 test results[b] (£)	Average cost per positive sample (£)	Average cost per true positive sample detected (£)	Total no. of positives undetected (false negatives) per 100[c]	Total no. of positives wrongly detected (false positives)[c]
Antigenaemia (pp65): diagnostic tests only	3217	1287	1287	8.0	0
Antigenaemia (pp65): screening regime	3217	2475	2475	2.7 (2.3)[c]	0 (0.1)
NASBA, Organon Teknika: screening regime	3389	1169	1540	0.9 (0.8)	0.7 (0.8)
Roche Amplicor Assay PCR: screening regime	2972	929	1858	1.0 (0.7)	1.6 (1.4)
Nested in-house PCR (single-round): screening regime	1191	425	518	0.6 (0.5)	0.6 (0.6)
Nested in-house PCR (two-round): screening regime	1537	154	615	0.4 (0.25)	7.5 (6.2)

[a] Screening regimes include diagnostic tests ordered as part of regime.
[b] Based on batch size of 5 samples and allowing for observed level of test failure.
[c] Figures in parentheses based on all completed questionnaires. Main figures based only on complete sets (i.e. request and results questionnaire).

all other screening regimes appear less cost-effective. The latter pattern is not unexpected because one would expect that when a diagnostic test is ordered there would be a greater likelihood of a positive test result and, therefore, a more favourable cost-effectiveness ratio; for in-house PCR it is the lower testing costs that outweigh this.

- In haematology patients, the latter pattern is observed with the cost-effectiveness ratios much higher for all regimes, including for the diagnostic testing regime (£1287 per true positive detected).

If one compares the various **CMV screening regimes**, then the following conclusions can be drawn:

- Single-round nested in-house PCR appears to be more cost-effective (£116 and £518 per true positive detected for renal and haematology patients, respectively) than all other screening regimes (range £120–643 per true positive for renal and £615–2,475 for haematology patients with other tests).
- Two-round nested in-house PCR produces by far the highest number of false positives in both renal and haematology patient samples, with the Roche Amplicor PCR Assay also exhibiting a higher rate than the remaining tests.
- pp65 antigenaemia produces by far the highest number of undetected positives in both renal and haematology patient samples.

For renal patients, if a screening regime were to use the antigenaemia test, this is estimated to be the least cost-effective regime (£643 per true positive detected), owing partly to the relatively high cost of the test and also to the large number of positives undetected. A similar conclusion is drawn for CMV screening in haematology transplant patients, but with an even more unfavourable cost-effectiveness ratio (antigenaemia £2475 per true positive detected).

Sensitivity analysis varying batch size and excluding diagnostic tests

In order to consider the likely effect, on the cost-effectiveness ratios calculated, of varying the assumptions made, a sensitivity analysis was conducted. This examined the impact upon cost-effectiveness ratios of increased test batch size (the major influence on test costs) and limiting the analysis only to tests ordered purely as part of the screening protocol, that is, excluding any diagnostic tests (the major influence on likelihood of a test positive).

As described above, the average throughput figure observed in Cardiff was 9.4 samples per week. Test costs were calculated for this larger average batch size were a single batch to be run weekly. *Tables 80* and *81* in Appendix 15 show the impact of this larger batch size on the cost per positive detected and per true positive detected. As might be expected, tests exhibit an improved cost-effectiveness ratio with larger batch size, owing to a fall in the cost of producing an individual test result. However, this effect is relatively slight. Comparing the various screening tests at both batch sizes indicates that in terms of the cost per true positive detected, single-round nested in-house PCR remains more cost-effective than all other screening regimes, including two-round PCR.

In terms of generalisability to other laboratories, the average figure of N10 samples per week observed in Cardiff is, in fact, only one-third of the average weekly throughput reported by other UK laboratories (34.6 CMV tests per week, as shown in Chapter 4). However, batch sizes of this magnitude are not feasible for certain tests (see Appendix 12) and it also more likely that a laboratory would run more frequent, smaller batch sizes in such a case. Higher throughputs occur because most other UK centres report screening protocols that involve much more frequent screening tests than in the present study [see the sections 'Renal screening and diagnostic testing for CMV' (p. 67) and 'Haematology screening and diagnostic testing for CMV' (p. 71) for renal and haematology transplant patients, respectively].

The sensitivity analysis indicates that

- As batch size doubles, the cost per true positive detected falls. Therefore, it appears that single-round nested in-house PCR will be even more cost-effective if batch sizes of >10 are practicable in a laboratory.
- Increased batch size does not, however, change the relative positions of the other screening tests.

The sensitivity of the findings to limiting the analysis to screening tests only (i.e. excluding any diagnostic tests ordered during the screening period) is shown in Appendix 15 (*Tables 82* and *83*). Cost-effectiveness ratios are presented for smaller and larger batch sizes; the cost-effectiveness ratios are limited to **true** positives only (excluding the less robust cost per positive values). These ratios are consistently higher than the ratios in *Tables 64* and *65* owing to the lesser likelihood of detecting a true positive when there is no clinical reason to order a CMV test (especially for haematology transplant patients). The ratios demonstrate a similar pattern to that shown in *Tables 64* and *65*, with nested in-house PCR estimated to have the most favourable cost-effectiveness ratio, although the two-round nested PCR test is now estimated to be slightly more cost-effective than the one-round test for both throughputs and both patient groups. However, once again this test continues to identify far more false positives in these samples than any other screening test and might be rejected on this basis. Bearing in mind that diagnostic and screening tests could not be easily separated (as discussed above), with this caveat in mind, Appendix 15 demonstrates a similar pattern for screening tests only to that shown in *Tables 64* and *65*.

Incremental cost-effectiveness ratios (ICERs) for UHW CMV screening strategies

Tables 66 and *67* provide a comparison of average and incremental costs and benefits (relative to antigenaemia screening) for different tests used in a CMV screening regime, like that adopted in UHW, for renal and haematology patients, respectively. Costs in these tables are based on laboratory costs only. Comparisons are based on all tests processed as part of the screening regime (i.e. including diagnostic tests). All molecular tests detect more true positives than antigenaemia when used in a screening regime, and all except NASBA are less expensive per test (more details are provided in Appendix 16, *Tables 84* and *85*). All molecular tests exhibit a negative ICER (except for NASBA). The larger this negative ICER is, the more cost-effective is the test relative to antigenaemia; single-round in-house PCR is therefore most cost-effective.

As mentioned above, *Table 66* shows that, based on the **average cost** per true positive, the use of single-round nested in-house PCR remains the most cost-effective molecular screening regime for renal transplant patients. Hence the ICERs presented provide further evidence to support the use of single-round nested in-house PCR in screening as the most cost-effective strategy. *Table 67* similarly indicates that, for haematology transplant patients, a molecular screening regime using single-round nested in-house PCR is also the most cost-effective.

In conclusion, based on the direct laboratory testing cost and the number of true positives detected, *Tables 64–67* (and Appendices 15 and 16) appear to demonstrate that PCR (single-

TABLE 66 *Incremental[a] cost-effectiveness of CMV screening regimes[b] for renal transplant patients (direct test costs only)*

CMV screening regime	Average cost per true positive sample detected (£)	Incremental[a] cost per additional true positive sample detected (£)	Total no. of positives undetected (false negatives)	Total no. of positives wrongly detected (false positives)
Antigenaemia (pp65)	643	–	6.5	0.4
NASBA, Organon Teknika	458	+72	2.3	1.1
Roche Amplicor Assay PCR	256	–37	2.0	2.7
Nested in-house PCR (single-round)	116	–382	3.0	0.2
Nested in-house PCR (two-round)	120	–215	0.4	10.1

[a] Relative to pp65 antigenaemia.
[b] Full regime in which diagnostic tests ordered as part of screening regime are included.

TABLE 67 *Incremental[a] cost-effectiveness of CMV screening regimes[b] for haematology patients (direct test costs only)*

CMV screening regime	Average cost per true positive sample detected (£)	Incremental[a] cost per additional true positive sample detected (£)	Total no. of positives undetected (false negatives)	Total no. of positives wrongly detected (false positives)
Antigenaemia (pp65)	2475	–	2.7	0
NASBA, Organon Teknika	1540	+191	0.9	0.7
Roche Amplicor Assay PCR	1858	–817	1.0	1.6
Nested in-house PCR (single-round)	518	–2026	0.6	0.6
Nested in-house PCR (two-round)	615	–1400	0.4	7.5

[a] Relative to pp65 antigenaemia.
[b] Full regime in which diagnostic tests ordered as part of screening regime are included.

round) is the most cost-effective CMV screening test for renal and haematology patients in terms of both average and incremental cost per true positive detected. Cost-effectiveness ratios for the commercial tests are two to three times less favourable.

In addition, there were a large number of false positives associated with the use of other tests such as two-round PCR and (to a lesser extent) the Amplicor Assay. These may have resource consequences (associated/concomitant costs) and also disbenefits to patients from the toxic side-effects that might result from unnecessary use of ganciclovir treatment [see the section 'Ganciclovir treatment' (p. 5)].

Furthermore, undetected positives may also have major disbenefits, unless a subsequent test identifies these cases correctly. It appears that antigenaemia exhibits the highest level of false negatives and two-round nested in-house PCR the lowest level; other tests have largely similar (low) levels.

Associated (non-test) costs and impact on cost-effectiveness

Ideally, the cost-effectiveness ratios calculated should include the **total** cost associated with a particular test, that is, incorporating any concomitant costs associated with the test information provided, in addition to the laboratory cost of testing itself. Such associated costs may include repeat CMV tests requested to confirm results, plus any treatment or investigation costs associated with false-negative or false-positive test results. Appendix 17 presents estimates of the maximum levels of such associated costs predicted in the UHW trial for renal transplant and haematology patients (*Tables 86*, and *87*, respectively), separately for screening and for diagnostic tests. These figures are based on the *ex ante* reported likely impact of a positive or negative test result on therapy and further investigations, and the type of result produced by a particular test for that sample (whether it was reported or not). The cost placed on these investigations or treatments is that incurred in UHW during the period of the study. Individual figures cannot be

TABLE 68 *Cost-effectiveness of CMV screening regimes[a] for renal transplant patients (includes associated/concomitant costs)*

CMV screening regime	Average direct and associated cost per true positive sample detected[b] (£)	Incremental direct and associated cost per additional true positive sample detected (£)	Total no. of positives undetected (false negatives)	Total no. of positives wrongly detected (false positives)
Antigenaemia (pp65)	697	–	6.5	0.4
NASBA, Organon Teknika	491	+62	2.3	1.1
Roche Amplicor Assay PCR	346	+80	2.0	2.7
Nested in-house PCR (single-round)	116	–433	3.0	0.2
Nested in-house PCR (two-round)	437	+271	0.4	10.1

[a] Include diagnostic tests ordered as part of screening regime.
[b] Based on batch size of five samples and allowing for observed level of test failure.

summed because they do not necessarily all relate to the same patient.

Appendix 17 demonstrates that, in general, a higher level of concomitant costs is predicted in haematology patients (regardless of the test) than that reported for renal patients.

For renal patients (*Table 86*, Appendix 17), the single largest such cost is linked to false-positive test results and associated with prescribing of CMV treatment. This type of associated cost is likely to be higher for screening samples since the likelihood of a false positive is higher. There is also a (limited) predicted impact in terms of other investigations, such as bronchoscopy. For renal patients, false negatives were predicted to have very few indirect cost consequences, mainly linked to X-ray and bronchoscopy. False positives and negatives were not predicted to have any significant impact on other forms of prescribing (e.g. antibiotics), imaging (CT, etc.) or ordering of repeat CMV tests.

For haematology patients (*Table 87*, Appendix 17), once again a major indirect cost consequence linked to false-positive test results would be prescribing of CMV treatment. There is also a significant reported effect in terms of IVIg costs associated with false positives for haematology patients. The predicted impact of false positives on bronchoscopy and imaging costs is low. False negatives are once again reported to have limited indirect cost consequence, principally on IVIg therapy costs and further imaging. As was the case for renal patients, no associated costs were predicted by clinicians in terms of other prescribing (e.g. antibiotics), and only very limited effects in terms of repeat CMV testing.

If these concomitant costs are calculated for actual screening tests in the UHW trial, the pattern of associated costs will be generally higher in haematology patients (see *Tables 88* and *89* in Appendix 17, for renal and haematology patients, respectively).

Comparing the various molecular tests, nested in-house PCR (two-round) is the most likely to lead to associated/concomitant costs, in both groups of patients, followed by the Roche Amplicor PCR Assay. The highest costs are associated with the two tests that demonstrated the highest level of false positives and the lowest positive predictive values (see *Table 21*).

Cost-effectiveness ratios can be recalculated to include associated costs. *Tables 68* and *69* present estimates of the average and incremental cost per true positive detected, once these associated/concomitant cost consequences are taken into account (more details are provided in Appendix 17, *Tables 90* and *91*).

These tables show that, for both renal and haematology transplant patients, comparison of average and incremental values demonstrates that

- The screening strategy using single-round in-house PCR clearly emerges as by far the most cost-effective testing regime based on average and incremental values, once associated costs are incorporated.

- Once associated costs are included, the cost-effectiveness analysis demonstrates that two-round PCR now becomes far less favourable than it appeared to be when only laboratory testing costs were considered (see *Tables 66* and *67*).

TABLE 69 *Cost-effectiveness of CMV screening regimes[a] for haematology transplant patients (includes associated/concomitant costs)*

CMV screening regime	Average direct and associated cost per true positive sample detected (£)[b]	Incremental direct and associated cost per additional true positive sample detected (£)	Total no. of positives undetected (false negatives)	Total no. of positives wrongly detected (false positives)
Antigenaemia (pp65)	2537	–	2.4	0
NASBA, Organon Teknika	1580	+199	0.9	0.7
Roche Amplicor Assay PCR	3954	+10093	1.0	1.6
Nested in-house PCR (single-round)	727	–1626	0.6	0.6
Nested in-house PCR (two-round)	3051	+3608	0.4	7.5

[a] Include diagnostic tests ordered as part of screening regime.
[b] Based on batch size of 5 samples and allowing for observed level of test failure.

TABLE 70 *Cost-effectiveness ratios for single-round in-house PCR*

CMV screening regime: nested in-house PCR (single-round)	Renal patients		Haematology patients	
	Cost per diagnostic change	Cost per patient management change[a]	Cost per diagnostic change	Cost per patient management change[a]
Direct laboratory costs only	£284	£993	£95	£361
Direct laboratory costs and associated costs	£284	£993	£134	£507

[a] Based on changes in patient management following change in diagnostic certainty.

Alternative cost-effectiveness ratios

It is also possible to consider the cost-effectiveness of CMV screening tests in terms of outcomes such as changes in diagnostic certainty or changes in patient management. Examining the test that has been identified as most cost-effective above (i.e. single-round in-house PCR), cost-effectiveness ratios are compared in *Table 70*.

For renal patients, the cost per increase in diagnostic certainty (£284) is of a slightly higher order than the cost per true positive detected (£116), but the cost per change to patient management, a more 'concrete' effect, is much higher at £993.

In the case of haematology patients, because CMV disease is rare, the direct cost per true positive detected is relatively high (£518), although the cost per change in diagnostic certainty (£95) and per change in patient management (£361) is lower. This demonstrates that in terms of their impact on diagnosis and on patient management, CMV screening tests are more cost-effective in haematology transplant patients than in renal transplant patients. If associated costs (which are higher in haematology patients) are included, these ratios rise to £727 per true positive sample versus £134 per change in diagnostic certainty and £507 per change in patient management, so screening remains relatively cost-effective in this patient group.

Cost-effectiveness of alternative CMV screening strategies

A number of alternative scenarios, other than a universal screening strategy or a one-test strategy, as assumed above, might be envisaged for CMV screening.

One possible strategy would be the use of a combination of tests such as antigenaemia plus a molecular test (add-on tests). This would add to the cost of testing but might reduce associated/concomitant costs if it resulted in fewer false positives/negatives being reported to

clinicians. Interestingly, the UK laboratory survey found no evidence of the routine use of two tests in CMV screening (see Chapter 4). In the present trial, two tests were used for comparison purposes. Where laboratories nationally report the use of a second test, this is principally as a back-up, or as an alternative first-line test for particular types of patients. Hence there is no evidence that different tests are being used in combination (add-on) in CMV screening strategies for haematology or renal transplant patients. A scenario in which more than one test is carried out routinely on screening samples was therefore not considered further.

However, a national survey of laboratories and transplant centres, as detailed in Chapters 4 and 7, respectively, did identify targeted CMV screening strategies, particularly for haematology patients. From the survey responses, it appears that centres may not screen all their transplant patients, but instead they may stratify them based on the perceived risk of CMV disease.

We therefore modelled the likely impact on cost-effectiveness ratios of screening strategies in which transplant patients are differentiated as follows:

- *'High'-risk cases* – those individuals identified to have an enhanced risk of CMV disease prior to transplant, arising from the CMV status of the recipient and/or donor. In these patients CMV screening would be undertaken.
- *'Low'-risk cases* – those individuals where the risk of CMV disease is judged to be low, and screening would not be undertaken.

We first identified which patient groups **might** be targeted as high risk. Patient groups were categorised as at 'high', 'medium', 'low' or 'insignificant' risk of CMV based on disease levels reported in the literature 4,5 **and** based on our study findings (see *Tables 43* and *44*). Next, these categorisations were compared, and groups that were rated as at insignificant or low risk in our study **and** not identified as high risk in the literature were disregarded; the remainder were labelled as 'possibly' high risk (see *Table 71*).

The 'possibly high-risk' groups (marked 'Possibly' in *Table 71*) were then compared with responses to the UK surveys of renal and haematology transplant units (see Chapter 7). Thus, the renal group labelled as possibly high-risk (R–D+) accorded with responses to the national survey. UK renal transplant units specifically mentioned that, where risk stratification occurs, R–D+ patients are selected for CMV screening. The R–D– patients were consistently identified as a group that is **not** screened and R+D+ and R+D– groups were not mentioned by any respondent. For haematology transplant patients, all three groups labelled as 'possibly' high risk were compatible with UK survey responses. All bone marrow transplant centres undertaking risk stratification reported that they exclude autologous transplants from CMV screening. Furthermore, there was a high level of consistency in the types of allogeneic transplants included; screening was usually reported to take place only if either patient or donor was CMV positive, although two centres did report that they screen all allogeneic transplants (i.e. including R–D–).

Likely impact of risk stratification on cost-effectiveness of CMV screening

In order to model the likely impact on cost-effectiveness of CMV screening strategies targeted at 'high-risk' patients, two screening regimes were assessed:

- *Targeted renal screening* – screening of R–D+ group only
- *Targeted haematology screening* – screening of allogeneic transplants only, excluding R–D–.

Cost-effectiveness ratios were calculated for both of these targeted screening strategies.

Cost calculations included associated/concomitant costs and also the direct cost of laboratory testing; associated costs were based on the *ex ante* reported likely impact of a positive or negative test result on therapy and further investigations for the high-risk patients only in the Cardiff cohort. The pattern of concomitant costs predicted in these high-risk groups is shown in Appendix 18 (*Tables 92* and *93*). Comparison with Appendix 17 (*Tables 88* and *89*) shows that for renal transplant patients the total associated costs (first column) are expected to be slightly higher for the targeted strategy, especially for two-round nested in-house PCR (£49.88 versus £40.60 per test). For targeted screening of high-risk haematology patients, associated costs are calculated to be nearly double those of universal screening, especially for tests such as two-round nested in-house PCR (£117.26 versus £60.90 per test) and Roche Amplicor PCR (£74.95 versus £33.54 per test).

Hence there is no evidence that the introduction of a CMV-targeted 'high-risk' screening regime will reduce associated costs per test significantly; rather the reverse, which would make the cost-

TABLE 71 *High-risk groups for CMV screening (renal and haematology)*

CMV status	Level of risk in literature[a]	Level in study site[b]	Interim 'high risk'	UK surveys indicate this group screened	Selected as 'high risk' for cost-effectiveness analysis
Renal transplants					
R–D–	Insig. (0%)	Insig. (0%)			
R–D+	High (44%*)	High (43%)	Possibly	Yes	Yes
R+D–	Low (6%)	Insig. (0%)			
R+D+	Medium (25%*)	Low (3%)			
Haematology allogeneic transplants					
R–D–	Low (3%)	Insig. (0%)			
R–D+	Low (4%)	Medium (25%)	Possibly	Yes	Yes
R+D–	High (44%*)	Medium (25%)	Possibly	Yes	Yes
R+D+	High (12%)	Medium (14%)	Possibly	Yes	Yes
Haematology autologous transplants					
R–	Insig. (0%)	Insig. (0%)			
R+	Low (5%)	Low (2%)			

[a] Likelihood of CMV infection; data from Fox *et al*. (1995)[4] and Emery *et al*. (2000).[5]
[b] Based on study patients who had at least one true positive CMV test.

TABLE 72 *Cost-effectiveness of CMV 'high-risk' screening regimes[a] for renal transplant patients (includes associated/concomitant costs)*

CMV screening regime	Average cost per true positive sample detected (£)		Targeted screening strategy	
	Targeted	Universal	False negatives	False positives
Antigenaemia (pp65)	562	697	7.9	0.2
NASBA, Organon Teknika	397	491	2.7	1.4
Roche Amplicor Assay PCR	313	346	2.4	3.3
Nested in-house PCR (single-round)	98	116	3.6	0.3
Nested in-house PCR (two-round)	426	437	0.6	12.2

[a] Full regime in which diagnostic tests ordered as part of screening regime are included.

effectiveness ratio less favourable. However, the diagnostic yield of these targeted strategies (i.e. likelihood of a positive test result) will be higher, which should make the cost-effectiveness ratio more favourable.

Cost-effectiveness ratios calculated for targeted and universal CMV screening strategies are shown in *Tables 72* and *73*, based on the renal and haematology patients observed in Cardiff. These ratios include direct and associated costs (more details are provided in Appendix 18, *Tables 94* and *95*). The tables demonstrate that single-round nested PCR remains the more cost-effective test to use in targeted screening strategies. Comparison with universal screening shows that targeted strategies would appear to be more cost-effective; this is true regardless of the type of test or patient. However, the difference is much more striking for the haematology group. Once again, single-round PCR is more cost-effective than all other screening tests.

Other benefits from CMV screening tests

The analyses above clearly identify single-round in-house PCR as the most cost-effective test to use in CMV screening (of the five tests assessed), and also that use of targeted screening will have a much greater impact on cost-effectiveness for haematology patients than for renal patients. However, cost-effectiveness analysis is unable to indicate whether CMV screening is worthwhile, that is, whether £98–170 per true positive (for targeted screening in renal and haematology patients, respectively) is value for money.

TABLE 73 *Cost-effectiveness of CMV 'high-risk' screening regimes[a] for haematology patients (direct test costs only)*

CMV screening regime	Average cost per true positive sample detected (£)		Targeted screening strategy	
	Targeted	Universal	False negatives	False positives
Antigenaemia (pp65)	1285	2537	6.7	0
NASBA, Organon Teknika	487	1580	2.1	1.3
Roche Amplicor Assay PCR	2553	3954	3.5	4.2
Nested in-house PCR (single-round)	170	727	1.2	0.6
Nested in-house PCR (two-round)	1842	3051	1.2	12.9

[a] Full regime in which diagnostic tests ordered as part of screening regime are included.

At the same time, it appeared anecdotally that an important benefit provided by screening might be general reassurance that provisional diagnosis and planned patient management are correct. Hence there would be no **measurable** impact on diagnostic certainty or patient management. Therefore, screening costs were also compared with the number of tests judged to be of benefit by clinicians (rather than the number of positive results).

Overall, 63% of haematology test results and 72% of renal test results were identified by clinicians as being of benefit, and therefore might be considered to have a measurable reassurance effect.[62] For renal transplant patients, the most favourable 'cost per beneficial result' ratio (including associated costs) is estimated to be £16.54 for in-house PCR (single-round); the corresponding figure for haematology transplant patients is estimated to be slightly higher at £26.54 for single-round in-house PCR. Therefore, in terms of general benefits, CMV screening appears to be more cost-effective in renal transplant patients, but in both cases the cost per 'beneficial' result is low (£16–26). The same ratio is most unfavourable for two-round (nested) PCR, at £78 for renal transplant patients and £121 for haematology patients. The two commercial molecular tests and antigenaemia produce ratios that range from £48 to £56 in the case of renal patients and from £52 to £100 for haematology transplant patients.

Finally, it is unclear to what extent the benefits reported in the present study would have been influenced by an increase in the frequency of CMV screening tests. Overall testing costs would, of course, be much higher if three times as many tests were performed annually by a laboratory, even though the cost of an individual test might fall because of larger batch sizes. However, if the increase in overall testing cost did not produce an equally significant increased clinical impact (compared with that recorded in the present study), then the cost-effectiveness ratio would be expected to become less favourable for regimes that include more frequent testing (such as those reported nationally).

Chapter 9
Discussion

Introduction

Prior to 1996, the virology laboratory at Cardiff offered CMV serological diagnostic tests for use in diagnosis of CMV disease in **immunocompetent** individuals. CMV isolation in cell culture was available and particularly useful for the diagnosis of congenital CMV in neonates. However, for **immunocompromised** patients, neither assay was appropriate and instead the laboratory offered pp65 antigenaemia tests done on a 'same day' basis.

Although costly, this assay performed well on samples from renal transplant patients who had only a moderate degree of immunosuppression and plentiful functioning circulating white blood cells. Haematology patients post stem cell transplant were much more immunosuppressed and at the time of risk of CMV had very few circulating white blood cells. In addition, the cells that were present were immature and not functionally normal. Under these circumstances, pp65 antigenaemia becomes very unreliable. Therefore, a negative test result was regarded with lack of confidence by both laboratory and clinical staff. This led to clinicians adding ganciclovir to already complex, costly and toxic treatment regimes in this group of patients 'just in case' CMV disease was present but not diagnosed. Patients with end-stage HIV–AIDS presented a similar difficulty. They too had very few circulating white blood cells, and any that were present might not be functional; again, a negative pp65 antigenaemia test was regarded as unreliable. In both of these patient groups, a positive pp65 antigenaemia test was, however, generally accepted as a true result and led to therapy initiation.

At this time, manufacturers were marketing a number of molecular tests for the detection of CMV nucleic acid (either DNA or RNA). An increasing number of NHS laboratories were using these molecular tests to replace or complement non-molecular methods. In addition, there was widespread use by laboratories of locally developed in-house molecular methods that had not yet been subjected to rigorous evaluation but which were substantially cheaper than manufactured 'kit-based' assays.

However, there had been very little consistency in the molecular-based methods and strategies utilised by different UK centres for CMV screening and diagnosis in immunosuppressed patients.

In all three groups of patients at risk of severe CMV disease (renal and stem cell transplant recipients and patients with AIDS), asymptomatic reactivation of CMV may occur with low levels of virus replication and no tissue damage. The challenge for the health service was to distinguish this type of innocuous presence of persistent virus from its active replication and disease production, particularly in BMT and HIV-infected patients where clinical consequences are rapid and severe if CMV disease is not detected and treated. Therefore, comparison of the clinical utility of screening and diagnostic tests for CMV in different 'at risk' patient groups, and the best use of screening assays in predicting CMV disease and enabling pre-emptive therapy, presented an important area for study.

Pressures to introduce molecular tests for CMV screening

Clinicians and laboratory scientists in the UK were aware of the growing use of molecular tests to detect CMV DNA, particularly in centres with a strong track record of CMV research. Many clinicians wished to have such tests available to their patients; they particularly wanted access to 'PCR', which had become almost a talisman of best practice. There was little recognition or understanding of the range and variety of available molecular tests, or that few had been shown to have rigorous clinical utility in predicting CMV disease. Screening well patients for low-level CMV replication was being used to enable pre-emptive therapy in some centres without robust evidence that low-level viraemia led to overt CMV disease.

There was, and is, very little laboratory standardisation of methods in areas so close to new research, and centres tended to use whatever tests their local laboratory offered. Virtually no comparisons had been made between methods, or even between different technological approaches to molecular assays. There was no validated

external quality assessment scheme at the time and results from different laboratories were not comparable. At least two such schemes are now available to laboratories, although they test assay accuracy in terms of copy number of CMV DNA molecules rather than disease prediction, which remains an ongoing difficulty.

All molecular assays were and are costly, and those groups relying on laboratory tests of extreme sensitivity might experience more toxicity from ganciclovir than CMV disease. As a result, there remains a lack of consensus and considerable concern about how best to manage these vulnerable patients.

Outline of the research

The aim of the present study was to provide reliable evidence based on which clinicians, laboratory scientists and managers can make more rational choices about the use of CMV tests for diagnosis and screening in immunosuppressed patients. Within this overall aim, the objectives of the research were to compare the technical performance, costs and benefits of various molecular tests versus the most commonly used non-molecular test.

The four main tests evaluated in the study were

1. CMV pp65 antigenaemia assay – widely accepted, non-molecular method
2. semi-quantitative (single-round and nested) in-house PCR – molecular test diffusing into practice
3. qualitative PCR (Roche Amplicor Assay) – new/emerging, commercial molecular test
4. qualitative pp67 NASBA assay (Organon Teknika) – new/emerging, commercial molecular test.

The study design adopted was a prospective trial of CMV screening regimes introduced in a routine service setting. This involved the phased introduction of different molecular tests with the aim of determining the extent to which their routine use as screening assays was reliable, effective and cost-effective compared with testing using pp65 antigenaemia assay.

The evaluation framework used in the study was based on the hierarchy formulated by Fineberg and colleagues[30] and others[57] for diagnostic technologies. An adapted form was developed for CMV testing with the following six levels:

- Technical capacity – did the test perform reliably and deliver accurate (i.e. precise) information?
- Diagnostic accuracy – did it contribute to accurate diagnosis and/or prediction of CMV disease?
- Diagnostic impact – did the test replace other diagnostic tests or procedures?
- Therapeutic impact – did the test result influence the selection and delivery of treatment?
- Patient outcome – did the test contribute to improved health for the patient?
- Cost-effectiveness – did use of the molecular test improve the cost-effectiveness of healthcare compared with alternative interventions?

The research consisted of two stages. In stage one, the technical performance of all three molecular methods was assessed through an independent masked comparison of each molecular test against the established (antigenaemia) test. For all samples, the scientist performing a particular test was blind to the other test results for that sample. During this stage of the study, in-house PCR results (single-round) were provided to clinicians along with antigenaemia results. Any definitive diagnoses of CMV disease, all CMV therapy and any other reported impact on patient management were recorded. At the end of this first stage, the remaining molecular tests (NASBA, Organon Teknika; Roche Amplicor Monitor PCR; and nested, two-round in-house PCR) were compared in terms of their cost and the available information on technical performance. On the basis of these criteria, one test was selected (NASBA) for use in stage two.

During stage two of the study, NASBA results were provided to clinicians along with the established (antigenaemia) test result. Once again, any impact on diagnosis, CMV therapy or patient management was recorded. Towards the end of the study a survey of all UK virology laboratories was undertaken to identify CMV screening practice. In addition, all UK renal transplant surgeons and haematology (BMT and PBSCT) transplant centres were surveyed in order to identify current clinical practice and perceptions of the benefits of CMV screening.

Technical performance of molecular assays studied

Nested in-house PCR

The nested in-house PCR technique is highly

sensitive, since it can theoretically amplify DNA from a single copy of the target sequence. Because of its high sensitivity there is a danger that this technique will detect latent virus DNA in the absence of replication or disease. Nevertheless, this type of qualitative PCR test was already beginning to diffuse into clinical practice at the outset of the study.

At the same time, several reports were appearing which claimed that **quantitative** assessment of CMV DNA ('viral load') could give a better prediction of overt disease than qualitative assessment. Hence it was clearly important to include some form of quantitative assessment of CMV DNA in the study. The research team selected an **in-house** PCR test for evaluation. This test had been developed in Cardiff and the technique included a nested approach and produced a semi-quantitative result. The results obtained were semi-quantitative in that a sample which was positive for CMV DNA after a single round of PCR had more DNA present than a sample which only yielded detectable CMV DNA after two rounds of PCR (i.e. a nested reaction). Samples that were positive for CMV DNA after a nested reaction but not after a single round had very little DNA present, perhaps only a small number of copies.

At the end of stage one of the study, analysis of test performance demonstrated that nested in-house PCR (two-round) produced a large number of false positives and it was therefore not selected for use in stage two of the study. This was not an unexpected result as this assay had the highest sensitivity. The assay undoubtedly detected CMV DNA at very low levels, which in many patients did not predict disease, and this low level of reactivation of CMV resolved spontaneously. This means that in-house nested PCR proved to be of low specificity and low positive predictive value.

In-house assays are, on the whole, considerably less costly than commercial assays and have been popular within the NHS for that reason. Under the new EC Directive on In Vitro Diagnostic Devices[64] there is a requirement to CE mark or self-certify any in-house assays used within the NHS, unless these assays are used only on the premises of their manufacture. As a result, it is probable that in-house assays will become far less prominent since, once developed, few NHS laboratories will have the resources to CE mark their in-house assay so that it can be used in other laboratories. This could lead to the withdrawal of many in-house molecular assays and adoption of commercial assays which have been CE marked instead, with a consequent increase in cost to the NHS and possible decrease in test performance. As an example, the in-house assay used in the present study will not be CE marked in Cardiff. A commercial assay, which bears a CE mark, has been substituted.

Roche Amplicor Assay

The in-house molecular test described above used whole blood, rather than plasma, as the sample. Whole blood includes circulating cells, in addition to serum/plasma, and the DNA in the cells may include latent CMV DNA which is doing no harm. It may be argued, therefore, that detection of this DNA should not be used to initiate therapy. If this hypothesis is true, looking for CMV DNA in plasma might be a more reliable method of detecting replicating and potentially disease-causing CMV virus and a more reliable indicator of the need to start therapy.

The study team therefore assessed a second (commercial) PCR test that used plasma, rather than whole blood. The Roche Amplicor Assay detected cell-free CMV DNA by PCR. This was a new commercial test that was expected to diffuse widely into practice and might potentially offer more effective diagnosis, leading to improved clinical outcome.

The performance of the Roche Amplicor Assay was found to be satisfactory and comparable to that of the other commercial molecular assay (NASBA, Organon Teknika). The major drawback of this assay was cost, although a secondary consideration was the higher level of technical skill required. The assay is not quantitative and was found to be relatively insensitive. It was, however, fairly specific and might offer a useful signal for pre-emptive therapy. Since the completion of the study, however, the development and roll-out of real-time quantitative PCR assays, which are quicker and easier to perform, and perceived to be 'cheaper', has led to the abandonment of the Roche Amplicor Assay for CMV in the UK.

NASBA, Organon Teknika

The third type of molecular test that the study team assessed was one based on nucleic acid sequence-based amplification (NASBA) of late CMV messenger RNA (pp67). This method differed from the PCR-based tests in that, rather than detection of CMV DNA, the test detected production of CMV messenger RNA, suggesting that the virus had become 'switched on' and was

starting to make proteins and to replicate. That is, the virus was no longer latent but was starting to become active and potentially disease inducing. There was some evidence in the literature that such a test might lead to improved diagnosis and clinical outcome.

Although commercial kits for this method were being marketed by Organon Teknika, there had been no systematic assessment of these. The study team therefore identified this company's assay for qualitative NASBA of late CMV messenger RNA (mRNA) as the third molecular test for evaluation.

Detailed comparison of the two commercial molecular tests (NASBA and Roche Amplicor Assay) demonstrated that NASBA was the preferred test in terms of technical performance. Also, comparison of test costs indicated that the NASBA test would not be significantly more expensive than the Roche Amplicor Assay.

The performance of the NASBA assay during the second stage of the study continued to be satisfactory. However, this assay was perceived as much less flexible than real-time PCR and also thought to be more costly. It has consequently not been adopted by any laboratory in the UK. For these reasons, the assay is no longer offered in Cardiff.

National surveys

CMV screening tests used nationally

By late 2002, all but one UK virology laboratory reported some processing of CMV screening samples for transplant patients, and the remaining laboratory planned to introduce screening. Over three-quarters of laboratories' CMV testing workloads consisted of screening samples. Only 58% of laboratories stated that they had been undertaking CMV screening for haematology transplant patients in late 1998 (when the study started) and 66% for renal transplant patients. Hence there had been a rapid diffusion of CMV screening during the course of the HTA study.

The highest CMV test workload reported by laboratories was for haematology transplant patients (average 23 CMV tests per week, 71% screening tests) as opposed to renal transplant patients (average 6.5 tests per week, 78% screening). CMV testing for HIV patients was less prominent in laboratory workloads (the average reported by laboratories was three tests per week) with much lower levels of screening tests (<50%). Individual laboratory throughput for CMV tests varied by a factor of 300 (18–6776 samples, 2001 figures).

For the laboratories that processed CMV samples on-site, 28% reported the use of antigenaemia and the remainder all used some form of PCR-based test. Clinicians who responded to the national survey similarly reported the use of a PCR test in 75% of renal transplant centres (with only 21% reporting the use of antigenaemia); 100% of haematologists who replied also reported the use of PCR. A few laboratories indicated the occasional use of two assays, with one assay as a second line test. Some used a qualitative PCR test first, followed by more expensive quantitative PCR on samples that tested positive.

In terms of their test preferences for CMV screening of immunocompromised patients, only 16% of laboratories expressed a preference for antigenaemia and the remainder for some form of PCR test; 31% of the latter preferred real-time PCR, 31% some other quantitative PCR test and 38% a qualitative PCR test.

Therefore, from the laboratory perspective, CMV screening had diffused rapidly with screening tests representing three-quarters of CMV tests undertaken in the transplant patient groups studied. Although laboratories reported a clear preference for use of PCR tests in screening (rather than antigenaemia), they appeared to be divided between real-time, other quantitative or qualitative PCR tests.

National CMV screening and prescribing protocols

When UK laboratories were asked for details of the CMV screening protocols in place locally, these were found to exhibit certain similarities, but there was no clear consistency nationally.

For renal transplant patients, the period of screening instituted in the present study (16 weeks) was broadly similar to that reported nationally; no UK laboratory reported a screening follow-up period of <12 weeks or >24 weeks. However, screening tests were undertaken far less frequently (every 4 weeks) in the study protocol than was reported nationally, where weekly screening was most common. The information provided by the survey of laboratories was confirmed in the survey of renal transplant centres. Two-thirds (68%) of centres replied that they undertook CMV screening; 12–14 weeks was

the most common period of screening follow-up, with a test every 7 days the most likely frequency during this period. When asked about selection of patients for screening, nearly two-thirds of centres reported that all their renal transplant patients were being screened, as in the study site. However, one-third of centres reported that they stratified patients using a predefined protocol; all of these centres stated that they would screen R–D+ transplants, with half reporting use of CMV prophylaxis in addition to screening.

For haematology patients, the national picture was slightly more confused in terms of the period of screening follow-up. No laboratory reported screening for <12 weeks. Almost equal numbers of laboratories reported screening up to 24 weeks (the study screening period) as screened for 16 weeks, with a very small number reporting screening for >24 weeks. Haematology centres were more likely than renal centres (82% versus 68%) to reply that they undertook CMV screening and, when they did, to test more frequently. Most centres reported that screening tests were undertaken every 7 days or even more frequently, unlike the study protocol where tests were every 2 weeks at first and then every 4 weeks. There was also more widespread evidence of selection of patients for screening. Most centres (90%) reported that patients were stratified for screening; most of these centres (80%) stratified using a predefined protocol and the remainder selected on a case-by-case basis. Only one centre reported that it undertook universal screening, as implemented in the Cardiff study protocol. In all cases where a predefined protocol existed, it was reported that autologous transplant patients were not screened. In 60% of centres, R–D– allogeneic transplants were also not screened, although the remaining centres screened all allogeneic transplants.

When renal clinicians were asked whether protocols existed to guide their ganciclovir prescribing following CMV screening test results, 57% reported use of some form of protocol, with two-thirds of these being formal protocols. Most renal protocols specified that clinicians should intervene if CMV disease was **clinically** suspected, although only one in eight required a particular test result as a trigger, such as two consecutive positive results or viral load above a preset level. The remaining protocols were more complex, several including a link to prophylactic treatment. However, overall, over half of renal centres had no formal protocol in place. In contrast, virtually all haematology transplant centres (90%) reported a formal protocol.

In most cases where details were provided, protocols required two positive CMV screening tests before prescribing. However, when clinicians were asked in what situations they would **personally** prescribe anti-CMV therapy if a transplant patient tested positive after previously testing negative for CMV, two-thirds of renal clinicians said they would prescribe ganciclovir if the patient was an R–D+ transplant. For all other types of renal transplant (R+D–, R+D+ and R–D–) and for patients on antibody induction therapy, only one in three clinicians reported that they would prescribe based on a single positive result. Similarly, most haematologists reported that they would prescribe based on a single positive result for an allograft patient (80%), although only a minority (20%) would prescribe for autografts, with a further 44% possibly prescribing. The survey responses appear to indicate that individual clinicians would apply any 'two positive tests' protocol rule selectively, depending on the particular case. This appears to confirm the pattern observed in the trial, where clinicians varied in terms of whether they prescribed following a single positive screening test result (see the next section).

Hence the relationship between CMV screening test results and the prescribing of anti-CMV therapy is complex. The national survey responses indicated that in some cases this is further complicated by the use of CMV prophylaxis. As explained previously, prophylaxis was not used in Cardiff during the course of the study. However, it was reported to be in widespread use in UK renal transplant centres. Most clinicians (90%) indicated that they would give prophylaxis to R–D+ renal transplant patients in their centre, and over half to patients on antibody induction therapy, although fewer than one quarter would prescribe for R+D+ or R+D– and none for R–D– transplants. The national picture was less clear for haematology transplant patients. No centre reported that it would give prophylaxis to autografts; also, no centre would give CMV prophylaxis to R–D– allografts, but 20% might give prophylaxis to other allografts.

When asked if they were satisfied with existing CMV screening, only one in five renal clinicians reported that they were not satisfied, and half of these specifically mentioned that they were unhappy with follow-up screening tests post-discharge. In contrast, virtually all haematologists reported that they were satisfied with existing CMV screening, with no-one mentioning similar problems with post-discharge CMV screening tests, presumably because patients return to the transplant centre for follow-up.

Clinical utility of molecular assays studied

The clinical utility of CMV screening test results was assessed in the prospective Cardiff study based on the analysis of 2554 responses. This demonstrated that clinical signs or symptoms when a screening test was requested (e.g. fever, chest symptoms, raised white blood cell count, high platelets, raised creatinine) did not discriminate patients whose samples were found to be positive. In neither patient group were recorded symptoms related to whether CMV disease would be identified in the sample, except for pyrexia ($p < 0.05$). This is not unexpected for screening samples, but emphasises the poor prognostic value of clinical symptoms in the patients being screened.

The majority of CMV test results were judged to have been of benefit to the patient, although approximately one in three were not. Renal clinicians were significantly more likely ($p < 0.01$) to report that CMV results had been of benefit than were haematology clinicians (72% versus 63%), but this effect was no longer significant if diagnostic tests requested during the screening period were excluded.

Although most screening tests were judged to have been of benefit to the patient, actual recorded impact on diagnostic certainty or patient management was relatively infrequent. There was a significant difference between the two patient groups in terms of reported impact on diagnostic certainty; in haematology cases, 13% of results increased diagnostic certainty, but only 4% in renal transplant patients ($p < 0.01$). Changes in patient management were far less likely than reported impact on diagnostic certainty. Changes were recorded following <5% of test results; start of CMV therapy was triggered by 4% of results and further investigations by 3% of tests. Test results were more likely ($p < 0.01$) to lead to further investigations in haematology than in renal patients. Therefore, although a majority of screening test results were judged to be of benefit to the patient, very few led to a recorded increase in diagnostic certainty and even fewer were reported to have influenced patient management.

More detailed examination of patterns of CMV prescribing failed to demonstrate a clear link between screening test results and prescribing. For renal patients, one in four in whom a screening test had identified CMV disease were not prescribed ganciclovir and, conversely, one in 10 patients with no positive samples received ganciclovir (in 44% of the latter cases clinicians also requested diagnostic tests, so CMV disease was suspected). For haematology patients, all patients with a positive CMV result were prescribed ganciclovir, but 5% of patients with only negative samples also received ganciclovir (in three-quarters of these cases the clinician also requested diagnostic tests). It therefore appears that clinicians considered screening test results in the light of other factors such as clinical signs and symptoms and the relative risk of treating/not treating a particular patient. Renal clinicians are probably less likely to treat well individuals with a laboratory diagnosis of CMV since they know that ganciclovir is toxic to the transplanted kidney, hence they may have preferred to wait until there were definite signs of disease before instituting treatment. This is reasonable for renal patients who respond well to therapy. For haematology patients, pre-emptive therapy, before a patient becomes unwell, was more likely to be considered. This behaviour pattern mirrored clinicians' responses to the national surveys.

The clinical utility of the CMV screening regime was contrasted with a diagnostic CMV testing strategy (as previously used in Cardiff) by comparing a historical, consecutive series of transplant patients with the patients recruited to the prospective CMV screening trial. No significant differences could be identified for outcomes such as the level of CMV disease detected (historically 13% for renal and 2.2% for haematology patients and 13% and 3.6%, respectively, in the trial groups). The slight (non-significant) increase observed in haematology patients might be due to differences in the types of patients treated since, unlike for renal historical controls, it could not be confirmed that the two groups of haematology patients were not significantly different in their CMV risk status or demographics. Similarly, although there was a significant difference in outcomes for renal patients (deaths, transplant failures) at 9 months, this effect appeared to be linked to organisational changes rather than improved detection of CMV disease. There was only one CMV-related death recorded during the screening period (a haematology patient), and therefore comparison of prospective and historical control data was not able to provide any evidence that CMV screening had led to reductions in CMV deaths. Similarly, there was no evidence that screening had improved levels of CMV detection and disease, or that it had a positive effect on transplant success rates.

However, comparison of study patients and historical controls did show that the introduction of CMV screening had produced a significant increase in the number of CMV diagnostic tests requested for patients (both renal and haematology). There was also some indication that a lower percentage of diagnostic tests were positive once screening was introduced, possibly linked to a lowering of clinicians' thresholds for ordering diagnostic tests, but this effect was not significant. In addition, any benefits due to reductions in inappropriate CMV therapy appeared to be minimal (<0.5% of screening tests led to planned CMV therapy being avoided in the prospective study).

In the prospective trial, comparison of the two transplant groups demonstrated that *ex ante* haematologists were significantly ($p < 0.01$) more likely to report that a positive test result would lead to a repeat CMV test request and to other investigations being ordered (e.g. X-rays, CT/MRI, bronchoscopy). Hence these clinicians were more likely to request further investigations as a result of a positive screening test. At the same time, the likelihood that a positive test result would lead to prescribing of CMV therapy was also significantly higher in haematology patients ($p < 0.01$), but in neither patient group was it 100% certain. As discussed earlier, the actual impact on prescribing was complex because clinicians were likely to consider an individual screening test result in the light of other factors.

Cost-effectiveness of molecular assays studied

A key aim of the study was to assess the optimum use of screening assays in predicting CMV disease and enabling pre-emptive therapy. In order to do this, the cost and clinical effectiveness of different testing strategies were compared.

In addition to the laboratory costs of the various CMV tests, any other NHS costs directly associated with false-positive or false-negative test results were also considered. Laboratory test costs will vary depending on factors such as staff grade, extraction method used and batch size. Sensitivity analysis, taking these factors into account, showed that in-house semi-quantitative PCR is the least expensive molecular test, with single-round PCR less expensive (£7.80–13.70) than two-round PCR (£9.50–17.30).

Qualitative, commercial molecular assays (NASBA and Roche Amplicor Assay) were both more expensive, incurring similar costs (£22.50–34.70 for NASBA and £23.20–29.20 for Roche Amplicor Assay). Nationally, laboratories reported very similar cost figures for their qualitative PCR tests; these ranged from £22 to £40 per sample tested when all costs were included (i.e. labour, consumables, overheads, etc.)

The cost of an antigenaemia test in the study laboratory was estimated to be £21.00–27.40 for a batch size of five and £12.50–15.16 for larger batch sizes. Antigenaemia costs reported nationally, by laboratories whose figure included all cost elements, were similar to those measured in Cardiff (£15 and £22 per sample tested).

In the present study, quantitative PCR (COBAS) proved to be the most expensive of all tests at about £50 per sample. Only one laboratory nationally reported a cost for this test; at £65 this was similar to the cost measured in Cardiff.

Finally, although we were unable to include real-time quantitative PCR tests in the study, laboratories using this technology provided an estimate of costs for LightCycler (£20–40) and TAQMAN £32.25 (not including accommodation and hospital overhead costs). Hence real-time PCR would appear to be as costly as the commercial kits (NASBA and Roche Amplicor Assay) assessed in the study.

The present research was not able to identify any significant differences in final outcomes for patients (e.g. deaths, transplant failures) linked to the introduction of a CMV screening regime in Cardiff (as explained above). However, it was possible to identify differences in interim outcomes (e.g. increases in diagnostic certainty, changes to patient management) when diagnostic tests and screening tests ordered by clinicians during the trial period were compared. This was a somewhat artificial comparison since the introduction of CMV screening tests had been shown to produce a significant ($p < 0.01$) increase in the number of CMV diagnostic tests requested. There was also some indication that the likelihood of a positive result in these diagnostic samples was lower. With these caveats in mind, it was possible to compare the costs and outcomes for diagnostic tests with those for CMV screening tests.

A screening regime using single-round in-house PCR was calculated to be more cost-effective (£116 per true positive detected for renal transplant patients and £518 for haematology patients) than antigenaemia diagnostic testing (£130 and £1287

respectively). All other screening regimes were estimated to be **less** cost-effective than a diagnostic regime in both groups of patients, apart from the two-round (nested) in-house PCR test. However, the latter test produced by far the highest number of false positives, in addition to true positives, and therefore the single-round PCR test would clearly be preferred. The study findings also demonstrated that the least cost-effective test to use in screening was antigenaemia (£643 per true positive detected for renal transplant patients and £2475 for haematology patients). Sensitivity analysis indicated that in-house PCR remained the most cost-effective test for CMV screening even if the assumptions made were varied.

Inclusion of wider NHS costs beyond the laboratory (i.e. costs associated with false positives and false negatives such as unnecessary repeat CMV tests, further investigations or inappropriate therapy) confirmed that single-round in-house PCR remained the most cost-effective test to use for screening (£116 per true positive detected for renal transplant patients and £727 for haematology patients). However, two-round (nested) in-house PCR was now the **least** cost-effective of all tests owing to the high level of costs associated with the false positives produced by this test.

Estimation of the **incremental** costs and benefits of screening using the various molecular tests (relative to antigenaemia screening) produced a similar picture. Based on laboratory testing costs and the number of true positives detected, single-round in-house PCR remained the most cost-effective CMV screening test for both renal and haematology patients. ICERs for the commercial tests were two to three times less favourable, although all molecular tests remained more cost-effective than antigenaemia screening. Of the two commercial tests, Roche Amplicor Monitor PCR was calculated to be more cost-effective on the basis of this ratio than NASBA.

The analyses above appear to indicate that the CMV screening protocols introduced in Cardiff were more cost-effective in renal transplant patients than in haematology patients, with lower cost-effectiveness ratios in the former. However, if alternative outcome measures are used for comparison purposes (e.g. the cost per change in diagnostic certainty or per change in patient management) then screening of haematology transplant patients can be viewed in a more favourable light. Cost-effectiveness ratios based on both of these interim outcome measures still identify single-round PCR as the most cost-effective test. However, ratios are now £284 for renal and £134 for haematology patients for the cost **per change in diagnostic certainty** (laboratory costs and associated costs included). Similarly, ratios for the cost **per change in patient management** are £993 for renal and £507 for haematology patients. Therefore, in terms of diagnostic impact and reported concrete changes to patient management, CMV screening appears to be more favourable in haematology patients.

Finally, when the cost per beneficial result (as reported by clinicians) was calculated, then the cost-effectiveness ratios were **much** more favourable in both patient groups; for single-round in-house PCR they were £16.54 per beneficial result (including associated costs) for renal patients and £26.54 for haematology patients. However, it is not possible to judge from any of the findings above whether the use of screening assays *per se* is worthwhile in either patient group.

All the analyses discussed so far have been based on the CMV screening protocols introduced in Cardiff. The national surveys had indicated that several transplant centres first stratified patients in terms of their CMV risk and then only screened selected subgroups. Cost-effectiveness analyses were therefore also undertaken modelling the likely impact of the following targeted screening regimes reported to be in use nationally, using data for these same sub-groups from the Cardiff data set:

- *Targeted renal screening* – screening of R–D+ group only
- *Targeted haematology screening* – screening of allogeneic transplants only, excluding R–D–.

The cost-effectiveness ratios calculated for both these targeted screening strategies indicated that single-round in-house PCR remained the most cost-effective test to use in both types of transplant patient. Furthermore, the differential impact of targeted screening on the renal group was rather limited (e.g. the cost per true positive detected fell from £116 to £98), but there was estimated to be a much greater advantage resulting from using targeted screening in the haematology patient group (the cost per true positive detected was predicted to fall from £727 to £170).

In conclusion, comparison of the ICERs of the various tests assessed indicates that the optimum test to use is single-round in-house PCR. This test

is less costly to perform and is also predicted to result in lower associated costs linked to false positives and false negatives than other tests. If a CMV screening strategy that targets only high-risk patients is used, rather than whole population screening, this is once again estimated to be most cost-effective if the single-round PCR test is used. It is also clear that, although targeted screening is more cost-effective than universal screening for both patient groups, this is especially true in the case of haematology transplant patients. Finally, it is difficult to draw definite conclusions about the cost-effectiveness of screening *per se*, as opposed to use of a diagnostic testing regime, from our results. This is because the reported effects of diagnostic and screening tests recorded during the prospective trial were essentially interim outcome measures (e.g. changes in diagnostic certainty, alterations to patient management) rather than patient outcomes. Also, since the historical patient data were incomplete, a robust comparison of patient outcomes for a CMV diagnostic testing strategy versus a CMV screening regime could not be undertaken based on historical controls.

Further technical developments

Since the start of this study, the development and roll-out of quantitative PCR assays has changed the national picture. In particular, the introduction of real-time PCR assays which are quicker and easier to perform is leading to an abandonment of the non-quantitative and semi-quantitative assays evaluated in the present study. All the assays studied (apart from COBAS) had an end-point that was either positive or negative. In contrast, real-time PCR provides a dynamic picture of what is happening in the assay tube; assays are carried out in very small volumes and results are available more quickly than with traditional molecular methods that are tube- or plate-based and involve larger reaction volumes. Although our study measured the cost of a quantitative PCR test (COBAS), we were not able to estimate its likely clinical impact because so few tests were performed. This test was, however, the most expensive of all the molecular tests, estimated at £50 per sample. Furthermore, even though we were not able to include real-time quantitative PCR tests in our costing study, cost estimates were provided by some UK laboratories in response to the national survey. These indicated that the cost per sample ranged from £20 to £40 (LightCycler) and £32 (TAQMAN), although the latter figure is likely to be low because it did not include accommodation and hospital overhead costs. With the caveat that these cost estimates may not be robust, real-time PCR assays would appear to be much more expensive than in-house semi-quantitative PCR (£7.80–13.70). Its cost is closer to that calculated for the commercial qualitative CMV assays assessed (NASBA £22.50–34.70 and Roche Amplicor Assay £23.20–29.20). Therefore, unless real-time PCR test performance is vastly superior to that observed for single-round in-house PCR, it is unlikely that these new tests will be more cost-effective than the most cost-effective test observed in the Cardiff setting.

Developments in molecular technology are continuing. Real-time PCR is likely to be superseded by 'chip'-based technology, which is a highly miniaturised format enabling large numbers of reactions to be performed in minutes. This changing context emphasises the challenge for health technology assessments with a long lead-in time to remain technically relevant in a fast-moving area. Comprehensive assessments, which can take years to undertake, may delay any conclusions beyond the time when the findings are of benefit to providers and purchasers alike. This exemplifies a long-recognised conundrum: "Buxton's Law of Technology Evaluation: it's always too early until, suddenly, it's too late."[65]

The impact of legislation regarding *in vitro* diagnostic devices

The present study has clearly demonstrated that an in-house PCR assay is the most cost-effective test to use as part of a CMV screening strategy, outperforming commercial molecular assays on cost and test performance. The use of antigenaemia pp65 for screening, as reported by a number of laboratories in the UK survey, is clearly less cost-effective than all three molecular tests evaluated.

At the same time, the new EC Directive on *in vitro* diagnostic services, recently incorporated into UK law and interpreted by the Medicines and Healthcare Products Regulatory Agency (MHRA), will influence laboratories' choice of test. In future, all commercial *in vitro* diagnostic assays used by NHS healthcare providers must be CE marked. Commercial suppliers will need to decide whether or not to CE mark their assays, recognising that only if they do so will they be able to market their product in the UK. For small-usage assays this will not be commercially viable and these assays may no longer be available to UK patients. Hence it was unclear at the end of this project whether it

was the intention of Roche to CE mark their CMV assay. Organon Teknika had been taken over by Biomerieux, and it was similarly unclear whether Biomerieux would CE mark the CMV NASBA assay.

In-house assays are, in some circumstances, exempted from the need for CE marking (Balmer K, IVDAC Secretariat, MRHA, London: personal communication, 2004). If, however, an in-house assay is used to examine samples from patients outside the institution in which the assay was developed, the exemption will not apply. Most molecular diagnostic units are based in tertiary referral centres which offer a diagnostic service to a large catchment area and test samples sent from patients in other hospitals and primary care settings, as was the case in the present study. Indeed, Pathology Modernisation has encouraged the establishment of discrete centres of excellence offering complex tests on samples referred from less specialised units. Under these circumstances, the in-house exemption does not apply and any unit offering an in-house assay will have to achieve CE marking or self-certification with the Competent Authority (MHRA).

Few units have the resource to CE mark their assays and it is as yet unclear what self-certification will require. The implication of these legislative changes is that units will tend to move away from relatively inexpensive in-house assays in favour of more costly CE marked commercial tests, where these are available. For CMV diagnosis there are currently available real-time PCR molecular assays, which are CE marked, and which will inevitably become the market leaders. Unfortunately, none of these assays has undergone the kind of rigorous assessment of performance and clinical utility undertaken in the present study for the molecular tests examined.

Conclusions

The following are the main conclusions drawn from this technology assessment of use of molecular tests in CMV screening of immunocompromised patients:

- In individuals who have severely reduced immunity, CMV can cause serious and even fatal infection. Those at greatest risk from CMV infection include renal transplant recipients and patients who receive stem cell harvests or bone marrow transplant recipients.
- The study findings offer some evidence that in both patient groups a universal CMV screening regime is more cost-effective than diagnostic testing alone, based on the cost per true positive detected. The same conclusion is reached if interim outcome measures are examined (e.g. increases in diagnostic certainty, changes to patient management). However, the study was unable to demonstrate any benefit in longer term patient outcomes (e.g. deaths, transplant failures).
- If CMV screening is introduced, the use of antigenaemia pp65, as reported by a number of UK laboratories, is clearly less cost-effective than the use of the molecular tests assessed.
- The study identified the optimum test for a CMV screening regime as an in-house, semi-quantitative molecular test (single-round PCR test). This test was less costly to perform and also resulted in lower costs linked to false positives and negatives than other tests. The in-house test was two to three times more cost-effective than the commercial molecular tests assessed.
- The use of targeted screening (limiting CMV screening to specific transplant patient sub-groups), as opposed to universal screening, is calculated to offer a significant improvement in cost-effectiveness for haematology transplant patients, but has limited impact in the case of renal transplants.
- It may be difficult to use the findings of this study (i.e. that in-house, semi-quantitative PCR is the most cost-effective screening test) to inform UK healthcare practice except in general terms because of changes in the legal framework for *in vitro* diagnostic testing.
- In future, health technology assessments may need to be confined to commercially available CE marked diagnostic kits since it will be a challenge for NHS providers to develop any in-house assays to a point where they can be assessed. Hence it is unclear whether a similar study to this one, looking at later developments in in-house testing, will be practicable.
- Clinicians involved in the current study placed a high value on the CMV screening results received, even though their prescribing seemed to disregard the test results in some patients. This high regard is demonstrated by the willingness of clinicians to continue to purchase CMV molecular screening tests (real-time commercial PCR) long after the study was complete.

Recommendations for further research

- In a rapidly changing area, such as the introduction of new molecular diagnostic tests

for CMV screening, health technology assessment requires careful thought.
- Research is required to investigate subgroup-specific disease groups across a larger population. Access to such a comprehensive data set would allow more accurate modelling of the impact of CMV screening on disease progression.
- The economic analysis should be expanded to model the cost-effectiveness of more frequent screening tests (as reported nationally), and the likely impact of CMV screening in other 'at risk' groups (e.g. patients with advanced HIV infection, lung transplant patients).
- Because of changes in European legislation, it may be difficult for in-house molecular assays to be used by the NHS in future. Thought should therefore be given to including funding in any future health technology assessments for CE marking of in-house assays that are found to be cost-effective.
- A 'fast track' assessment approach may also be required in areas of rapid technological advance, such as the one studied. This should cover not only rapid funding but also the development of rapid, robust assessment methods so that results are available in a timely fashion. Otherwise, advances in technology may compel the use of CMV assays for which robust performance and clinical and cost-effectiveness data are unavailable.
- There is a need for studies of CMV screening programmes that address a range of outcome measures, including patient outcomes. The STARD checklist is limited to the measurement of diagnostic accuracy and does not consider the clinical utility of test results. Interim outcome measures, such as those used in the present study, also require further development and refinement.

Acknowledgements

Contribution of authors

A Szczepura (Professor of Health Services Research) was responsible for the health technology assessment methodology, coordination and undertook writing the final report.
D Westmoreland (scientific author) was responsible for the coordination of the scientific and clinical work and contributed to writing the final report.
Y Vinogradova (statistician) undertook the data analysis. J Fox (scientific author) coordinated the laboratory work and contributed to the final report. M Clark (health economist) undertook the cost analysis, national surveys of laboratories and clinicians and contributed to writing the final report.

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Appendix 1

Overview of molecular test selection for evaluation study

Nested in-house PCR

It had been reported that molecular analysis of whole blood for CMV nucleic acid (DNA) could offer a potentially more sensitive test than pp65 antigenaemia assay. At the outset of the study, the molecular method most likely to be used for this purpose was detection of cell-associated CMV DNA by PCR. The PCR technique is highly sensitive and PCR can theoretically amplify DNA from a single copy of the target sequence. Because of its high sensitivity, there may therefore be a danger that this technique will detect latent virus DNA in the absence of replication or disease. In fact, an initial study had reported positive predictive values as low as 28%. Nevertheless, such qualitative PCR tests were already beginning to diffuse into clinical practice at the outset of the study.

At the same time, several reports were claiming that *quantitative* assessment of CMV DNA ('viral load') could give a better prediction of overt disease than **qualitative** assessment. Therefore, it was clearly important to include some form of quantitative assessment of CMV DNA in the evaluation. However, the commercial assays that were available at the time of the study were very expensive and not validated. Therefore, the study team selected an **in-house** PCR test for evaluation. This had been developed in Cardiff and the technique included a nested approach and produced a semi-quantitative result. The results obtained are semi-quantitative in that a sample which is positive for CMV DNA after a single round of PCR has more DNA present than a sample which only yields detectable CMV DNA after two rounds of PCR (i.e. a nested reaction). Samples that are positive for CMV DNA after a nested reaction, but not after a single round, have very little DNA present, perhaps only a small number of copies.

Roche Amplicor Assay

The in-house molecular test described above used whole blood, rather than plasma, as the sample. Whole blood includes circulating cells, in addition to serum/plasma, and the DNA in the cells may include latent CMV DNA which is doing no harm. It may be argued, therefore, that detection of this DNA should not be used to initiate therapy. If this hypothesis is true, looking for CMV DNA in plasma might be a more reliable method of detecting replicating and potentially disease-causing CMV virus and a more reliable indicator of the need to start therapy.

The study team therefore identified a second (commercial) PCR test that used plasma, rather than whole blood. This was qualitative cell-free CMV DNA detection by PCR (Roche Amplicor Assay), a new commercial test that promised to diffuse widely into practice and might potentially offer more effective diagnosis, leading to improved clinical outcome.

NASBA Organon Teknika

The third type of molecular test that the study team selected was one based on nucleic acid sequence-based amplification (NASBA) of late CMV messenger RNA (pp67). This method differed from the PCR-based tests in that, rather than detection of CMV DNA, the test detected production of CMV mRNA, suggesting that the virus had become 'switched on' and was starting to make proteins and to replicate. That is, the virus was no longer latent but was starting to become active and potentially disease inducing. There was some evidence in the literature that such a test might lead to improved diagnosis and clinical outcome.

Although commercial kits for this method were being marketed in 1997 by Organon Teknika, there had been no systematic assessment of these. The study team therefore identified the Organon Teknika assay for qualitative NASBA of late CMV (mRNA) as the third molecular test for evaluation.

Molecular test added to study protocol

In addition, because of the recognition that **quantitative** PCR assessment of **cell-free** CMV

DNA might be of value in predicting the onset of CMV-related disease, when the opportunity arose, towards the end of the study, to obtain 100 commercial quantitative CMV DNA assays from the manufacturer (Roche) free of charge, these were accepted for inclusion in the evaluation.

Hence the study was able to include quantitative cell-free CMV DNA detection by quantitative PCR (COBAS Amplicor Monitor Assay by Roche) as a fourth and final molecular test. However, this could only be assessed in terms of its technical performance on selected (archived) samples owing to the limited number of assays provided.

Appendix 2

Detail on study stages

Stage one (months 1–14)

During stage one, a total of 150 patients were to be recruited and monitored prospectively using the molecular screening tests and antigenaemia. Following a 2-month bedding-in period, patients would be recruited consecutively over 12 months to include renal transplant patients (40 patients), bone marrow transplant or peripheral blood stem cell transplant patients (70 patients) and patients with advanced HIV infection (40 patients).

The technical performance of all three molecular methods would be assessed through an independent masked comparison of each molecular test against the established (antigenaemia) test for all 150 patients. Test 2 (semi-quantitative nested in-house PCR) would be undertaken on whole blood, with two possible levels of analysis, the first based on 'high-level' CMV DNA (positive after a single round of PCR) and the second on 'low-level' CMV DNA (positive only after two rounds of PCR).

All samples, where sufficient residual fluid was available after allocation for antigenaemia testing underwent the three molecular tests; in addition, some (archived) samples subsequently underwent additional molecular testing with the COBAS Amplicor Monitor Assay (Test 5). For all samples, the scientist performing the molecular test was blind to the antigenaemia test result, and vice versa. During stage one of the study, Test 2 (semi-quantitative in-house PCR) results were provided to clinicians along with antigenaemia results; only high-level results (first round positive) were presented to clinicians. A detailed costing study of the new molecular methods and the established antigenaemia test was performed.

For each patient entered into the study, a number of structured questionnaires were completed by clinicians to record: at recruitment, baseline data on the patient; at the point of requesting a screening testing, information on clinical status, current drug therapy, *ex ante* likelihood of CMV disease, *ex ante* likely impact of positive or negative test result on therapy and further investigations; on receipt of the test result, information on impact of result on diagnostic certainty, actual changes to planned patient management (e.g. therapy and investigations), and perceived benefit. Any definitive diagnosis of CMV disease and any CMV therapy were also recorded separately, as was the 'final' patient outcome at the end of the screening period specified by the protocol and any longer-term outcome information available at the end of the study.

At the end of this first stage, the two new/emerging molecular tests (Test 3 and Test 4) were compared in terms of their cost and the available information on technical performance and one selected (Test Y) for use in stage two.

Stage two (months 15–32)

During the second stage of the study, a further 110 consecutive patients (40 renal transplants and 70 bone marrow/stem cell transplants) were to be recruited over 12 months, plus a 4–6-month follow-up CMV screening period for the renal and haematology patients, respectively making the planned length of this stage 18 months. During stage two, the original 40 HIV patients were to continue to be monitored for CMV using the screening protocol.

The most promising new/emerging test (Test Y), selected at the end of stage one, was to be evaluated further for stage two patients. All samples were to undergo only this molecular test, in addition to antigenaemia. During this stage of the study, Test Y results would be provided to clinicians along with the established (antigenaemia) test results, instead of the semi-quantitative first-round in-house PCR (Test 2) results provided in stage one. Once again, any definitive diagnosis of CMV disease, any CMV therapy and reported impact on patient management were recorded.

During this stage of the study also, a historical control group of patients undergoing transplants immediately before the commencement of the research (for whom only diagnostic tests were requested) was to be identified for renal and haematology transplants. Patients were to be matched for procedure/condition and then basic

process and outcomes data extracted for the historical control groups.

In addition, towards the end of the study, a survey of all UK virology laboratories was to be undertaken to identify whether CMV screening tests had been introduced; the type(s) of tests used and testing protocols; test throughputs and turnround times; and test prices. For laboratories that had not yet introduced screening, questions were to be asked about any planned introduction of screening tests, together with the preferences of laboratory staff, and any constraints on screening. In addition, a questionnaire survey of UK renal transplant surgeons and haematology (BMT and PBSCT) transplant centres was also to be carried out in order to identify current clinical practice and perceptions of the benefits of CMV screening.

Appendix 3

Renal transplants: CMV testing patterns (all tests)

CMV testing patterns (all tests)

CMV status	No. of patients (%)	Probability of CMV (%)[a]	Median no. of tests per patient	No. of tests per patient (range and quartiles)				Median follow-up (days) (weeks)
				Min.	Lower quartile	Upper quartile	Max.	
R–D– (cadaveric)	17 (17.3%)	0	6	5	6	7	9	160 (23 weeks)
R–D– (live)	5 (5.1%)	0	5	5	5	5	6	119 (17 weeks)
R–D+ (cadaveric)	25 (25.5%)	44[b]	8	5	6	9	14	157 (22 weeks)
R–D+ (live)	3 (3.1%)	44[b]	11	6	6	11	11	392 (56 weeks)
R+D– (cadaveric)	17 (17.3%)	6	7	5	6	7	9	169 (24 weeks)
R+D– (live)	1 (18.4%)	6	7	–	–	–	–	154 (22 weeks)
R+D+ (cadaveric)	23 (1.0%)	25[b]	6	1	5	6	9	141 (20 weeks)
R+D+ (live)	7 (7.1%)	25[b]	7	5	6	7	8	153 (22 weeks)
Total	98 (100%)		6	1	6	8	14	152.5 (22 weeks)

D, donor; R, recipient.
[a] Likelihood of CMV infection in patient group, assuming no differences between live and cadaveric transplants.
[b] Significantly different from D–R–.
Data from Fox *et al*. (1995)[4] and Emery *et al*. (2000).[5]

CMV testing patterns (screening tests)

CMV status	No. of patients (%)	Probability of CMV (%)[a]	Median no. of tests per patient	No. of tests per patient (range and quartiles)				Median follow-up (days) (weeks)
				Min.	Lower quartile	Upper quartile	Max.	
R–D– (cadaveric)	10 (18.5%)	0	6	5	6	6	7	146.5 (21 weeks)
R–D– (live)	5 (9%)	0	5	5	5	5	6	119 (17 weeks)
R–D+ (cadaveric)	10 (18.5%)	44[b]	6	5	6	7	10	146.5 (21 weeks)
R–D+ (live)	0	44[b]	–	–	–	–	–	–
R+D– (cadaveric)	10 (18.5%)	6	6.5	5	6	7	7	168.5 (24 weeks)
R+D– (live)	1 (2%)	6	7	–	–	–	–	154 (22 weeks)
R+D+ (cadaveric)	15 (28%)	25[b]	6	1	5	6	6	139 (20 weeks)
R+D+ (live)	3 (5.6%)	25[b]	6	5	5	7	7	140 (20 weeks)
Total	54 (55%)		6	1	5	6	10	144.5 (21 weeks)

D, donor; R, recipient.
[a] Likelihood of CMV infection in patient group, assuming no significant differences between live and cadaveric transplants.
[b] Significantly different from D–R–.
Data from Fox *et al*. (1995)[4] and Emery *et al*. (2000).[5]

Appendix 4

Technical background to tests used

Technical background to pp65 antigenaemia assay

The main steps in the pp65 antigenaemia test are given in *Figure 4*. Buffy coat cells for antigenaemia assay were prepared from EDTA blood according to the manufacturers' instructions (CMV-vue kit, DiaSorin, Soluggia, Italy) and as described previously.[66] Polymorphonuclear leucocyctes (PML) were first isolated by dextran sedimentation and cells were counted using a haemocytometer. Cells were fixed to a slide in two spots (each 0.5×10^5 cells) and stained by application of a cocktail of monoclonal antibodies directed against the major matrix protein pp65 using an indirect immunoperoxidase technique. After counterstaining with haematoxylin, infected cells were identified by their red appearance and typical lobular staining pattern.

The skill level of the person interpreting the test was also critical as some non-specific staining was noted in occasional samples from haematology patients during engraftment. It required technically experienced personnel to differentiate the typical, specific staining in PML from that seen in some other cells. The subjective nature of the assay interpretation led us to have two independent observers evaluate any samples giving possible pp65 staining.

The assay required access to general laboratory equipment (bench centrifuge, microscope and haemocytometer), but no specialised items.

Technical background to nested In-house PCR (semi-quantitative cell-associated CMV DNA detection by PCR)

The main steps in the nested in-house PCR test are given in *Figure 5*. Nucleic acid was extracted from 100 µl of whole blood using the silica-based extraction method described previously by Boom and colleagues.[67] The method used for semi-quantitative CMV DNA detection in these whole blood extracts was a modification of a procedure used previously for cerebrospinal fluid.[68]

In brief, 10 µl of total extracted DNA were analysed in a 50 µl first-round PCR. After 35 cycles of amplification, 1µl of each first-round product was then added to the nested PCR mix in a total volume of 25µl. Twenty-five cycles of second-round amplification were carried out in a similar fashion to the first-round. Products of amplification were analysed by agarose gel electrophoresis. The outer set of primers, used in the first-round PCR, amplified a 150-bp region of the CMV gB while the inner primer set, used in the nested reaction, amplified a 100-bp region of the CMV gB. The primers for first-round PCR amplification had been validated previously for amplification of CMV[69] and were the basis for development of a published quantitative CMV DNA assays[4,70–72] and have been used for diagnosis of congenital CMV infections.[73] The nested (second-round) primers were initially designed, and validated, for sensitive detection of CMV DNA in ocular samples.[74] Hence this in-house assay uses procedures and approaches that have been well validated by ourselves and others. Typical results are given in *Figure 6*.

The method required a general molecular laboratory set-up with separate pre- and post-amplification areas, PCR machine (thermocycler) and gel analysis equipment.

Technical background to Roche Amplicor Assay (qualitative cell-free CMV DNA detection by PCR)

The main steps in the Roche Amplicor assay test are given in *Figure 7*. The assay includes an internal control that identifies inhibitors of PCR amplification and controls for specimen preparation. This control is useful for validation of negative results. The internal control is amplified with the same primers as the wild-type target but has a different internal sequence. Thus, after amplification, the product is detected with wild-type-specific and internal control-specific probes in a plate hybridisation reaction.

The method required a general molecular laboratory set-up with separate pre- and post-amplification areas. A plate washer and

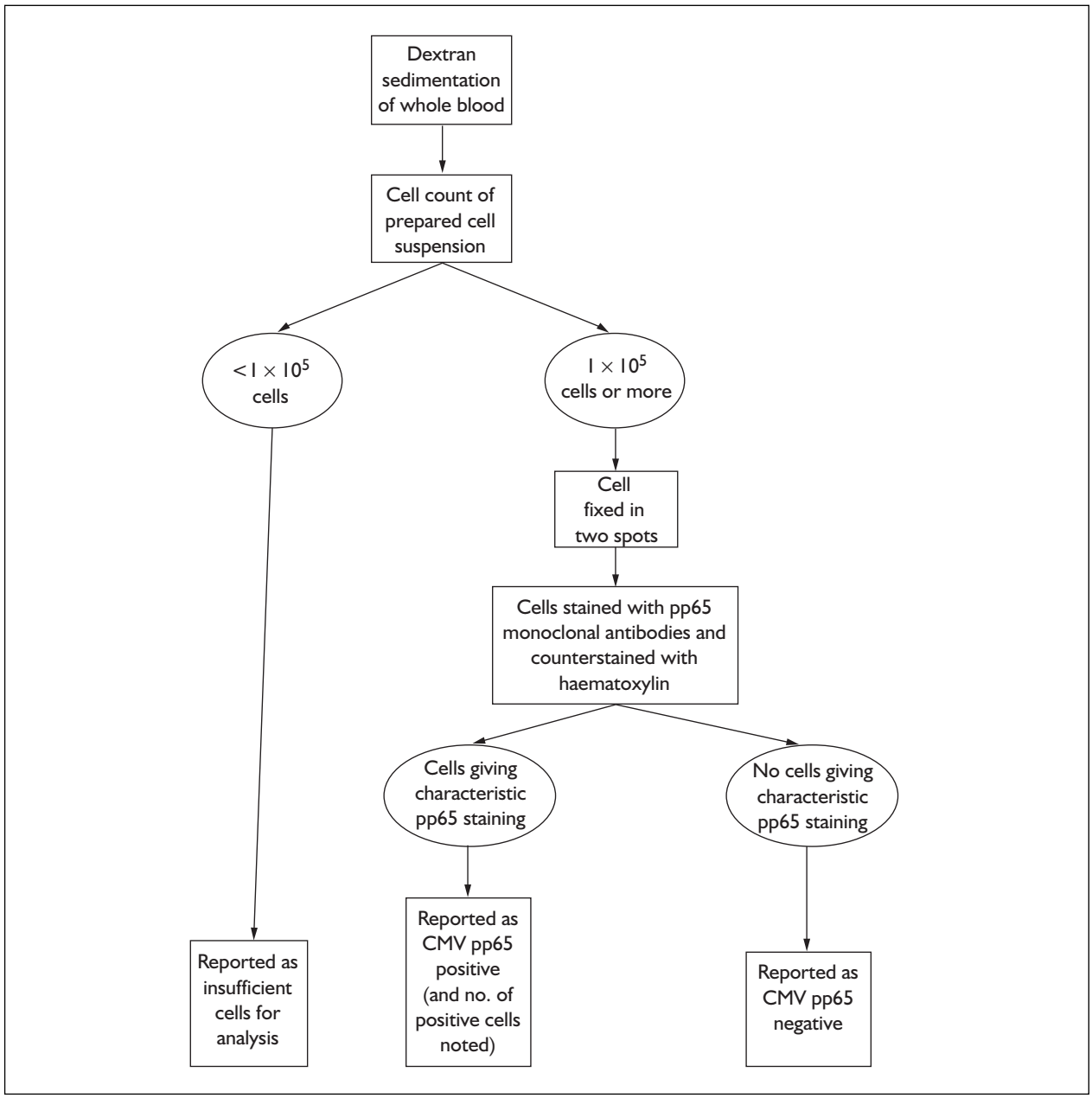

FIGURE 4 *Steps in the pp65 antigenaemia assay*

spectrophotometer were utilised for determination of optical density for wild-type and internal control hybridisations. The method requires a specialised thermocycler (9600 plate-format PCR machine, Applied Biosystems).

Technical background to qualitative NASBA analysis of late CMV messenger RNA (NASBA, Organon Teknika)

The main steps in the NucliSens, CMV pp67 test are given in *Figure 8*. The assay was used following the manufacturer's instructions and as published previously.[75] Nucleic acid was extracted from 100 µl of whole blood using the silica-based extraction method described by Boom and colleagues.[67] To exclude false-negative results due to the presence of inhibitors, the NucliSens®, pp67 kit included an internal CMV System Control mRNA that was added to the lysed-whole-blood suspension prior to nucleic acid isolation.

The method required a general molecular laboratory set-up with separate pre- and post-amplification areas. The only specialised piece of equipment dedicated to this assay was an

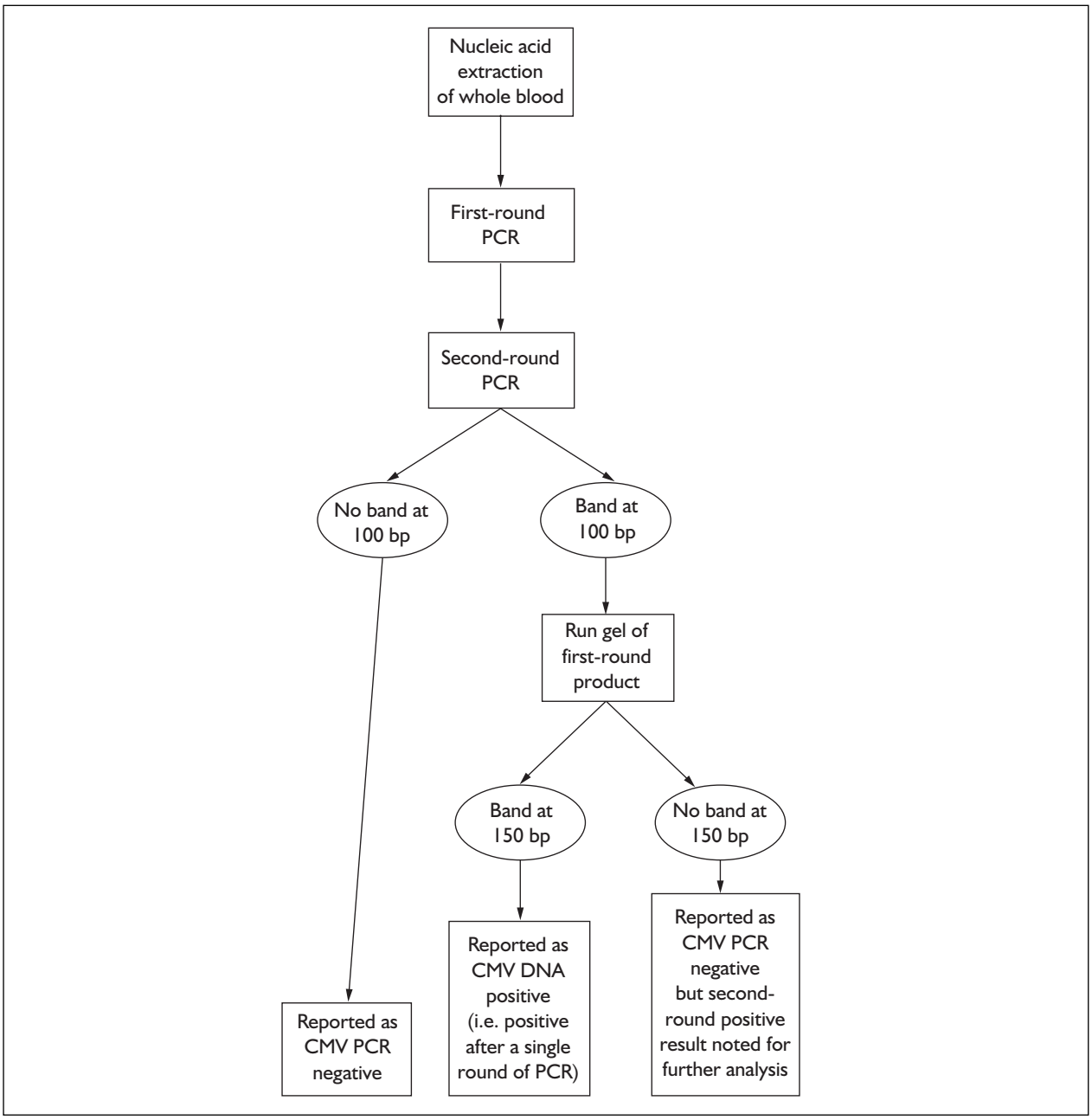

FIGURE 5 *Nested PCR for semi-quantitative assessment of cellular CMV DNA presence and level in whole blood samples*

electrochemiluminescence reader for probe-specific detection of wild-type and system control products.

Technical background to Roche COBAS Amplicor CMV Monitor Assay (quantitative cell-free CMV DNA detection by PCR)

The main steps in the Roche COBAS Amplicor CMV Monitor test are given in *Figure 9*. The assay is similar to the Roche Amplicor assay (*Figure 7*) and the same 365-bp portion of the CMV polymerase gene UL54 is amplified.[37] The internal control is modified as a quantitative standard (QS), which identifies inhibitors of PCR amplification and controls for specimen preparation. This standard is useful for validation of negative results and is amplified with the same primers as the wild-type target but has a different internal sequence. Thus, after amplification, the products are detected with wild-type-specific and QS-specific probes in a plate hybridisation reaction. The optical

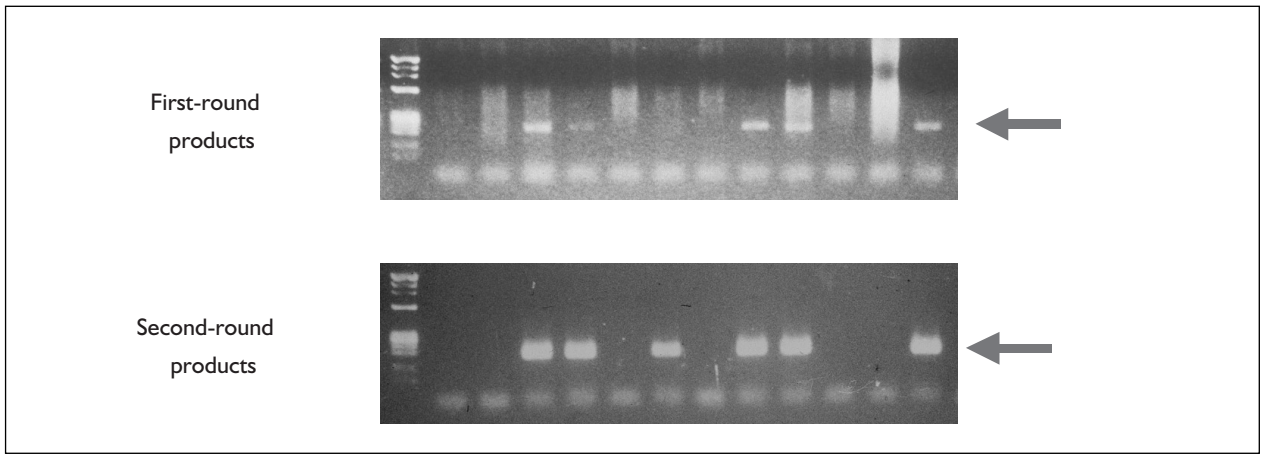

FIGURE 6 *Example results for semi-quantitative CMV DNA PCR*

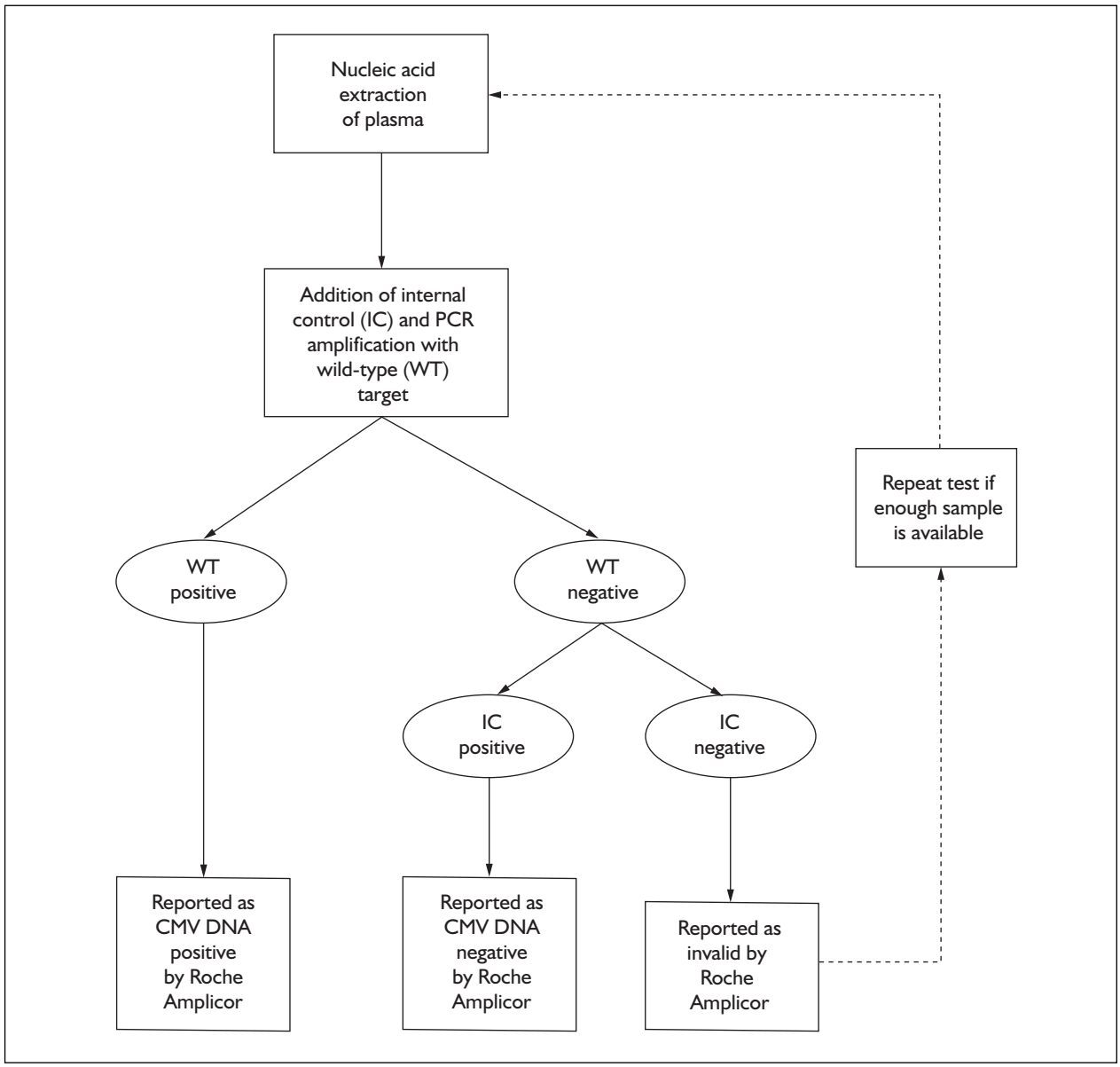

FIGURE 7 *Roche Amplicor Assay for assessment of cellular CMV DNA presence and level in plasma samples*

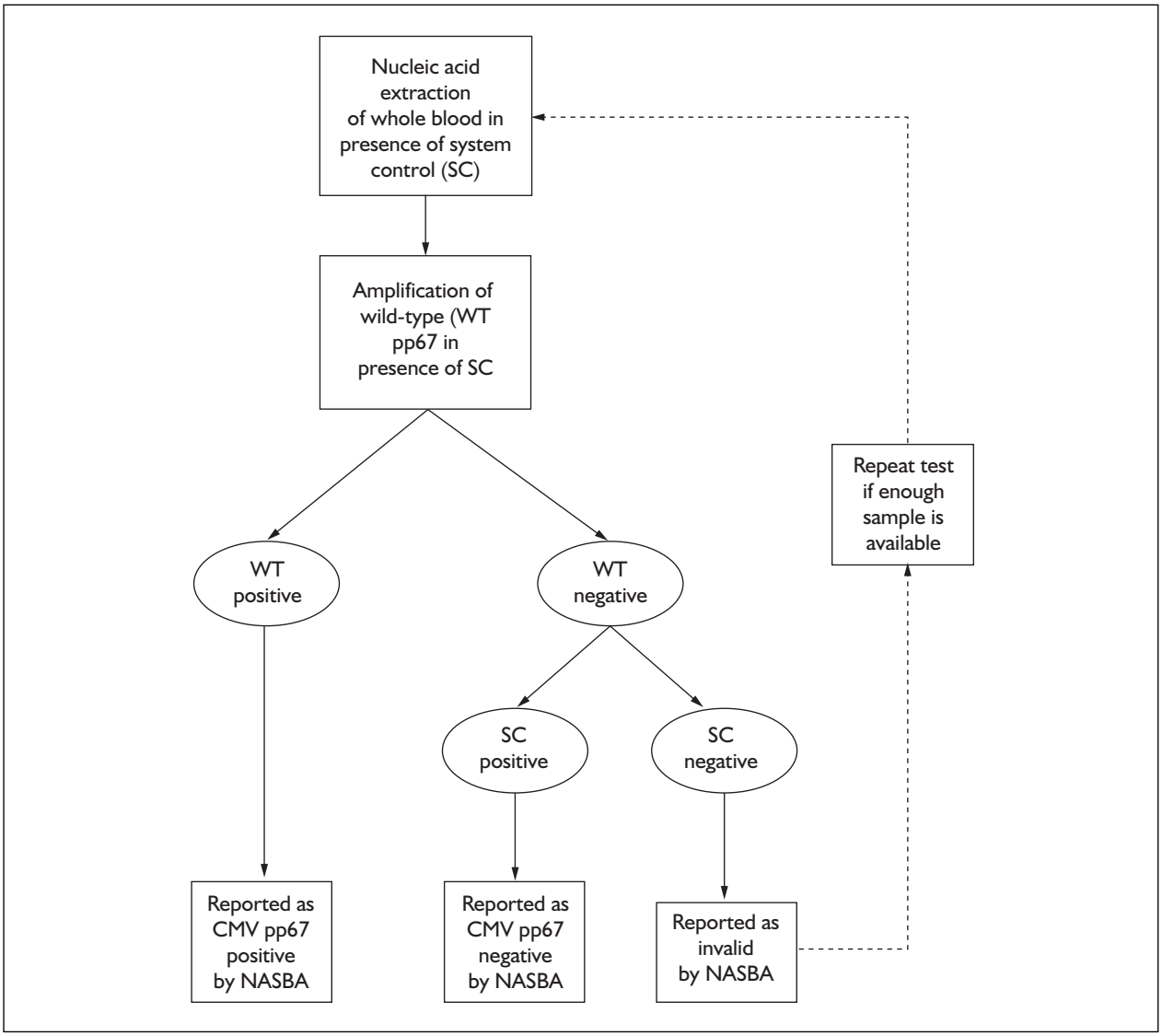

FIGURE 8 *NASBA for detection of CMV pp67 mRNA in whole blood*

density for the QS (the copy number input for which is known and constant) is compared with that for the wild-type target to determine the relative amounts of wild-type CMV DNA amplified.

The main steps in the procedure are given in *Figure 9*. The set-up of the assay is as described for the qualitative version but amplification and detection of hybridisation products uses the semi-automated COBAS instrument. A modified protocol using whole blood is available for the assay, but in this health technology assessment study we used the extraction module of the assay which is suitable only for plasma. Thus valid positive results were expressed as CMV DNA copies/ml of plasma.

The COBAS Amplicor Analyzer automates the amplification and detection steps of the PCR process on a single instrument, thus minimising hands-on time. The instrument provides high-throughput testing and is supplied by the manufacturer as part of a reagent/machine rental agreement. Relatively junior staff were able to undertake the method once adequate training had been given in handling of samples for molecular amplification assays, and specific COBAS training (for use of the instrument) had been completed. The kit-based nature of the assay made quality control and interpretation of results uncomplicated. Despite the semi-automated nature of the method, separate pre- and post-amplification areas were still required for specimen handling and amplification/analysis, respectively.

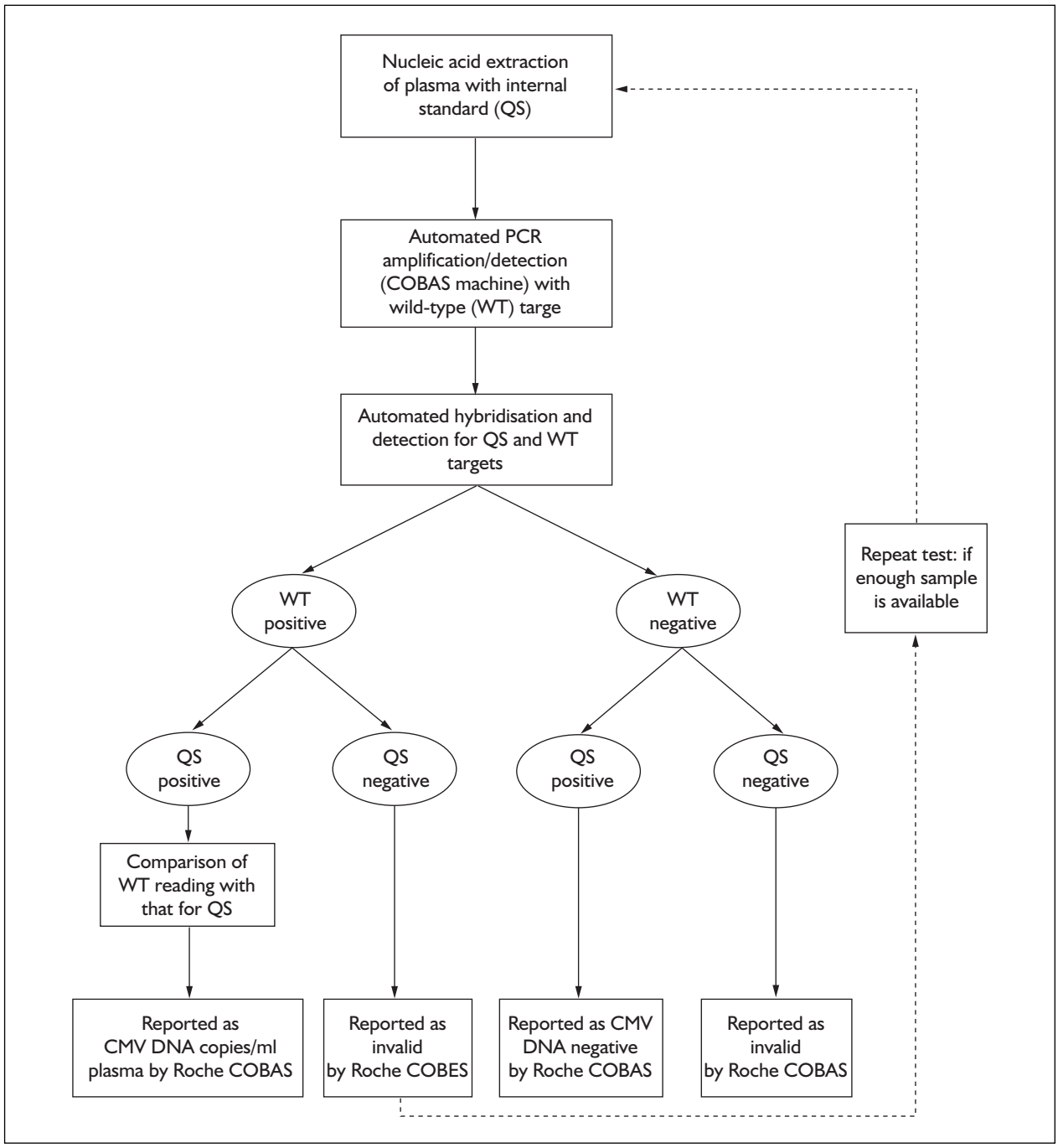

FIGURE 9 *Roche COBAS Amplicor CMV Monitor assay for assessment of cellular CMV DNA presence and level in plasma*

Appendix 5

Laboratory feasibility study

Following submission of the original outline proposal, a feasibility study was undertaken in Cardiff in order to examine further the molecular tests proposed for the main study. The aims of this pilot were (1) to finalise technical details for all the molecular techniques to be used in the study in order to ensure that they could be carried out effectively and (2) to examine initial results for the chosen techniques in order to confirm that these molecular methods were appropriate for inclusion in the study.

A pilot study was carried out focusing on renal transplant patients. A total of 29 samples from transplant recipients were sent for routine diagnostic detection of CMV antigens (pp65 antigenaemia assay). Samples were also analysed for CMV using the three molecular methods proposed; pp67 mRNA by NASBA, cell-associated CMV DNA by nested PCR and cell-free plasma CMV DNA by nested PCR. For 19 samples no positive result was obtained by any of the methods. A summary of the results for the 10 samples found positive by one or more of the methods is given in *Table 74*.

The correlation between pp65 antigenaemia assay and NASBA was reasonably good. Three of the samples analysed in the pilot study were antigenaemia positive and NASBA picked up one additional sample. For cell-associated PCR on whole blood, only three of the samples contained detectable CMV DNA after a single round of PCR. However, this test proved very sensitive after two rounds of amplification and 10 of the 29 samples contained detectable CMV DNA. These results suggest that many of the nested PCR-positive blood samples contained only low-level CMV DNA. Cell-free nested PCR on 10 µl plasma missed some samples that were antigenaemia and NASBA positive but picked up two additional samples.

In a separate part of the pilot study, blood samples from 25 healthy individuals were also analysed in order to determine whether it was possible to detect cell-associated or cell-free CMV DNA in these samples by nested PCR, or CMV mRNA by NASBA. In no case was it possible to detect CMV DNA or CMV mRNA in the equivalent of 5 µl of whole blood, nor did any plasma sample (10 µl analysed) yield detectable CMV DNA after PCR amplification. We conclude from these results that the methods we are proposing to evaluate for detection of CMV transcripts, cell-associated or plasma CMV DNA are appropriate and are unlikely to pick up the low-level CMV infection present in healthy individuals who have not reactivated the virus recently.

The pilot study results therefore demonstrate that if CMV PCR is to be used for monitoring patients at risk from CMV disease, the level of detectable

TABLE 74 *Samples positive by one or more method for markers of CMV infection*

Sample	pp65 antigenaemia assay	CMV late mRNA by NASBA	Cell-free/plasma CMV DNA by nested PCR	Cell-associated/whole blood CMV DNA by nested PCR
1	+	+	+	+
2	+	+	–	+
3	+	+	–	+
4	–	+	–	[a]
5	–	–	[a]	[a]
6	–	–	[a]	[a]
7	–	–	–	[a]
8	–	–	–	[a]
9	–	–	–	[a]
10	–	–	–	[a]

[a] PCR positive after a second round of amplification.

CMV DNA in whole blood extracts is critical. It is also clear from the results that the use of different molecular methods and strategies for monitoring CMV activation in individuals at risk from significant CMV-related disease requires further direct comparative studies on longitudinal samples. Findings from this pilot study have been published.[36]

Appendix 6

Test diagnostic accuracy (including STARD checklist)

Test diagnostic accuracy is usually presented in terms of sensitivity and specificity, or the ability of different tests to detect positive (abnormal) and negative (normal) results correctly. Sensitivity is defined as the ability of a test to detect disease when disease is present [Equation (1)], hence this parameter measures the proportion of diseased patients with a positive test. Specificity [Equation (2)] is the ability of the test to exclude correctly disease in non-diseased populations; hence it measures the proportion of non-diseased patients with a negative test. Sensitivity and specificity have been adopted widely because they are considered to be stable properties of a diagnostic test when derived for a broad spectrum of diseased and non-diseased patients. Under such circumstances, their values are not expected to change significantly when applied in populations with different prevalence, severity or presentation of disease.

$$\text{Sensitivity} = \text{proportion of diseased patients with a positive test} = \frac{\text{true positives}}{\text{true positives} + \text{false negatives}} \quad (1)$$

$$\text{Specificity} = \text{proportion of non-diseased patients with a negative test} = \frac{\text{true negatives}}{\text{true negatives} + \text{false positives}} \quad (2)$$

Taken alone, sensitivity and specificity do not reveal the probability that an individual really has a disease or condition if the test is positive, or the probability that an individual does not have the condition if the test is negative. These probabilities are captured by two other characteristics. Positive predictive value (PPV) [Equation (3)] is the proportion of those individuals with a positive test result who actually have the condition. Negative predictive value (NPV) [Equation (4)] is the proportion of individuals with a negative test result who actually do not have the condition. Accurately predicting absence of disease is important in the present instance since treatment can have such adverse effects. At the same time, accurately predicting disease is even more important because of the severe consequences of untreated CMV disease in these patients.

$$\text{PPV} = \text{proportion of positive test patients with CMV disease} = \frac{\text{true positives}}{\text{true positives} + \text{false positives}} \quad (3)$$

$$\text{NPV} = \text{proportion of negative test patients with no CMV disease} = \frac{\text{true negatives}}{\text{true negatives} + \text{false negatives}} \quad (4)$$

Limitations in reported assessments of test performance

Currently, new diagnostic technologies are usually evaluated adequately with respect to sensitivity and specificity. However, it is often common practice to exclude indeterminate or uninterpretable results from published evaluations of tests. Such results may occur because of technical factors (e.g. a new molecular test may be inappropriate for certain types of sample) or because the patient does not cooperate with a diagnostic procedure (e.g. inability to undergo MRI due to claustrophobia). If these and similar cases are excluded from published results, the reported findings will overstate the diagnostic test's actual performance once in a clinical setting (i.e. report an ideal efficacy rather than its effectiveness). Studies also rarely report other aspects of technical performance such as test replicability.

Equally importantly, the reported performance of a new diagnostic test may not be reliable because of an element of bias in the study design. For example, the range of patients on whom a test is evaluated may be inadequate. Often a test is first assessed on patients with advanced disease and compared with young healthy controls. A test may perform well under these conditions but may not be able to discriminate patients with less advanced or severe disease. Such selection bias will result in inferior test performance once the test is used in a broader range of patients.

Another important limitation in assessment of test performance is the fact that some element of interpretation may be part of the process of producing a test result. Hence the diagnostic

performance of a new virology test may depend not only on the technical quality of the equipment but also on the expertise of the person interpreting the output. Reported health technology assessments usually do not differentiate these two elements and instead address the test–interpreter combination. However, when evaluating new diagnostic technologies it may be important to separate inadequacies in the technology itself from deficiencies or difficulties inherent in interpretation, because either might be improved separately.

A further limitation when considering the performance characteristics of a test may be the lack of an appropriate gold standard (reference test). For many diseases or conditions, even the best available test (reference or gold standard) will still have some level of error, and therefore will not in fact have a sensitivity and specificity of 1.000. Evaluating a new test against an imperfect reference standard will obviously result in test performance measures which are not absolute. Ideally, any reference or gold standard should also be independent of the technology being evaluated. However, in certain circumstances the reference standard can involve expert judgement, which in its turn sometimes needs to be based, in part, on the technology being assessed. Furthermore, in some instances the reference standard used may involve subsequent confirmation at surgery or examination of tissue samples. In this situation, case selection bias can be a problem, since not all patients included in the study will necessarily have surgical or pathology reports. The evaluation results obtained may therefore not be repeatable or generalisable to the broader spectrum of patients. In some cases clinical follow-up is used as the reference standard. Clearly, such an outcome measure may be influenced by subsequent therapy.

The STARD Initiative

Recently, a group of scientists and editors have formed the STARD (Standards for Reporting of Diagnostic Accuracy) initiative, to provide a method of improving the quality of reporting of studies of diagnostic accuracy (http://www.consort-statement.org/standardstatement.htm). The group has produced a checklist (*Table 75*) which the HTA Editorial Board have agreed should be included in the Appendices of HTA reports.

TABLE 75 *STARD checklist for the reporting of studies of diagnostic accuracy.*

Section and topic	Item no.		On page no.
TITLE/ABSTRACT/ KEYWORDS	1	Identify the article as a study on diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity')	ix (Executive Summary)
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups	7–8
METHODS			
Participants	3	Describe the study population: the inclusion and exclusion criteria, setting and locations where the data were collected	12
	4	Describe participant recruitment: was recruitment based on presenting symptoms, results from previous tests or the fact that the participants had received the index tests or the reference standard?	12
	5	Describe participant sampling: was the study population a consecutive series of participants defined by selection criteria in items 3 and 4? If not, specify how participants were further selected	12 (consecutive series)
	6	Describe data collection: was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	9 (prospective for clinical data collection, retrospective for technical – because of nature of reference standard)

continued

TABLE 75 *STARD checklist for the reporting of studies of diagnostic accuracy. (cont'd)*

Section and topic	Item no.		On page no.
Test method	7	Describe the reference standard and its rationale	24–25
	8	Describe technical specification of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard	Appendix 4
	9	Describe definition and rationale for the units, cut-offs and/or categories of the results of the index test(s) and the reference standard	Cut-offs n/a (qualitative tests). Reference standard categories see p. 25
	10	Describe the number, training and expertise of the persons executing and reading the index tests and the reference standard	Appendix 4
	11	Describe whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers	25 (masked)
Statistical methods	12	Describe methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals)	Appendix 6 quantifying uncertainty n/a (qualitative tests)
	13	Describe methods for calculating test reproducibility, if done	Not performed
RESULTS			
Participants	14	Report when study was done, including beginning and ending dates of recruitment	12
	15	Report clinical and demographic characteristics of the study population (e.g. age, sex, spectrum of presenting symptoms, co-morbidity, current treatments, recruitment centres)	13
	16	Report the number of participants satisfying the criteria for inclusion that did or did not undergo the index tests and/or the reference standard; describe why participants failed to receive either test (a flow diagram is strongly recommended).	n/a (all participants received the tests)
Test results	17	Report time interval from the index tests to the reference standard, and any treatment administered between	n/a (see p. 25)
	18	Report distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition	2–5, 13 (criterion not directly applicable to study patients)
	19	Report a cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard	22–24, 26–28
	20	Report any adverse events of index tests and reference standard	n/a
	21	Report estimates of diagnostic accuracy and measures of statistical certainty (e.g. 95% confidence intervals)	24–30, Appendix 7
	22	Report how indeterminate results, missing responses and outliers of index tests were handled.	Indeterminate 24–25; missing responses, i.e. failed, 22, 26; outliers of index test n/a (qualitative)
	23	Report estimates of variability of diagnostic accuracy between subgroups of participants, readers or centres, if done	27–30
	24	Report estimates of test reproducibility, if done	Not done
DISCUSSION	25	Discuss the clinical applicability of the study findings	47–66, 94–95

Appendix 7

95% confidence intervals

TABLE 76 *All patients: 95% CI for sensitivity/specificity of diagnostic versus screening tests*

Test method	Failed to complete test (%)	Diagnostic tests [screening tests][a]			
		Sensitivity	Specificity	PPV	NPV
1. Antigenaemia (pp65)	19.8	0.388 to 0.698 [0.203 to 0.464]	0.970 to 1 [0.992 to 0.99]	0.822 to 1 [0.611 to 0.96]	0.834 to 0.932 [0.957 to 0.978]
2. Nested in-house PCR (single-round)	< 1	0.736 to 0.947 [0.616 to 0.872]	0.949 to 0.996 [0.99 to 0.999]	0.803 to 0.982 [0.754 to 0.967]	0.927 to 0.987 [0.981 to 0.995]
3. Nested in-house PCR (two-round)	< 1	0.843 to 0.993 [0.843 to 0.993]	0.765 to 0.88 [0.915 to 0.946]	0.473 to 0.702 [0.298 to 0.479]	0.949 to 0.998 [0.992 to 1]
4. Roche Amplicor Assay	3.6	0.759 to 0.968 [0.625 to 0.883]	0.92 to 0.985 [0.97 to 0.988]	0.714 to 0.942 [0.506 to 0.77]	0.936 to 0.992 [0.981 to 0.995]
5. NASBA, Organon Teknika	2.5	0.586 to 0.852 [0.637 to 0.88]	0.965 to 1 [0.982 to 0.994]	0.834 to 0.999 [0.581 to 0.831]	0.89 to 0.965 [0.986 to 0.996]

[a] Calculated separately for diagnostic specimens and [screening specimens].

TABLE 77 *Haematology: 95% CI for sensitivity/specificity of diagnostic versus screening tests*

Test method	Failed to complete test (%)	Diagnostic tests [screening tests][a]			
		Sensitivity	Specificity	PPV	NPV
1. Antigenaemia (pp65)	25	0.078 to 0.502 [0.181 to 0.754]	0.955 to 1 [0.991 to 1]	0.396 to 1 [0.365 to 0.991]	0.811 to 0.936 [0.98 to 0.996]
2. Nested in-house PCR (single-round)	< 1	0.682 to 0.983 [0.17 to 0.927]	0.938 to 0.997 [0.986 to 0.999]	0.682 to 0.983 [0.139 to 0.861]	0.938 to 0.997 [0.988 to 0.999]
3. Nested in-house PCR (two-round)	< 1	0.741 to 0.998 [0.299 to 0.989]	0.788 to 0.916 [0.929 to 0.964]	0.371 to 0.702 [0.034 to 0.257]	0.943 to 1 [0.99 to 1]
4. Roche Amplicor Assay	3	0.655 to 0.982 [0.073 to 0.83]	0.92 to 0.993 [0.974 to 0.994]	0.611 to 0.96 [0.035 to 0.558]	0.932 to 0.997 [0.985 to 0.999]
5. NASBA, Organon Teknika	2	0.477 to 0.878 [0.478 to 0.968]	0.949 to 1 [0.982 to 0.996]	0.677 to 0.997 [0.285 to 0.761]	0.897 to 0.981 [0.991 to 1]

[a] Calculated separately for diagnostic specimens and [screening specimens].

TABLE 78 *Renal: 95% CI for sensitivity/specificity of diagnostic versus screening tests*

Test method	Failed to complete test (%)	Diagnostic tests [screening tests][a]			
		Sensitivity	Specificity	PPV	NPV
1. Antigenaemia (pp65)	12	0.545 to 0.898 [0.162 to 0.448]	0.919 to 1 [0.983 to 0.999]	0.791 to 1 [0.562 to 0.975]	0.791 to 0.959 [0.913 to 0.958]
2. Nested in-house PCR (single-round)	1	0.643 to 0.95 [0.628 to 0.892]	0.894 to 0.999 [0.983 to 1]	0.76 to 0.998 [0.829 to 0.998]	0.83 to 0.978 [0.956 to 0.989]
3. Nested in-house PCR (two-round)	1	0.784 to 0.998 [0.859 to 0.999]	0.62 to 0.855 [0.867 to 0.929]	0.472 to 0.783 [0.404 to 0.632]	0.865 to 0.999 [0.982 to 1]
4. Roche Amplicor Assay	4	0.694 to 0.984 [0.666 to 0.921]	0.834 to 0.985 [0.945 to 0.984]	0.653 to 0.966 [0.594 to 0.863]	0.857 to 0.993 [0.958 to 0.991]
5. NASBA, Organon Teknika	3	0.545 to 0.898 [0.603 to 0.883]	0.919 to 1 [0.971 to 0.994]	0.791 to 1 [0.643 to 0.914]	0.791 to 0.959 [0.967 to 0.992]

[a] Calculated separately for diagnostic specimens and [screening specimens].

Appendix 8

Test results COBAS Amplicor Monitor Assay by Roche

Renal samples

CMV reference result	No. of samples tested	No. of valid readings	COBAS reading		
			Min.	Median	Max.
CMV-positive	37	37	922	11,400	275,000
CMV-negative	4	1[a]	632	632	632
Overall	41	34	632	10,500	275,000

[a] 3 samples gave reading 'below lower level'.

Haematology samples

CMV reference result	No of samples tested	No of valid readings	COBAS reading		
			Min.	Median	Max.
CMV-positive	22	22	648	12,900	95,200
CMV-negative	5	3[a]	1,730	5,310	8,500
Overall	27	25	648	10,000	95,200

[a]2 samples gave reading 'below lower level'.

Appendix 9

UK laboratories: respondents

Aberdeen: Microbiology Department, Aberdeen Royal Infirmary (Institute of Virology)

Belfast: Department of Microbiology, Royal Victoria Hospital

Birmingham: Public Health Laboratory, Birmingham Heartlands Hospital

Bristol: Public Health Laboratory

Dundee: Virology Department, Ninewells Hospital Medical School.

Glasgow: Institute of Virology.

Hull: Public Health Laboratory, Hull Royal Infirmary

Ipswich: Public Health Laboratory, Ipswich Hospital

Leicester: Department of Virology, Leicester Royal Infirmary

London: Virology Department, St Bartholomew's Hospital

London: Virology Section, Department of Infection, St Thomas's Hospital

London: Department of Virology for St Mary's, Chelsea and Westminster, and Hammersmith Hospitals

London: Virology Department, University College London Medical School

London: Virology Department, Royal Free Campus, UCL Medical School

London: Virology Department, Dulwich Hospital

London: Department of Microbiology, St George's Hospital

Manchester: Public Health Laboratory, Withington Hospital

Newcastle-upon-Tyne: Public Health Laboratory, Newcastle General Hospital

Oxford: Department of Microbiology, The John Radcliffe Hospital

Reading: Public Health Laboratory, Royal Berkshire Hospital

Sheffield: Public Health Laboratory

Southampton: Public Health Laboratory, Southampton General Hospital

Appendix 10

Levels of CMV tests reported by laboratories

Use of CMV tests for screening and diagnosis in haematology transplant patients

Av. no. of CMV tests (all centres providing data)[a]	Av. no. of CMV tests (just centres providing data on % screening tests also)	% of CMV tests for screening	Average estimated number of CMV *screening* tests	Average estimated number of CMV *diagnostic* tests
5	5	50	2.5	2.5
30	30	99	29.7	0.3
80	80	50	40	40
10	10	50	5	5
11	11	75	8.3	2.8
30	30	95	28.5	1.5
20	20	100	20	0
15	15	99	14.9	0.2
0.5	0.5	0.5	0	0.5
10	10	95	9.5	0.5
2.5	2.5	90	2.3	0.3
25	25	90	22.5	2.5
27.5	27.5	0	0	27.5
40	40	100	40	0
20	20	100	20	0
10	10	70	7	3
50	50	99	49.5	0.5

[a] No information: 5 centres.

Use of CMV tests for screening and diagnosis in renal transplant patients

Av. no. of CMV tests (all centres providing data)[a]	Av. no. of CMV tests (just centres providing data on % screening tests also)	% of CMV tests for screening	Average estimated number of CMV *screening* tests	Average estimated number of CMV *diagnostic* tests
10	10	50	5	5
2	2	99	2	0
15	15	50	7.5	7.5
2.5	2.5	20	0.5	2
5	5	0	0	5
4		No information	No information	No information
25	25	95	23.8	1.3
0.5	0.5	0.5	0	0.5
0.5		No information	No information	No information
1.5	1.5	0	0	1.5
5			No information	No information
1.5			No information	No information
13	13	100	13	0
1	1	100	1	0
4	4	10	0.4	3.6
12	12	99	11.9	0.1

[a] No information: 6 centres.

Use of CMV tests for screening and diagnosis in HIV patients

Av. no. of CMV tests (all centres providing data)[a]	Av. no. of CMV tests (just centres providing data on % screening tests also)	% of CMV tests for screening	Average estimated number of CMV *screening* tests	Average estimated number of CMV *diagnostic* tests
5	5	0	0	5
1	1	0	0	1
2.5	2.5	10	0.3	2.6
2	2	5	0.1	1.9
1			No information	No information
0.5	0.5	0.5	0	0.5
0.5		No information	No information	No information
0.5	0.5	0	0	0.5
5			No information	No information
1.5			No information	No information
16	16	100	16	0
1	1	0	0	1
2	2	0	0	2

[a] No information: 9 centres.

Appendix 11

Baseline analyses of CMV test costs

Baseline analysis: semi-variable (i.e. staffing costs and overheads) and variable (i.e. consumable costs)

CMV test: MLSO 1	Cost per sample (batch of 5) (£)	Range of cost per sample (lower) (£)	Range of cost per sample (upper) (£)
pp65 antigenaemia	22.36	1:73.60	20:13.03
Quantitative CMV PCR – manual extraction (nested in-house PCR) – 1 round	10.56	1:23.36	30:8.14
Quantitative CMV PCR – automated extraction (nested in-house PCR) – 1 round	12.49	1:23.18	30:10.50
Quantitative CMV PCR – manual extraction (nested in-house PCR) – 2nd round	13.46	1:32.32	30:9.89
Quantitative CMV PCR – automated extraction (nested in-house PCR) – 2nd round	15.17	1:31.22	30:12.19
Qualitative NASBA – manual extraction (Biomerieux)	30.27	5:30.27	30:22.96
Qualitative NASBA – automated extraction (Biomerieux)	31.20	5:31.20	30:25.50
Qualitative CMV PCR (Roche Amplicor)	27.47	5:27.47	32:23.36
COBAS CMV PCR (Roche COBAS Assay)[a]	Not available	12:49.86	24:48.68

[a] COBAS had to be costed using list prices supplied by Roche, because the actual kits were donated rather than charged for.

CMV test: MLSO 1	Cost per sample (batch of 5) (£)	Range of cost per sample (lower) (£)	Range of cost per sample (upper) (£)
Pp65 antigenaemia	25.80	1:87.99	20:14.47
Quantitative CMV PCR – manual extraction (nested in-house PCR) – 1 round	11.84	1:27.22	30:8.94
Quantitative CMV PCR – automated extraction (nested in-house PCR) – 1 round	13.33	1:25.60	30:11.05
Quantitative CMV PCR – manual extraction (nested in-house PCR) – 2nd round	15.28	1:37.80	30:11.03
Quantitative CMV PCR – automated extraction (nested in-house PCR) – 2nd round	16.16	1:35.37	30:13.13
Qualitative NASBA – manual extraction (Biomerieux)	33.04	5:33.04	30:24.16
Qualitative NASBA – automated extraction (Biomerieux)	33.58	5:33.58	30:26.67
Qualitative CMV PCR (Roche Amplicor Assay)	28.65	5:28.65	32:23.66
COBAS CMV PCR (Roche COBAS Assay)[a]	Not available	12:50.45	24:49.01

[a] COBAS had to be costed using list prices supplied by Roche, because the actual kits were donated rather than charged for.

Appendix 12

Sensitivity analyses of CMV test costs

Batch size of five samples and MLSO 1

CMV test: MLSO 1	Cost per sample (batch of 5) (£)	Cost per sample with –10% change in staff time (£)	Cost per sample with +10% change in staff time (£)
pp65 antigenaemia	22.36	21.07	23.66
Quantitative CMV PCR – manual extraction (nested in-house PCR) – 1 round	10.56	10.07	11.04
Quantitative CMV PCR – automated extraction (nested in-house PCR) – 1 round	12.49	12.17	12.80
Quantitative CMV PCR – manual extraction (nested in-house PCR) – 2nd round	13.46	12.77	14.15
Quantitative CMV PCR – automated extraction (nested in-house PCR) – 2nd round	15.17	14.63	15.71
Qualitative NASBA – manual extraction (Biomerieux)	30.27	29.23	31.31
Qualitative NASBA – automated extraction (Biomerieux)	31.20	30.30	32.10
Qualitative CMV PCR (Roche Amplicor Assay)	27.47	27.02	27.91
COBAS CMV PCR (Roche COBAS Assay)[a]	Not available	Not available	Not available

[a] COBAS had to be costed using list prices supplied by Roche, because the actual kits were donated rather than charged for.

Batch size of five samples and MLSO 2

CMV test: MLSO 2	Cost per sample (batch of 5) (£)	Cost per sample with –10% change in staff time (£)	Cost per sample with +10% change in staff time (£)
pp65 antigenaemia	25.80	24.16	27.44
Quantitative CMV PCR – manual extraction (nested in-house PCR) – 1 round	11.84	11.23	12.45
Quantitative CMV PCR – automated extraction (nested in-house PCR) – 1 round	13.33	12.93	13.72
Quantitative CMV PCR – manual extraction (nested in-house PCR) – 2nd round	15.28	14.41	16.15
Quantitative CMV PCR – automated extraction (nested in-house PCR) – 2nd round	16.60	15.92	17.29
Qualitative NASBA – manual extraction (Biomerieux)	33.04	31.72	34.35
Qualitative NASBA – automated extraction (Biomerieux)	33.58	32.44	34.71
Qualitative CMV PCR (Roche Amplicor Assay)	28.65	28.08	29.21
COBAS CMV PCR (Roche COBAS Assay)[a]	Not available	Not available	Not available

[a] COBAS had to be costed using list prices supplied by Roche, because the actual kits were donated rather than charged for.

Larger batch sizes and MLSO 1

CMV test: MLSO 1	Range of cost per sample (upper) (£)	Cost per sample with –10% change in staff time (£)	Cost per sample with +10% change in staff time (£)
pp65 antigenaemia	20:13.03	12.49	13.57
Quantitative CMV PCR – manual extraction (nested in-house PCR) – 1 round	30:8.14	7.84	8.44
Quantitative CMV PCR – automated extraction (nested in-house PCR) – 1 round	30:10.50	10.29	10.71
Quantitative CMV PCR – manual extraction (nested in-house PCR) – 2nd round	30:9.89	9.46	10.32
Quantitative CMV PCR – automated extraction (nested in-house PCR) – 2nd round	30:12.19	11.83	12.54
Qualitative NASBA – manual extraction (Biomerieux)	30:22.96	22.51	23.42
Qualitative NASBA – automated extraction (Biomerieux)	30:25.50	25.06	25.94
Qualitative CMV PCR (Roche Amplicor Assay)	32:23.36	23.25	23.47
COBAS CMV PCR (Roche COBAS Assay)[a]	24:48.68	43.81	53.54

[a] COBAS had to be costed using list prices supplied by Roche, because the actual kits were donated rather than charged for.

Larger batch sizes and MLSO 2

CMV test: MLSO 2	Range of cost per sample (upper) (£)	Cost per sample with –10% change in staff time (£)	Cost per sample with +10% change in staff time (£)
pp65 antigenaemia	20:14.47	13.79	15.16
Quantitative CMV PCR – manual extraction (nested in-house PCR) – 1 round	30:8.94	8.56	9.33
Quantitative CMV PCR – automated extraction (nested in-house PCR) – 1 round	30:11.05	10.79	11.32
Quantitative CMV PCR – manual extraction (nested in-house PCR) – 2nd round	30:11.03	10.48	11.57
Quantitative CMV PCR – automated extraction (nested in-house PCR) – 2nd round	30:13.13	12.68	13.59
Qualitative NASBA – manual extraction (Biomerieux)	30:24.16	23.59	24.74
Qualitative NASBA – Automated extraction (Biomerieux)	30:26.67	26.11	27.22
Qualitative CMV PCR (Roche Amplicor Assay)	32:23.66	23.52	23.80
COBAS CMV PCR (Roche COBAS Assay)[a]	24: 49.01	44.11	53.91

[a] COBAS had to be costed using list prices supplied by Roche, because the actual kits were donated rather than charged for.

Appendix 13

Haematology transplant centres' CMV screening protocols (three examples)

Centre 1

The protocol combines screening tests and CMV prophylaxis for high- and intermediate-risk patients. Low-risk patients are identified as autologous transplants and R-D- allogeneic transplants and receive no screening tests or prophylaxis. Prophylaxis is provided for high- and intermediate-risk patients in parallel with quantitative PCR surveillance. High-risk patients are identified as R+D– allogeneic transplants; and intermediate-risk patients as R+D+ and R–D+ transplants. High risk transplants have quantitative PCR for CMV DNA performed on peripheral blood twice each week, and medium-risk patients are tested every 7 days. From day 100 onwards, these patients have quantitative PCR performed at each outpatient visit owing to the risk of late CMV reactivation. In all cases CMV screening is carried out in order "to detect reactivation which would then permit the introduction of anti-viral therapy" although "as yet, a clinical relevant 'cut-off' of viral copy numbers has not been established, below which anti-viral therapy need not be instituted or could be discontinued".

Centre 2

The protocol screens patients for CMV based upon similar risk factors as Centre 1. Autologous transplants are not screened. For allogeneic stem cell transplants (SCTs), those who are R–D– are also not routinely screened. However, in the allogeneic group, if the donor or recipient is CMV positive screening is undertaken while an inpatient until 4–6 months.

Centre 3

The protocol states that screening is undertaken using quantitative PCR and that results may trigger ganciclovir treatment. It is indicated that all allografts and matched unrelated donor transplants (MUDs) are monitored post-transplant (including R–D–) by EDTA bloods and in-house quantitative PCR at the beginning of each week (Monday). Those testing positive have a repeat test for CMV that week (Thursday). A 1 log rise in CMV viral load between the two samples results in pre-emptive ganciclovir treatment, as does an initial CMV viral load for the first test of >10,000 copies/ml. During and after pre-emptive treatment patients are monitored twice weekly (Monday and Thursday) by in-house quantitative PCR. Pre-emptive treatment is normally continued for 14 days and is continued for longer if CMV viral load has not stabilised (variation <0.5 log difference) on the last two readings.

Appendix 14

Comprehensive evaluation of diagnostic tests

In addition to evaluating diagnostic accuracy, for which the STARD initiative has recently produced guidelines, any new diagnostic technology can also be assessed in terms of its impact on **process** measures such as utilisation rates of medical treatment, use of further investigations or patients' average hospital LOS. The assumption underlying use of such process measures as an indicator of benefit is that health services that meet certain process standards provide better care, especially if unnecessary hospital stays, invasive treatments or investigations are avoided. In addition, a new diagnostic technology may be assessed in terms of its impact on patient outcome in addition to process measures. Patient outcome refers to the health status and well-being of patients, especially as this is affected by healthcare, for example, traditional measures of mortality, morbidity and health-related quality of life measures. The assumption underlying the use of patient outcome measures is that healthcare that generates improved patient outcomes also provides better healthcare.

Health technology assessment is acknowledged to be more difficult for diagnostic technologies than for many other types of health technology. This is partly because diagnostic technologies are dealing with the production of information which is then mediated by an agent (e.g. a physician, surgeon or in some instances, the patient) and partly because the effects of a diagnostic test or procedure on patient outcome are typically less direct than for other interventions such as pharmaceuticals. Hence assessment of new diagnostic technologies, or extended uses of established technologies, for example developments based on improving detection of a viral infection, has to determine both whether a test provides significant new diagnostic information and whether the information provided and its impact on subsequent clinical care and patient outcome offset the costs and risks of the technology.

The impact of a diagnostic technology has therefore to be measured along a chain of inquiry such as that shown in *Table 79*. Clearly, if a diagnostic technology fails at any step in the chain, then it is unlikely to be successful at a later stage. More crucially, however, success at a particular level in the hierarchy does not guarantee success at the next. Hence an accurate test may or may not lead to more accurate diagnosis, which in its turn may or may not lead to better therapy, which may or may not result in better health for the patient, and the benefits may or may not outweigh the cost of the technology.

The immediate objective of a diagnostic technology should be to provide information about the presence, severity and sometimes the extent of a disease or other health condition. The diagnostic test or procedure should therefore be able to discriminate between individuals who have

TABLE 79 *Chain of inquiry for diagnostic technologies*

Level	Assessment	Information
1	Technical capacity	Does the technology perform reliably and deliver accurate information?
2	Diagnostic accuracy	Does the technology contribute to making an accurate diagnosis?
3	Diagnostic impact	Do the results influence the pattern of subsequent use of diagnostic technologies? Does it replace other diagnostic technologies?
4	Therapeutic impact	Do the findings influence the selection and delivery of treatment?
5	Patient outcome	Does use of the diagnostic technology contribute to improved health of the patient?
6	Cost-effectiveness	Does use of the diagnostic technology improve the cost-effectiveness of health care compared to alternative interventions?

Adapted from (i) Finebery HV, Bauman R, Sosman M. Computerized cranial tomography: effect on diagnostic and therapeutic plans. *JAMA* 1997;**238**:224–30; (ii) Institute of Medicine. *Assessing Medical Technologies*. Washington, DC: National Academy Press, 1985; (iii) Szczepura A, Kankaanpää J, editors. *Assessment of Health Care Technologies: Case Studies, Key Concepts and Strategic Issue*. Wiley: Chichester, 1996.

a particular disease or condition and those who do not, or should be capable of identifying the severity of the condition or discriminating among different extents of disease. Healthcare professionals will then be able to use this information to make decisions about the use of other interventions (including further diagnostic tests) that may in their turn affect patient health outcomes.

Many technologies used for diagnosis can also be used for population surveillance or screening. An important difference between diagnosis and screening is that, typically, diagnosis is carried out in 'symptomatic' patients who have approached the healthcare system, and screening is carried out in individuals who have not sought a diagnostic test and are most likely to be asymptomatic. For a given test, used for either diagnosis or screening, the actual prevalence will have an effect on the probability that someone with a positive or negative test result has a disease or other health condition.

For some disease or conditions, however, it is possible to have a marker which is *discrete*, rather than continuous, and is, in principle, capable of discriminating populations which have or do not have the disease or condition, for example a microbiological test for the TB bacterium or the qualitative molecular tests for CMV disease in the present study. In these cases, the technical performance of the test will be dependent on the ability of the diagnostic test used to accurately register the presence or absence of this marker, such as late CMV mRNA (pp67) in the NASBA test. The relationship between a marker and the presence or absence of disease is usually not clear cut.

In such a situation, clinicians and scientists will need to reach agreement about when disease can be assumed to be present. For an established diagnostic test with a **continuous** marker, scientists and clinicians have usually been able to achieve a level of consensus on the appropriate cut-off level, possibly refined for different populations (e.g. based on age or sex). Therefore, in such cases laboratories can report a test result for an individual patient as either 'positive' or 'negative'.

Finally, the continuously evolving nature of many diagnostic technologies can give rise to temporal bias. The results of early assessment may be questioned in the light of subsequent improvements in the technology, or early assessments may be applied inappropriately to an improved technology.

Higher level assessment of diagnostic tests (levels 3–6)

In terms of diagnostic impact, an important issue for new diagnostic tests is the propensity for clinicians to introduce these as an add-on to existing tests. This may occur even if the new test is superior because it may be viewed as providing additional information or other benefits rather than producing replacement information. Test replacement is more likely to occur, however, where the existing test is invasive or has poor test performance.

Measurement of therapeutic impact and effect of a test on patient outcome is more difficult to assess for diagnostic technologies. Diagnostic tests are often used in combination and even a carefully constructed study design may not be able to discriminate the separate contributions of an individual test to clinical decisions and patient outcomes. Ideally, a randomised controlled trial is required in which all diagnostic pathways can be assessed, but this is often not feasible or in some cases ethical. In fact, many published assessments of diagnostic technologies are typically confined to diagnostic performance and only rarely attempt to measure clinically important impacts of diagnostic tests such as the influence on choice of therapy or the clinical outcomes following therapy. Furthermore, it is sometimes appropriate to consider the social impact of a test, (e.g. screening tests), but this is not often examined.

Full economic evaluation of diagnostic tests may adopt any of the recognised forms of analysis: cost-minimisation analysis, cost-effectiveness analysis, cost–utility analysis or cost–benefit analysis. The most common is cost-effectiveness analysis, based on measures such as the 'cost per case detected'.

Appendix 15

Sensitivity analysis for UHW average cost-effectiveness ratios

TABLE 80 *Cost-effectiveness ratios[a] for higher batch size for renal patients (direct test costs only)*

CMV screening/ diagnostic test regime	Total direct cost per 100 test results[b] (£)	Average cost per positive sample detected (batch size 9.4) (£)	Average cost per true positive sample detected (batch size 9.4) (£)	Total no. of positives undetected (false negatives)	Total no. of positives wrongly detected (false positives)
Antigenaemia (pp65): diagnostic tests only	2803	113	113	9.9	0
Antigenaemia (pp65): screening regime	2803	529	562	6.5 *(6.2)*	0.4 *(0.3)*
NASBA, Organon Teknika: screening regime	3229	385	437	2.3 *(2.3)*	1.1 *(1.1)*
Roche Amplicor Assay PCR: screening regime	2888	203	249	2.0 *(1.9)*	2.7 *(2.9)*
Nested in-house PCR (single-round): screening regime	1140	109	111	3.0 *(2.5)*	0.2 *(0.4)*
Nested in-house PCR (two-round): screening regime	1462	63	114	0.4 *(0.4)*	10.1 *(10.1)*

[a] Include diagnostic tests ordered as part of screening regime.
[b] Based on an average batch size of 9.4 samples and allowing for observed level of test failure.

TABLE 81 *Cost-effectiveness ratios[a] for higher batch size for haematology patients (direct test costs only)*

CMV screening/ diagnostic test regime	Total direct cost per 100 test results[b] (£)	Average cost per positive sample detected (batch size 9.4) (£)	Average cost per true positive sample detected (batch size 9.4) (£)	Total no. of positives undetected (false negatives)	Total no. of positives wrongly detected (false positives)
Antigenaemia (pp65): diagnostic tests only	2803	1121	1121	8.0	0
Antigenaemia (pp65): screening regime	2803	2157	2157	2.7 *(2.3)*	0 *(0.1)*
NASBA, Organon Teknika: screening regime	3229	1114	1467	0.9 *(0.8)*	0.7 *(0.8)*
Roche Amplicor Assay PCR: screening regime	2888	903	1805	1.0 *(0.7)*	1.6 *(1.4)*
Nested in-house PCR (single-round): screening regime	1140	407	496	0.6 *(0.5)*	0.6 *(0.6)*
Nested in-house PCR (two-round): screening regime	1462	146	585	0.4 *(0.25)*	7.5 *(6.2)*

[a] Include diagnostic tests ordered as part of screening regime.
[b] Based on an average batch size of 9.4 samples and allowing for observed level of test failure.

TABLE 82 *Cost-effectiveness of CMV screening regimes (screening tests only) for renal transplant patients (direct test costs only)*

CMV screening tests	Average cost per positive sample detected (batch size 5)[a] (£)	Average cost per true positive sample (batch size 5)[a] (£)	Average cost per positive sample (higher batch size)[b] (£)	Average cost per true positive sample detected (higher batch size)[b] (£)	Total no. of positives undetected (false negatives)	Total no. of positives wrongly detected (false positives)
Antigenaemia (pp65): screening tests only	1178	1414	1027	1233	6.0	0.5
NASBA, Organon Teknika: screening tests only	518	640	494	609	1.6	1.2
Roche Amplicor Assay PCR: screening tests only	263	341	256	331	1.8	2.6
Nested in-house PCR (single-round): screening tests only	180	186	173	178	2.5	0.2
Nested in-house PCR (two-round): screening tests only	81	159	77	152	0.2	9.4

[a] Based on batch size of 5 samples and allowing for observed level of test failure.
[b] Based on batch size of 9.4 CMV samples, assuming once per week processing by study laboratory, rather than twice weekly.

TABLE 83 *Cost-effectiveness of CMV screening regimes (screening tests only) for haematology transplant patients (direct test costs only)*

CMV screening tests	Average cost per positive sample detected (batch size 5)[a] (£)	Average cost per true positive sample detected (batch size 5)[a] (£)	Average cost per positive sample (higher batch size)[b] (£)	Average cost per true positive sample detected (higher batch size)[b] (£)	Total no. of positives undetected (false negatives)	Total no. of positives wrongly detected (false positives)
Antigenaemia (pp65): screening tests only	2995	2995	2609	2609	1.7	0
NASBA, Organon Teknika: screening tests only	1520	2221	1448	2116	0.4	0.7
Roche Amplicor Assay PCR: screening tests only	1717	6011	1668	5841	0.7	1.2
Nested in-house PCR (single-round): screening tests only	1489	1985	1425	1900	0.5	0.2
Nested in-house PCR (two-round): screening tests only	233	1547	222	1471	0.2	5.6

[a] Based on batch size of 5 samples and allowing for observed level of test failure.
[b] Based on batch size of 9.4 CMV samples, assuming once per week processing by study laboratory, rather than twice weekly.

Appendix 16

Details of incremental cost-effectiveness ratios for UHW CMV screening strategies

TABLE 84 *Incremental[a] cost-effectiveness of CMV screening regimes[b] for renal transplant patients (direct test costs only)*

CMV screening/diagnostic test regime	Total direct cost per 100 test results[a] (£)	Additional[a] direct cost per 100 test results (£)	Total no. of true positives per 100 test results	Additional[a] no. of true positives per 100 test results	Average cost per true positive sample detected (£)	Incremental cost per additional true positive sample detected (£)	Total no. of positives undetected (false negatives)	Total no. of positives wrongly detected (false positives)
Antigenaemia (pp65): screening regime	3217	0	5.0	0	643	–	6.5	0.4
NASBA, Organon Teknika: screening regime	3389	+172	7.4	+2.4	458	+72	2.3	1.1
Roche Amplicor Assay PCR: screening regime	2972	–245	11.6	+6.6	256	–37	2.0	2.7
Nested in-house PCR (single-round): screening regime	1191	–2026	10.3	+5.3	116	–382	3.0	0.2
Nested in-house PCR (two-round): screening regime	1537	–1680	12.8	+7.8	120	–215	0.4	10.1

[a] Relative to pp65 antigenaemia.
[b] Full regime in which diagnostic tests ordered as part of screening regime are included.

TABLE 85 *Incremental[a] cost-effectiveness of CMV screening regimes[b] for haematology patients (direct test costs only)*

CMV screening/diagnostic test regime	Total direct cost per 100 test results[a] (£)	Additional[a] direct cost per 100 test results (£)	Total no. of true positives per 100 test results	Additional[a] no. of true positives per 100 test results	Average cost per true positive sample detected (£)	Incremental cost per additional true positive sample detected (£)	Total no. of positives undetected (false negatives)	Total no. of positives wrongly detected (false positives)
Antigenaemia (pp65): screening regime	3217	0	1.3	0	2475	–	2.7	0
NASBA, Organon Teknika: screening regime	3389	+172	2.2	+0.9	1540	+191	0.9	0.7
Roche Amplicor Assay PCR: screening regime	2972	–245	1.6	+0.3	1858	–817	1.0	1.6
Nested in-house PCR (single-round): screening regime	1191	–2026	2.3	+1.0	518	–2026	0.6	0.6
Nested in-house PCR (two-round): screening regime	1537	–1680	2.5	+1.2	615	–1400	0.4	7.5

[a] Relative to pp65 antigenaemia.
[b] Full regime in which diagnostic tests ordered as part of screening regime are included.

Appendix 17

Inclusion of non-test (associated) NHS costs in cost-effectiveness analysis of UHW CMV screening strategies

TABLE 86 *Renal patients: maximum associated/concomitant cost consequences*

Type of treatment/investigation	Cost per treatment/ investigation (£)	Max. cost associated with false positives (£)		Max. cost associated with false negatives (£)	
		Per 100 diagnostic tests	Per 100 screening tests	Per 100 diagnostic tests	Per 100 screening tests
CMV drug treatment (ganciclovir)	–612[a]	857	3060	–	–
X-rays	11.88	0	0	2.5	0
Bronchoscopy	712.522	142.5	0	142.5	0

[a] Based on mean cost per 3-week course of i.v. ganciclovir treatment in UHW during the study period.
[b] Assumed that will not require additional inpatient stay.

TABLE 87 *Haematology patients: maximum associated/concomitant cost consequences*

Type of treatment/investigation	Cost per treatment/ investigation (£)	Max. cost associated with false positives (£)		Max. cost associated with false negatives (£)	
		Per 100 diagnostic tests	Per 100 screening tests	Per 100 diagnostic tests	Per 100 screening tests
CMV drug treatment (ganciclovir)	612[b]	1163	1040	–	–
IVIg	3300[c]	1980	1320	0	660
X-rays	11.88	9.5	11	5	2.5
CT/MRI/US	92[d]	37	18	37	0
Bronchoscopy	712.52[e]	427.5	570	0	0

US, ultrasonography.
[a] Cost per test result (batch size 5 and allowing for test failures). Range: nested in-house PCR (one-round) £11.91 to NASBA £33.89.
[b] Based on mean cost per 3-week course of i.v. ganciclovir treatment in UHW, during the study period.
[c] Cost of IVIg treatment varies between patients and between indications; assumes treatment is 5 doses of 300 g, cost £3300 (UHW prices).
[d] Based on breakdown of imaging reported (mainly CT) and UHW imaging costs.
[e] Assumed that will not require additional inpatient stay.

TABLE 88 *Renal patients: associated/concomitant cost consequences of different CMV screening regimes*[a]

CMV screening/diagnostic test regime	Total associated cost[b] per 100 tests (£)	Renal: type of secondary impact (per 100 tests)					
		Ganciclovir[c]		X-rays		Bronchoscopy	
		False positives associated cost (£)	False negatives associated cost (£)	False positives associated cost (£)	False negatives associated cost (£)	False positives associated cost (£)	False negatives associated cost (£)
Antigenaemia (pp65): diagnostic testing only	1159	0	n/a	0	19	0	1140
Antigenaemia (pp65)	268	122	n/a	0	2.5	0	143
NASBA, Organon Teknika	245	245	n/a	0	0	0	0
Roche Amplicor Assay PCR	1040	1040	n/a	0	0	0	0
Nested in-house PCR (single-round)	0	0	n/a	0	0	0	0
Nested in-house PCR (two-round)	4060	3917	n/a	0	0	143	0

[a] Include diagnostic tests ordered as part of screening regime.
[b] Associated/concomitant costs linked to false-positive and false negative test results.
[c] Excludes instances where patient prescribed ganciclovir when test requested.

TABLE 89 *Haematology patients: associated/concomitant cost consequences of different CMV screening regimes*[a]

CMV screening/ diagnostic test regime	Total associated cost[b] per 100 tests (£)	Haematology: type of secondary impact (per 100 tests)											
		Repeat tests[c]		Ganciclovir[d]		IVIg		X-rays		CT/MRI/US		Bronchoscopy	
		False positives associated cost (£)	False negatives associated cost (£)	False positives associated cost (£)	False negative associated cost (£)	False positives associated cost (£)	False negatives associated cost (£)	False positives associated cost (£)	False negatives associated cost (£)	False positives associated cost (£)	False negatives associated cost (£)	False positives associated cost (£)	False negatives associated cost (£)
Antigenaemia: diagnostic testing only	379	0	119	0	n/a	0	0	0	30	0	230	0	0
Antigenaemia	741	0	39	0	n/a	0	660	0	5	0	37	0	0
NASBA	88	3.5	20.5	61	n/a	0	0	2.5	0	0	0	0	0
Roche	3354	0	0	734	n/a	1320	660	12	2.5	55	0	570	0
PCR single-round	481	0	476	0	n/a	0	0	2.5	2.5	0	0	0	0
PCR two-round	6090	15.5	3	1714	n/a	3300	0	20	2.5	37	0	998	0

[a] Include diagnostic tests ordered as part of screening regime.
[b] Associated/concomitant costs linked to false-positive and false-negative test results.
[c] Cost of repeat CMV test. Based on same test and batch size of 5 samples allowing for observed level of test failure.
[d] Excludes instances where patient prescribed ganciclovir when test requested.

TABLE 90 *Cost-effectiveness of CMV screening regimes[a] for renal transplant patients (includes associated/concomitant costs)*

CMV screening/diagnostic test regime	Total direct and associated cost per 100 test results (£)	Additional[b] direct and associated cost per 100 test results (£)	Total no. of true positives per 100 test results	Additional[b] no. of true positives per 100 test results	Average cost per true positive sample detected (£)	Incremental[b] cost per additional true positive sample detected (£)	Total no. of positives undetected (false negatives)	Total no. of positives wrongly detected (false positives)
Antigenaemia (pp65)	3485	0	5.0	0	697	–	6.5	0.4
NASBA, Organon Teknika	3634	+149	7.4	+2.4	491	+62	2.3	1.1
Roche Amplicor Assay PCR	4012	+527	11.6	+6.6	346	+80	2.0	2.7
Nested in-house PCR (single-round)	1191	–2294	10.3	+5.3	116	-433	3.0	0.2
Nested in-house PCR (two round)	5597	+2112	12.8	+7.8	437	+271	0.4	10.1

[a] Relative to pp65 antigenaemia.
[b] Full regime in which diagnostic tests ordered as part of screening regime are included.

TABLE 91 *Incremental cost-effectiveness of CMV screening regimes[a] for haematology transplant patients (includes associated/concomitant costs)*

CMV screening/diagnostic test regime	Total direct and associated cost per 100 test results (£)	Additional[b] direct and associated cost per 100 test results (£)	Total no. of true positives per 100 test results	Additional[b] no. of true positives per 100 test results	Average cost per true positive sample detected (£)	Incremental[b] cost per additional true positive sample detected (£)	Total no. of positives undetected (false negatives)	Total no. of positives wrongly detected (false positives)
Antigenaemia (pp65)	3298	0	1.3	0	2537	–	2.4	0
NASBA, Organon Teknika	3477	+179	2.2	+0.9	1580	+199	0.9	0.7
Roche Amplicor Assay PCR	6326	+3028	1.6	+0.3	3954	+10093	1.0	1.6
Nested in-house PCR (single-round)	1672	–1626	2.3	+1.0	727	–1626	0.6	0.6
Nested in-house PCR (two-round)	7627	+4329	2.5	+1.2	3051	+3608	0.4	7.5

[a] Full regime in which diagnostic tests ordered as part of screening regime are included.
[b] Relative to pp65 antigenaemia.

Appendix 18

Targeted CMV screening strategies: details of cost-effectiveness analyses

TABLE 92 *Renal patients: associated/concomitant cost consequences of different CMV 'high-risk' screening regimes*[a]

CMV screening/diagnostic test regime	Total associated cost[b] per 100 tests (£)	Renal: type of secondary impact (per 100 tests)					
		Ganciclovir[c]		X-rays		Bronchoscopy	
		False positives associated cost (£)	False negatives associated cost (£)	False positives associated cost (£)	False negatives associated cost (£)	False positives associated cost (£)	False negatives associated cost (£)
Antigenaemia (pp65): diagnostic testing only	1087	0	n/a	0	18	0	1069
Antigenaemia (pp65)	268	122	n/a	0	2.5	0	143
NASBA, Organon Teknika	306	306	n/a	0	0	0	0
Roche Amplicor Assay PCR	1285	1285	n/a	0	0	0	0
Nested in-house PCR (single-round)	0	0	n/a	0	0	0	0
Nested in-house PCR (two-round)	4988	4774	n/a	0	0	214	0

[a] Includes ordered as part of screening regime.
[b] Associated/concomitant costs linked to false-positive and false-negative test results.
[c] Excludes instances where patient prescribed Ganciclovir when test requested.

TABLE 93 *Haematology patients: associated/concomitant cost consequences of different CMV 'high-risk' screening regimes*[a]

CMV screening/ diagnostic test regime	Total associated cost[b] per 100 tests (£)	Haematology: type of secondary impact (per 100 tests)											
		Repeat tests[c]		Ganciclovir[d]		IVIg		X-rays		CT/MRI/US		Bronchoscopy	
		False positives associated cost (£)	False negatives associated cost (£)	False positives associated cost (£)	False negatives associated cost (£)	False positives associated cost (£)	False negatives associated cost (£)	False positives associated cost (£)	False negatives associated cost (£)	False positives associated cost (£)	False negatives associated cost (£)	False positives associated cost (£)	False negatives associated cost (£)
Antigenaemia: diagnostic testing only	504	0	161	0	n/a	0	0	0	39	0	304	0	0
Antigenaemia	2181	0	77	0	n/a	0	1980	0	14	0	110	0	0
NASBA	363	17	34	306	n/a	0	0	6	0	0	0	0	0
Roche	7495	0	0	2142	n/a	2310	2310	33	8	193	0	499	0
PCR single-round	14	0	7	0	n/a	0	0	0	7	0	0	0	0
PCR two-round	11726	29	9	3917	n/a	6270	0	30	7	110	0	1354	0

[a] Include diagnostic tests ordered as part of screening regime.
[b] Associated/concomitant costs linked to false-positive and false-negative test results
[c] Cost of repeat CMV test. Based on same test and batch size of 5 samples allowing for observed level of test failure.
[d] Excludes instances where patient prescribed ganciclovir when test requested.

TABLE 94 *Cost-effectiveness CMV 'high-risk' screening regimes[a] for renal transplant patients (includes associated/concomitant costs)*

CMV screening/diagnostic test regime	Total direct and associated cost per 100 test results (£)	Total no. of true positives per 100 test results	Average cost per true positive sample detected (£)	Total no. of positives undetected (false negatives)	Total no. of positives wrongly detected (false positives)
Antigenaemia (pp65): screening regime	3485	6.2	562	7.9	0.2
NASBA Organon Teknika: screening regime	3695	9.3	397	2.7	1.4
Roche Amplicor Assay PCR: screening regime	4260	13.6	313	2.4	3.3
Nested in-house PCR (single-round): screening regime	1191	12.2	98	3.6	0.3
Nested in-house PCR (two-round): Screening regime	6525	15.3	426	0.6	12.2

[a] Includes ordered as part of screening regime.

TABLE 95 *Cost-effectiveness of CMV 'high-risk' screening regimes[a] for haematology patients (direct test costs only)*

CMV screening/diagnostic test regime	Total direct and associated cost per 100 test results (£)	Total no. of true positives per 100 test results	Average cost per true positive sample detected (£)	Total no. of positives undetected (false negatives)	Total no. of positives wrongly detected (false positives)
Antigenaemia (pp65): screening regime	5398	4.2	1285	6.7	0
NASBA, Organon Teknika: screening regime	3752	7.7	487	2.1	1.3
Roche Amplicor Assay PCR: screening regime	10467	4.1	2553	3.5	4.2
Nested in-house PCR (single-round): screening regime	1205	7.1	170	1.2	0.6
Nested in-house PCR (two-round): screening regime	13263	7.1	1842	1.2	12.9

[a] Includes ordered as part of screening regime.

Appendix 19

Data points for *Figure 3*

id	tr_time	lnPtstay
254	0	16
288	0	14
369	0	12
329	0	16
332	0	11
247	0	9
337	0	13
231	0	21
225	1	25
243	1	12
221	1	9
256	1	12
211	1	17
252	1	10
273	1	13
334	1	16
318	2	11
306	2	12
218	2	16
320	2	11
389	2	11
384	2	11
236	2	16
201	2	10
310	3	23
316	3	18
335	3	13
336	3	15
246	3	14
297	3	13
394	3	18
266	3	28
354	3	18
277	5	19
290	5	24
321	5	11
319	6	14
307	6	10
398	6	13
352	6	12
377	6	17
330	6	14
300	6	15
207	7	36
312	7	12
333	7	12
358	7	13
235	7	13
278	7	13
294	8	15
260	8	12
375	8	12
208	8	9
292	8	15
323	8	19

id	tr_time	lnPtstay
242	8	20
289	9	15
342	9	21
340	10	14
350	10	14
283	11	24
309	11	11
341	11	24
262	12	14
209	12	12
214	13	15
202	13	18
222	13	29
296	13	12
315	13	33
210	14	21
285	14	22
223	14	11
357	14	14
276	15	28
264	15	13
215	15	18
366	15	22
280	15	10
374	16	16
259	16	
213	16	23
295	16	16
387	16	12
355	16	17
399	16	17
227	16	12
396	17	14
347	17	19
217	17	14
274	17	14
220	17	10
305	17	16
216	17	
230	17	11
286	18	15
379	18	22
205	18	53
258	18	57
239	19	21
390	19	16
371	19	16
382	19	14
331	20	14
304	20	16
206	20	65
284	20	16
212	21	23
271	21	15
293	21	16

id	tr_time	lnPtstay
326	21	19
219	21	9
229	21	21
228	21	
238	21	11
343	21	12
226	22	17
338	22	18
272	22	17
275	22	20
345	22	47
386	22	19
378	23	16
302	23	13
367	23	18
322	23	82
311	24	
224	24	22
282	24	22
373	24	9
385	24	23
344	24	6
265	24	
287	25	19
368	25	21
339	25	16
250	25	15
325	25	13
359	25	17
291	25	32
381	25	9
281	26	10
372	26	16
356	26	14
233	26	26
314	26	15
255	27	24
391	27	22
257	27	20
364	27	16
267	27	30
203	27	15
248	27	35
328	27	14
392	28	28
395	28	34
244	28	17
361	28	10
313	28	14
363	28	65
308	28	36
393	29	27
317	29	22
365	29	14
2001	37	24
2002	38	23
2003	38	29
2004	39	13
2005	39	27
2006	39	30
2007	39	26
2008	40	10
2009	41	14
2010	41	17
2011	41	14
2012	41	12
2013	41	17
2015	41	19
2016	42	45
2017	41	17
2018	43	17
2019	44	16
2020	44	12
2021	44	19
2022	44	53
2023	43	8
2024	45	12
2025	45	10
2026	45	16
2027	47	11
2028	48	14
2029	48	19
2030	48	11
2031	50	9
2032	50	11
2034	50	12
2035	50	11
2036	51	10
2037	51	13
2038	51	18
2039	51	18
2040	50	12
2041	53	9
2042	35	31
2043	53	9
2044	55	9
2045	54	11
2046	54	13
2047	54	16
2048	56	10
2049	55	20
2050	56	12
2051	56	10
2052	55	23
2054	55	18
2055	55	10
2056	54	17
2057	51	13
2058	51	10
2059	58	12
2060	57	10
2061	58	16
2062	57	12
2063	57	15
2064	58	10
2065	58	9
2066	59	20
2067	59	9
2068	59	11
2069	59	9
2070	59	10
2071	59	17
2072	59	9
2073	53	9
2074	60	8
2075	59	21

id	tr_time	lnPtstay
2076	61	8
2077	61	17
2078	59	12
2079	61	7
2080	61	19
2081	42	9
2082	60	17
2083	62	9
2084	62	14
2085	62	12
2086	62	15
2087	65	10
2088	65	13
2089	65	17
2091	63	26
2092	64	17
2093	65	20
2094	66	13
2095	66	11
2096	66	9
2097	66	10
2098	68	10
376	1	9
263	1	12
204	2	15
234	3	28
298	3	35
270	4	13
261	4	17
268	4	20

id	tr_time	lnPtstay
240	5	11
299	5	11
269	6	10
232	6	59
301	7	22
251	8	14
279	8	12
348	9	20
237	10	20
397	10	41
388	11	9
241	14	21
324	14	17
380	14	20
370	14	22
303	15	52
353	17	9
249	18	22
362	19	14
351	23	6
346	23	15
327	26	25
253	26	23
349	26	37
360	26	36
245	26	27
383	29	26
2014	42	55
2033	50	10
2053	56	14
2090	65	15

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We look forward to hearing from you.

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