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1. Title

The clinical accuracy and cost effectiveness of integrated multiplex PCR tests **(**xTAG Gastrointestinal Pathogen Panel, FilmArray Gastrointestinal Panel and Faecal Pathogens B assay) for rapidly identifying gastrointestinal pathogens in people with suspected infectious gastroenteritis

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3. Plain English Summary

Gastroenteritis is a common, short-lived disorder usually caused by infection with viruses, bacteria or parasites. Acute diarrhoea may occur which may be cared for in the community or in hospital.

Many microbiology laboratories use traditional culture and assay methods to identify the type of infection, although these tests can take up to 3 days to complete. Rapid diagnosis can be achieved using highly multiplex tests, which identify multiple viral, parasitic and bacterial pathogens directly from one stool sample. Such tests may increase laboratory costs but may reduce other costs arising from delays identifying the cause of infection.

To inform the decision whether the NHS should routinely use integrated multiplex tests for suspected infectious gastroenteritis, this assessment will evaluate the reliability, accuracy and cost-effectiveness of three integrated multiplex tests: The xTAG Gastrointestinal Pathogen Panel (Luminex), The FilmArray Gastrointestinal Panel (BioFire Diagnostics) and the The Faecal Pathogens B assay (AusDiagnostics). The performance and impact in certain higher risk groups such as infants, those travelling abroad, people with certain long-standing illnesses and those in hospital, will also be evaluated.

4. Decision problem

4.1 Purpose of the decision to be made

Gastroenteritis is a common, transient disorder usually caused by infection with viruses, bacteria or parasites. It is estimated that around 25% of people in the UK have a gastrointestinal infection each year (Tam et al. 2011). Gastroenteritis is characterised by acute onset of diarrhoea with or without vomiting (NICE Clinical Knowledge summary, 2015). Depending on the cause of the infection, the symptoms of gastroenteritis can take from a few hours to a few days to develop. The most commonly identified pathogens in England in the Second Infectious Intestinal Disease in the Community Study were norovirus, sapovirus, campylobacter and rotavirus (Tam et al. 2011). Gastroenteritis can also occur in people who are currently, or who have recently taken antibiotics. This is known as antibiotic associated diarrhoea, which is frequently caused by Clostridium difficile or less frequently by Clostridium perfringens, Staphylococcus aureus, Klebsiella oxytoca, Candida species and Salmonella species (Public Health England, 2014a).

Diarrhoea may have non-infectious causes such as inflammatory bowel disease and it is therefore desirable to be able to identify or rule-out infectious causes of gastroenteritis in people who present to health services with diarrhoea or vomiting. Differential diagnoses for gastroenteritis include non-gastrointestinal infections (for example pneumonia, urinary tract infection or HIV), irritable bowel syndrome, inflammatory bowel disease, coeliac disease, side effects of medications, endocrinopathy (for example diabetes or hyperthyroidism) and secretory tumours (NICE Clinical Knowledge Summary, 2015).

Patients who are in hospital and have suspected infectious diarrhoea may be nursed in an isolation bay or side room until an infection has been ruled out. Throughout this time they will be asked to remain in isolation and not enter other areas of the ward or hospital until members of a hospital infection control team have advised them otherwise. Procedures such as endoscopy may also be cancelled, if there is a risk of transmitting the infection. The use of rapid tests may therefore reduce the amount of time spent in isolation for some

patients. Additionally, for people presenting to primary care services who require faecal microbiology tests, the more rapid provision of test results may provide earlier information for people who are in regular contact with young children and older people to help them reduce the risk of transmission within their work or home environment. This could bring forward the timing of specific treatment for pathogens such as giardiasis or typhoid where empirical antimicrobial treatment is usual, as well as guide the type of treatment used in very ill or vulnerable patients where clinical assessment supports antimicrobial treatment. Early diagnosis may also modify exclusion advice to reduce risk of spread if typhoid, verocytotoxin producing pathogens or cryptosporidiosis are detected since these diagnoses can lead to prolonged exclusion.

The NICE Diagnostic Advisory Committee (DAC) will provide guidance to the NHS about the use of the named integrated multiplex tests for patients with acute diarrhoea with or without vomiting, thought to be due to infective gastroenteritis. To inform the DAC, the external assessment group (EAG) will provide an assessment of the clinical accuracy and cost effectiveness of the named multiplex panels as a replacement or adjunct for standard assessment procedures. The potential value of the multiplex tests is in rapidly determining the presence and nature of infection, which may be bacterial, viral or parasitic**.**

4.2 Clear definition of interventions

Three integrated multiplex tests will be evaluated as interventions: xTAG GPP (Luminex), FilmArray (Biofire Diagnostics) and Faecal Pathogens B (AusDiagnostics). Clinical judgment is used when interpreting multiplex test findings and may be further informed by current routine tests or other confirmatory testing.

i. xTAG Gastrointestinal Pathogen Panel

The xTAG Gastrointestinal Pathogen Panel (Luminex) is a CE marked qualitative highly multiplexed PCR test for the simultaneous detection and identification of nucleic acids from up to 15 gastroenteritis-causing viruses, parasites and bacteria (see Table 1). It can analyse human stool samples that are fresh, frozen, or in holding medium, and the results should be used in conjunction with other clinical and laboratory findings. It is intended to be used in a laboratory setting.

The assay uses reverse transcription polymerase chain reaction (RT-PCR) and the procedure includes 5 phases:

- Pre-treatment of the sample
- Nucleic acid extraction and purification using an automated nucleic acid extraction system
- Broad-range PCR reaction using a thermal cycler
- Bead hybridization and detection using a thermal cycler
- Data acquisition and analysis (using Luminex 100/200 or MAGPIX analyser)

Ten microlitres (µl) of purified sample is required for the initial broad-range PCR reaction which amplifies nucleic acids that are present in the sample. 5µl of the broad range PCR products is then added to a hybridization and detection reaction, where target nucleic acids bind to species specific tagged beads Where pathogen nucleic acid is present, fluorescence is emitted by a streptavidin and R-Phycoerythin conjugate which is included in the reaction.

Fluorescence intensity is measured by either the Luminex 100/200 or MAGPIX analyser to determine which bacterial, viral, or parasitic DNA is present in the sample. Positive and negative controls should be included in each test run. The company recommends that 3 negative controls (RNase-free water) and at least 1 positive control (known positive samples) should be included in each run. The assay also contains an internal control which is added to each sample prior to extraction and indicates whether the assay is functioning as intended.

The estimated turn-around time for the xTAG Gastrointestinal Pathogen Panel is 5 to 6 hours, including sample preparation time. Up to 96 samples (including controls) can be processed in one run, depending on the capacity of a laboratory's PCR thermo cyclers. The test does not provide any information on antimicrobial resistance genes or antimicrobial susceptibility.

Table 1: Pathogens detected by the xTAG GPP assay

Bacteria and bacterial toxins	**Viruses**
Campylobacter	Adenovirus 40/41
Clostridium difficile, Toxin A/B	Norovirus GI/GII (genogroup)
Escherichia coli O157	Rotavirus A
Enterotoxigenic *E. coli* (ETEC) LT/ST	
Shiga-like Toxin producing *E. coli* (STEC) stx1/stx 2	**Parasites**
Salmonella	*Cryptosporidium*
Shigella	*Entamoeba histolytica*
Vibrio cholerae	*Giardia*
Yersinia enterocolitica	

ii. FilmArray Gastrointestinal Panel

The FilmArray Gastrointestinal Panel (BioFire Diagnostics) is a CE marked qualitative highly multiplexed PCR test which can simultaneously detect and identify up to 22 pathogens (see Table 2) from stool samples in Cary Blair transport media. It is intended for use within a clinical laboratory and should be used in conjunction with other clinical and laboratory findings.

The FilmArray Gastrointestinal Panel is intended for use with FilmArray and FilmArray 2.0 integrated systems, which include automated sample preparation. The FilmArray system can process 1 sample per hour, and the FilmArray 2.0 system allows several FilmArray systems to be linked to process up to 8 samples per hour depending on how many modules are purchased (1 sample per hour per module). All reagents required for sample preparation, reverse transcription, PCR and detection are provided freeze-dried in a single use pouch. Prior to inserting the reagent pouch into the analyser the sample is combined with sample buffer and is injected into the pouch along with a hydration solution. The system automatically processes a sample through the following stages once a pouch has been inserted:

- Nucleic acid purification
- Reverse transcription and multiplex PCR
- Second stage 'nested' PCR with species specific primers
- Detection with melting curve analysis

The system extracts and purifies nucleic acids which then undergo reverse transcription and

are amplified in the first broad-range PCR reaction. A second nested PCR reaction containing species-specific primers is run to detect and identify any pathogens present in the sample by fluorescence. Each single use pouch also contains two internal controls, one RNA process control assay and one control assay for the second stage PCR. Both controls must be positive for the sample to be reported. Results are reported automatically using the FilmArray software. The estimated test turn-around time is 1 hour.

Table 2: Pathogens detected by the FilmArray GI Panel

Bacteria	**Viruses**
Campylobacter (jejuni, coli and upsaliensis)	Adenovirus F 40/41
Clostridium difficile (toxin A/B)	Astrovirus
Plesiomonas shigelloides	Norovirus GI/GII
Salmonella	Rotavirus A
Yersinia enterocolitica	Sapovirus (I, II IV and V)
Vibrio (parahaemolyticus, vulnificus and *cholerae)*	
Vibrio cholorae	**Parasites**
Enterroaggregative *E. coli* (EAEC)	*Cryptosporidium*
Enteropathogenic *E. coli* (EPEC)	*Cyclospora cayetanensis*
Enterotoxigenic *E. coli* (ETEC) lt/st	*Entamoeba histolytica*
Shiga-like toxin-producing *E. coli* (STEC) stx1/stx2	*Giardia lamblia*
E. coli O157	
Shigella/Enteroinvasive *E. coli (EIEC)*	

iii. Faecal Pathogens B (16Plex)

The Faecal Pathogens B assay (AusDiagnostics) is a CE marked highly multiplexed PCR test which can detect and identify up to 15 pathogens from nucleic acid extracted from fresh faecal samples. The pathogens detected by the assay are shown in Table 3. The assay is intended to be used in conjunction with the High-Plex Multiplex Tandem PCR system and Easy-Plex results software. The assay procedure includes the following processes:

- Nucleic acid extraction and purification
- Broad-range PCR (using High-Plex MultiPlex Tandem PCR system)
- Real-time PCR with species specific primers (using High-Plex MultiPlex Tandem PCR system)
- Detection with melting curve analysis

In the first PCR step broad range primers are used and the product of this reaction is diluted and divided in to a number of real-time PCR reactions, which use nested species-specific primers to detect and identify any pathogens present in the sample by fluorescence. Results are reported using the Easy Plex results software. Where multiple pathogens are present in a sample the software provides an indication of the relative quantitation between the targets which may allow the determination of the relative importance of each detected pathogen. Each tube used for the broad range PCR reaction includes an internal positive control (SPIKE), and the company advises that both positive and negative (water) controls are included in each run. Up to 24 samples can be processed in 1 run.

The estimated test turn-around time is 3 to 4 hours. The assay is intended to be used in conjunction with other clinical and laboratory findings.

Table 3: Pathogens detected by the Faecal Pathogens B assay

Bacteria	**Viruses**
Salmonella spp.	Rotavirus A
Shigella spp. and EIEC	Norovirus genogroup I and II
Campylobacter spp.	Adenovirus group F and group G
Clostridium difficile	Sapovirus
Shiga toxin 1 and 2	Astrovirus
Escherichia coli O157	
	Parasites
	Giardia lamblia (18s)
	Cryptosporidum (parvus and *hominis)*
	Entamoeba histolytica (not *dispar)*

4.3 Populations and relevant subgroups

The primary population is patients with suspected infectious gastroenteritis within community and hospital settings. Potential subgroups evaluated may include: patients in the community; patients in hospital; young children; and patients who are immunocompromised.

i. Management in hospitals

A key aspect of infection prevention and control in hospital is isolation or barrier nursing. Isolation and barrier nursing are often advised where it is desirable to stop infections spreading to other patients or to staff. It may also be advised for people who are immunocompromised to protect them from acquiring an infection whilst in hospital (reverse barrier nursing). Isolation nursing involves the patient being nursed in single room or side room of a ward. For suspected infectious diarrhoea and vomiting it is often advised that the patient is nursed in isolation until either negative microbiology results are available or the patient has been symptom free for 48 hours. Patients in isolation will be asked to remain in the room and not enter other areas of the ward or hospital until they have been advised otherwise by members of a hospital infection control team. Where side rooms are not available, barrier nursing may be undertaken on the main ward, but extra precautions are taken, for example staff wearing protective clothing such as gloves, apron and mask, to prevent the spread of an infection. Cohort nursing may also be employed where several patients who have been identified as having the same infection, for example Clostridium difficile, are nursed together in the same bay.

Where infection control measures are advised for a patient, some procedures which may not be classed as urgent, for example endoscopy, may be postponed until the infection has resolved. Communicable infections which may require isolation or barrier nursing are not restricted to gastrointestinal infections such as Clostridium difficile and norovirus and include infection with methicillin resistant staphylococcus aureus, and extended spectrum beta-lactamases, that is, E. coli and klebsiella that are resistant to penicillin and cephalosoporin antibiotics.

ii. Management in the community

Where infectious gastroenteritis is suspected in the community, people are often advised to absent themselves from work or in the case of children, from schools and nursery (Public

Health England, 2014c). Advice is also given on reducing the risk of transmission, particularly where highly transmissible pathogens such as norovirus and shigella are suspected. Infectious gastroenteritis can have particular implications for people in certain professions such as people who handle food and healthcare workers. Food handlers are typically advised to remain away from work until 48 hours after symptoms have resolved, however infections with certain pathogens, including Salmonella Typhi or Paratyphi, and E coli O157 may require negative microbiology results before the person is able to return to work (Food Standards Agency, 2009). In some cases the detection of suspected food-borne pathogens may result in public health teams initiating an outbreak investigation.

4.4 Place of the intervention in the treatment pathway(s)

The main clinical feature of gastrointestinal infection is diarrhoea, but other symptoms can include: nausea, sudden onset of vomiting, blood or mucus in stool or systemic features such as fever or malaise (NICE Clinical Knowledge Summary, 2015). In acute cases, diagnostic investigations are needed to confirm that an infection is present or to determine the causative pathogen. It is recommended that stool samples for microbiological diagnosis are taken when:

- There is persistent diarrhoea or malabsorption
- When there is blood, mucus or pus in the stool
- When there is a history of diarrhoea and/or vomiting, and the patient is systemically unwell
- When there is a history of recent hospitalisation
- When there is a history of antibiotic therapy

Where parasitic infections are suspected it is recommended that 3 samples are sent, 2 to 3 days apart as ova, cysts and parasites are shed intermittently (NICE Clinical Knowledge Summary, 2015). For hospital acquired gastroenteritis and diarrhoea, hospitals may employ three-day rules when deciding whether to send stool samples from inpatients to microbiology, although testing for Clostridium difficile should be done as soon as infective diarrhoea is suspected. The rule suggests that stool samples should not be sent to microbiology unless:

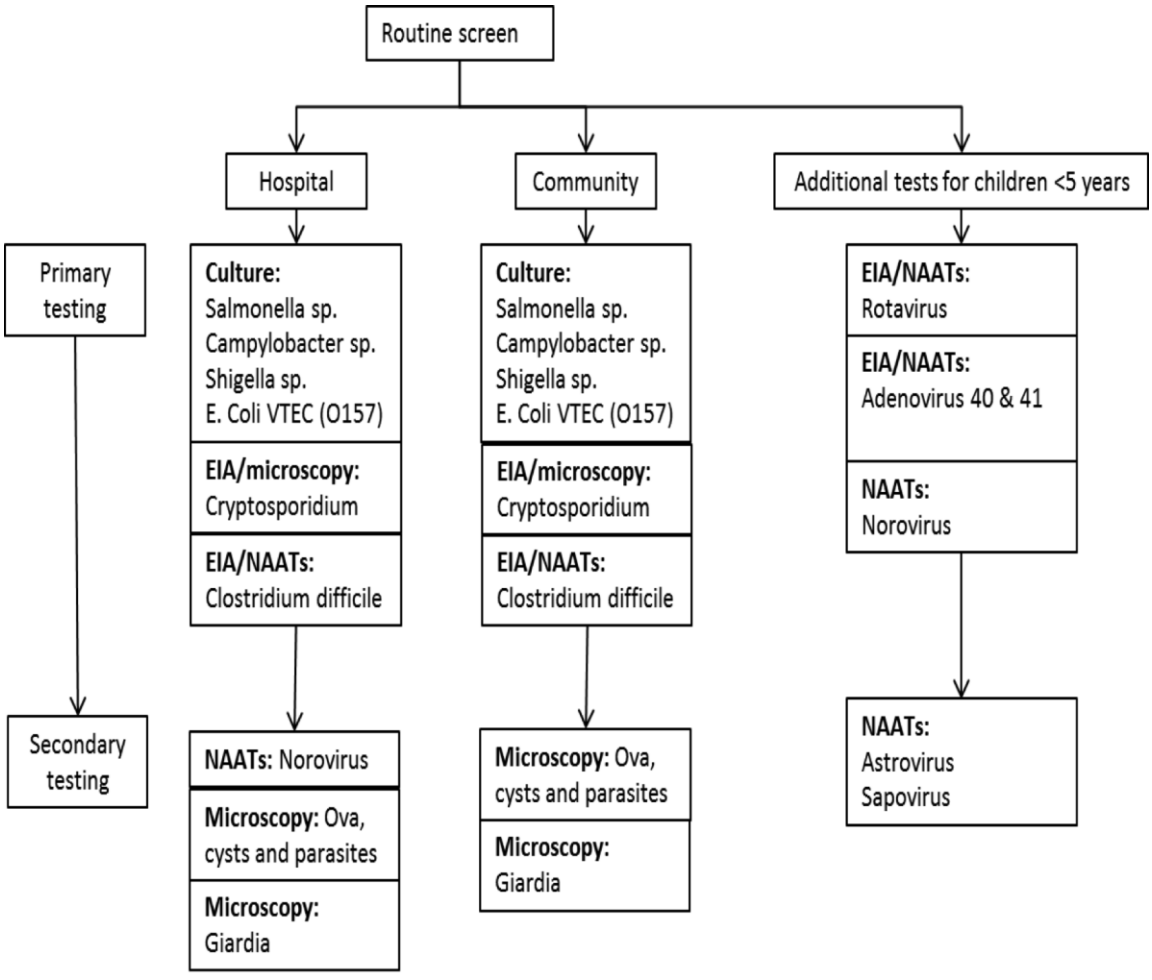
- Diarrhoea developed within 3 days of admission
- For adults with nosocomial diarrhoea if one of the following is present:
- Aged 65 or more with pre-existing disease causing permanently altered organ function
- HIV positive
- People with neutropenia
- Suspected nosocomial outbreak (e.g. Salmonella)
- Those with suspected non-diarrhoeal manifestations of enteric infections

Public Health England (PHE) advise that no infection-specific treatment is warranted in most patients. Management concerns strategies to maintain hydration and steps to prevent cross-infection. The setting for multiplex testing will include patients from community and hospital settings, with be microbiology laboratories receiving samples from primary and secondary care services. The use of findings will be in their clinical settings.

4.5 Relevant comparators

The comparator will be standard microbiology techniques, outlined in the Public Health England syndromic algorithm for routine testing in cases of Gastroenteritis and Diarrhoea (see Figure 1). People who have a history of recent travel (to areas other than Western Europe, North America, Australia or New Zealand) have additional primary testing for *Vibrio* and *Plesiomonas* species by bacterial culture. A two staged testing approach is currently recommended for *Clostridium difficile* which involves an initial testing step using either a nucleic acid amplification test or enzyme immunoassay for glutamate hydrogenase. Where the initial test is positive, a sensitive toxin enzyme inmmunoassay should be done to detect the presence of the toxins, which cause illness (Department of Health, 2012). The syndromic algorithm also notes that laboratories may opt to test for Norovirus only during cooler months when the peak incidence occurs (November to April). Blood cultures may also be taken if a patient is systemically unwell (Public Health England, 2013).

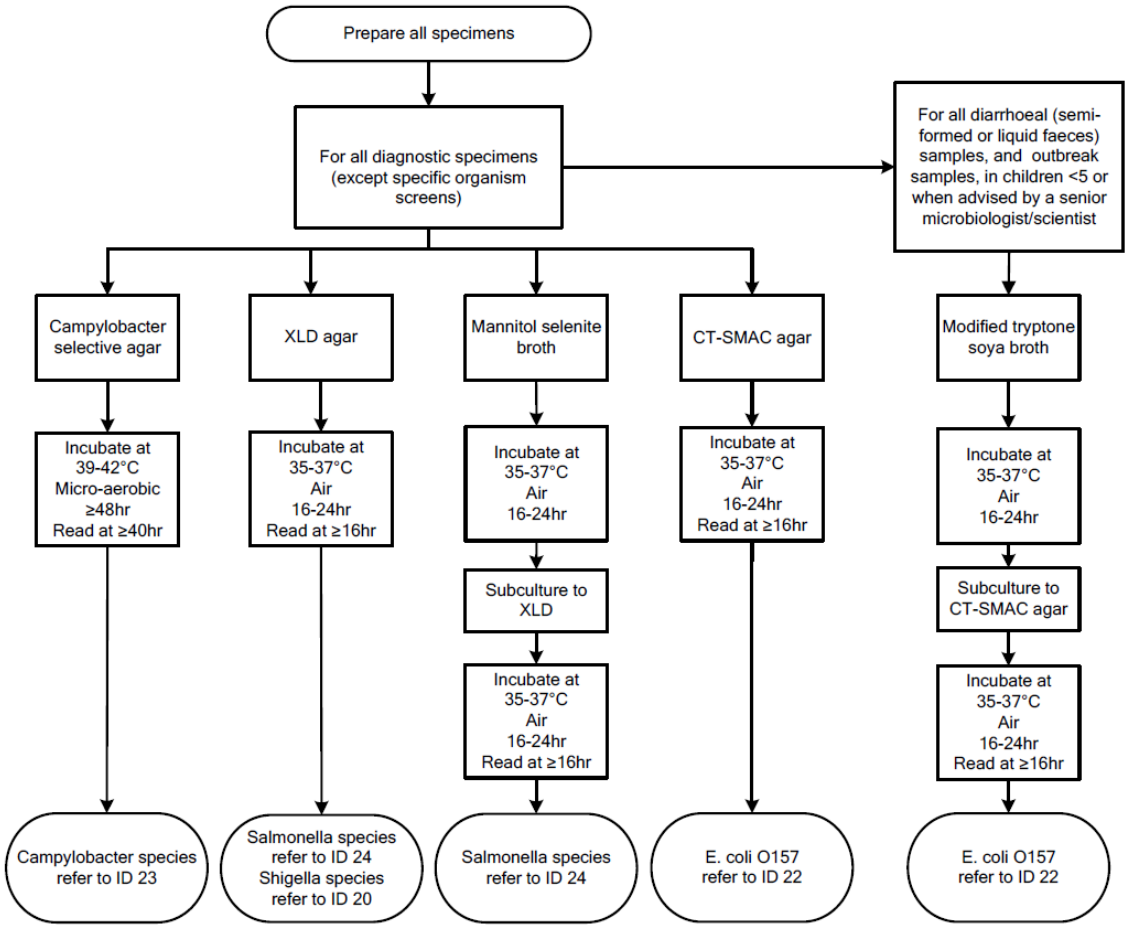
Figure 1: Current laboratory pathway for routine screening of stool samples for people with diarrhoea and vomiting (Source: Public Health England (2013)



Where bacterial culture is done, multiple types of media may be required, and it may take up to 3 days for incubation and pathogen detection. The current bacterial culture protocols recommended by Public Health England for the investigation of faecal specimens for routine bacterial pathogens (Public Health England, 2014b) are shown below in Figure 2. The standards also note that rapid diagnostic tests for the direct identification of bacteria

directly from faeces such as enzyme immunoassays and PCR are available. These tests are thought to be highly accurate for *Salmonella*, *Campylobacter* and *E. coli* O157, but less data is available on their effectiveness for detecting toxin producing bacteria such as *C. perfringens*, *Bacillus* species and *S. aureus* (Public Health England, 2014b).

Figure 2: Current laboratory pathway for routine screening of bacterial pathogens for people with diarrhoea and vomiting (Source: Public Health England (2013)



Issues to be considered regarding the standard algorithm comparator

It is apparent that standard microbiology techniques as described in the standard algorithm cannot provide a reference standard to evaluate multiplex tests.

- First-line tests are not 100% accurate and may require confirmatory PCR assays in the case of diagnostic doubt.
- A range of secondary PCR tests are used across the NHS. These cannot be included as comparators as they are neither used widely nor consistently across the NHS.
- Multiplex tests may identify substantially higher levels of certain pathogens than standard microbiology. Whether these additional findings are either correct or important (toxin-bearing) needs to be determined by an external reference standard.
- Scope to make traditional diagnostic accuracy assessments depends upon identifying evidence for both interventions and comparator against a common and adequate

reference standard

4.6 Key factors to be addressed

The appraisal will determine outcomes, costs and cost-effectiveness of multiplex tests as available evidence and scope for modelling permits.

Intermediate measures considered may include: diagnostic accuracy; discordant results with standard microbiology tests; time to test results; test failure rates; changes to treatment and management plans; changes to infection control decisions; length of stay in isolation rooms; and duration of barrier or cohort nursing

Clinical outcomes considered may include morbidity and mortality. Patient-reported outcomes considered may include health-related quality of life.

Costing will take an NHS and Personal Social Services perspective. Costs considered may include: costs of equipment, reagents and consumables and autoclaving of culture plates; costs of staff and associated training; costs associated with treatment; medical costs arising from testing and care such as hospital stay and isolation room use; medical costs arising from adverse events, including those associated with false test results and inappropriate treatment.

If evidence permits, cost-effectiveness of interventions may be expressed in terms of incremental cost per quality-adjusted life year, taking a time horizon sufficiently long to reflect any differences in costs or outcomes between the technologies being compared. If such modelling is infeasible a simple costs and consequences framework may be necessary, as has been the case for previous researchers working in this field.

Additionally, evidence for the public health impact of replacing or supplementing standard testing with panel testing may be described.

4.7 Areas outside the scope

Agreed areas at the scoping workshop, outside the scope of the assessment, included the value of other modular or partial multiplex tests; diagnosis during outbreaks; and routine management of chronic conditions.

The technologies included in the scope for this assessment were identified and discussed, with stakeholders and specialist committee members during scoping. Integrated multiplex PCR systems, which simultaneously detect bacteria, parasites and viruses, and have a similar clinical purpose to the xTAG Gastrointestinal Pathogen Panel have been included. NICE is aware that separate multiplex PCR assays for detecting bacteria, viruses and parasites are currently in use in some NHS laboratories; however, it was determined that there is variation in how the multiplex assays are used, meaning they cannot considered current practice and serve as comparators in this assessment.

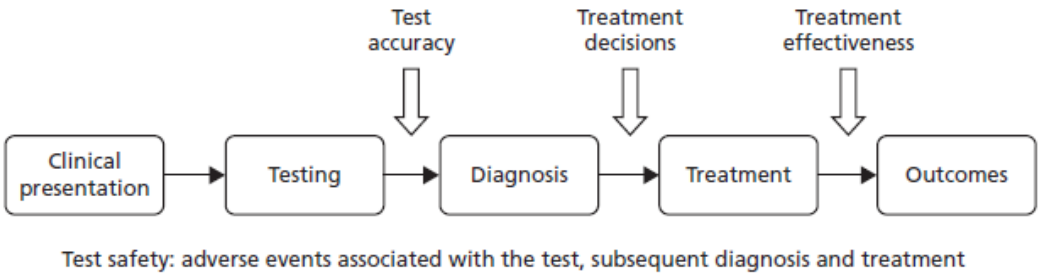
5. Methods for assessing outcomes arising from the use of multiplex tests

For the xTAG Gastrointestinal Pathogen Panel, FilmArray Gastrointestinal Panel and Faecal Pathogens B the assessment will: systematically review evidence for clinical-effectiveness;

systematically review existing economic evaluations; and develop a *de novo* economic model to assess cost-effectiveness.

Initial review scoping has identified no end-to-end studies that might characterize the value of multiplex testing in the patient population. Thus, assessment will require a systematic review of the determinants of clinical and cost-effectiveness and seek to link these determinants through modelling (see Figure 3).

Figure 3: Determinants of the clinical effectiveness of diagnostic technologies: Medical Services Advisory Committee (MSAC), 2005



5.1 Population

Use of multiplex testing will be evaluated for patients with acute diarrhoea with or without vomiting, thought to be due to infective gastroenteritis, with test referrals from hospital and community. Subgroups evaluated may include: people in the community; people in hospital; children aged younger than 5 years; people with recent foreign travel; and people who are immunocompromised. Clinicians may request more tests and empirical treatment in these subgroups.

5.2 Interventions

xTAG GPP (Luminex), FilmArray (Biofire Diagnostics) and Faecal Panel B (AusDiagnostics) will be evaluated.

5.3 Comparators

The comparator will be standard microbiology techniques, outlined in the Public Health England syndromic algorithm for routine testing in cases of Gastroenteritis and Diarrhoea.

Panel tests may replace or be used in conjunction with:

- Faecal culture
- Microscopy
- Enzyme immunoassays
- Nucleic acid amplification tests

The current Public Health England (PHE) algorithm serves as the extant standard of diagnosis in England although it is not always followed by clinicians or used as a comparator in published

studies. All intervention and comparator tests are used in conjunction with clinical judgement.

Findings will be reported for all pathogens tested by the three intervention tests. However, comparative performance will be limited to the common pathogens required within the PHE algorithm.

i Reference Standard

A complexity of evaluating multiplex testing lies in determining whether an adequate reference standard can be identified from published studies. For example, if discrepant pathogen results within a study comparing intervention and comparator are re-evaluated using single PCR (to deal with potential multiplex issues) and test a different genome sequence (to avoid duplication) then this might provide a pragmatic reference standard. Such pragmatic reference standards may vary between studies and will be different for the different pathogens under consideration. If not, it may be possible to compare intervention and comparator using a 'fair umpire' test using some measure of exposure or outcome, which characterizes the discordant results (Glasziou et al., 2008). Finally, summary measures may be constrained to levels of agreement, such as kappa statistics, and traditional test accuracy measures may not be possible.

5.4 Outcomes

The following intermediate, clinical and patient outcomes as well as costs will be subject to systematic review of original studies.

Intermediate measures for consideration may include:

- Diagnostic accuracy
- Discordant results with standard microbiology tests
- Time to test result
- Test failure rates
- Changes to treatment and management plans
- Changes to infection control decisions
- Length of stay in isolation rooms
- Length of hospital stay
- Duration of barrier or cohort nursing

Clinical outcomes for consideration following diagnosis may include:

- Morbidity (from the underlying condition and treatment side-effects)
- Mortality

Patient-reported outcomes for consideration following diagnosis may include:

- Health-related quality of life

Costs will be considered from an NHS and Personal Social Services perspective. Costs for consideration may include:

- Cost of equipment, reagents and consumables, and autoclaving of culture plates
- Cost of staff and associated training
- Costs associated with treatment
- Medical costs arising from testing and care such as hospital stay and isolation room use

- Medical costs arising from adverse events, including those associated with false test results and inappropriate treatment.

5.5 Study design

We will apply a common framework for determining the clinical effectiveness of diagnostic technologies (see Figure 3). We will systematically assess available evidence at each stage, and assess modelling methods by which the stages may be linked epidemiologically. Reviews will follow standard methods as described by Centre for Reviews & Dissemination (CRD): CRD's guidance for undertaking reviews in health care: Systematic Reviews (3rd Edition), 2008.

Any clinical diagnostic accuracy study that compares interventions with the comparator or an adequate reference standard will be included. Systematic reviews will be retrieved in order to check their reference lists for potentially relevant studies

5.6 Search strategy

The Medline search strategy below will be run and adapted as appropriate for other databases to search for clinical effectiveness studies (See Appendix 1). Epidemiological and interventional studies for acute diarrhoea may also be identified to inform modelling strategies (see Appendix 2).

Literature will be identified from the following sources

- Biomedical databases including MEDLINE, EMBASE, Cochrane Library, Web of Science, CRD databases (DARE, NHS EED and HTA), CEA database.
- Web sites (including NICE, Public Health England, FDA, manufacturers).
- Grey literature and meeting abstracts.
- Checking of reference lists.
- Contact with experts in the field.
- Research in progress databases including: Clinical trials.gov, UKCRN website, and WHO International Clinical Trials Registry Platform (ICTRP).

Additionally completed and ongoing studies will be identified by searches of the following registries:

- NIH ClinicalTrials.gov (http://www.clinicaltrials.gov/)
- Current Controlled Trials (http://www.controlled-trials.com/)
- WHO International Clinical Trials Registry Platform (ICTRP) (http://www.who.int/ictrp/en/)

All Identified references will be downloaded in a bibliographic database for further assessment and handling. References in retrieved articles will be checked for additional studies.

i. Inclusion criteria

Studies will be included that meet the criteria for population, target condition, interventions, comparator (complete, partial or selected) and/or reference standard, setting and outcomes. Studies will also be included which directly compare intervention tests. Inclusion will be restricted to studies published in the English language and using fresh or frozen stool samples. Full text papers and conference abstracts published from 2000 onwards will be retrieved,

reflecting CE marking and publication of evidence.

ii. Exclusion criteria

Reviews of studies will not be included in the analysis, but will be used to identify additional studies. The following study types will be excluded: biological studies, case reports, narrative reviews, editorials and opinions, poster presentations without supporting abstracts, non-English language reports, reports published as meeting abstracts only with insufficient details to permit critical appraisal. Studies limited to use of 'spiked' samples, swab-testing or non-representative populations will not be included. Studies using other modular or partial multiplex tests, using the index tests during outbreaks or for the routine management of chronic conditions will be excluded

Details of all full text excluded papers (including non-English language citation) will also be provided in the review.

5.7 Data extraction strategy

Selection of studies and extraction of study findings will be conducted independently by two reviewers using predesigned and piloted data extraction forms. Disagreements between reviewers will be resolved by consensus. The form will collect information on study design, methods, participants, testing procedures and test accuracy. Two reviewers will enter extracted data from selected studies independently into separate Excel spreadsheets. Reviewers will not be blinded to the names of study authors, institutions or publications. Where raw outcome data cannot be extracted directly, authors will be contacted.

5.8 Quality assessment strategy

A single reviewer will determine the methodological quality of included studies and a second reviewer will check findings. Any disagreements will be resolved by consensus or arbitration.

The methodological quality of included studies will be assessed according to the (possibly modified) QUADAS tool (Whiting *et al.,* 2011).

5.9 Methods of analysis/synthesis

The External Assessment Group (EAG) may apply a range of statistical methods according to available data and the decision problem.

As evidence permits, subgroups evaluated may include: people in the community; people in hospital; children aged younger than 5 years; people with recent foreign travel; and people who are immunocompromised.

If traditional measures of test accuracy can be determined then we will use Review Manager, using its section for diagnostic reviews, to generate coupled forest plots and ROC curves. We will also use MedCalc for producing figures. RevMan may not provide all the statistical analysis required, if so Stata will be used for more complex analysis. If network meta-analysis is possible this will be performed using R-Studio and JAGS.

An important value of rapid testing is to rule out serious infections requiring specific treatment and/or isolation. Thus the key performance parameter will be high sensitivity, an intrinsic test value, which provides a contextual high negative predictive value (NPV). If appropriate, predictive values will be imputed from intrinsic values (sensitivity and specificity) at a range of prevalence values.

Results will take account of country of origin since the prevalence and spectrum of different species may vary considerably. Although reviews will not be constrained by nationality, industrialised country settings are likely to be most relevant.

Heterogeneity will be examined by visual inspection of coupled forest plots of sensitivity and specificity. Formal testing will differentiate variability due to chance (many diagnostic studies have small sample sizes) and variation due to study populations, study methods or design deficiencies. If possible, and as recommended in Leeflang et al. 2009, we will investigate and identify potential sources of bias and limit the effects of these biases on the estimates and the conclusions of the test accuracy using sensitivity analysis, subgroup analysis or meta-regression: STATA or RStudio/JAGS platforms will be used since meta-regression cannot be performed using Review Manager.

We will report statistics used in diagnostic test accuracy studies: sensitivity and specificity, positive and negative predictive values, likelihood ratios; and Receiver Operating Characteristic (ROC) curves.

If appropriate, we may also explore fitting random effects in hierarchical models: the hierarchical summary ROC model and the bivariate random effects model. These models give a valid estimation of the underlying ROC curve and the average operating point. Addition of covariates to the models, or application of separate models to different subgroups may enable heterogeneity to be explored. Both models can be fitted with STATA, fitting mixed models. Any such analyses will be exploratory to aid understanding of test performance; the assessment provided for the DAC will address CE-marked test cut-offs.

A potential problem lies with published studies in the use of inadequate reference standards to assess test accuracy. For example, when a multiplex test is being compared with current practice, the multiplex test may have greater accuracy and identify more pathology than current routine tests, but the additionally identified pathology may of uncertain importance. If it is not possible to define an adequate reference standard with which to interpret studies then we will report simple agreement statistics providing narrative interpretations of individual studies, and appropriate statistical summaries. Summary measures may be constrained to levels of agreement, such as kappa statistics, and traditional test accuracy measures may not be possible. In this instance we may adopt a ‘fair umpire’ approach of assessing the characteristics of the discordant test results, using an imperfect but unbiased alternative test (Glasziou et al., 2008). The kind of reporting will be determined by the design of studies.

5.10 Methods for estimating quality of life

We will search systematically for extant quality-of-life studies relating to the decision problem, using strategies and methods similar to the search for test performance (see Appendix 3). The clinical effectiveness search will be extended to identify economic and quality-of-life studies (see below). A search will also be made of the Cost Effectiveness Analysis (CEA) registry.

6. Methods for synthesising evidence of cost effectiveness

6.1 Identifying and systematically reviewing published cost-effectiveness studies

We will search systematically for economic analyses relating to the decision problem, using strategies and methods similar to the search for test performance. The review will include studies identified by the scoping review, i.e. Goldenberg et al. (2015), Pankhurst et al. (2014) and Abubakar et al. (2007). We will review all models identified, examining the scope to refine and update them. The structure of economic models, use of evidence and assumptions, and findings together with deterministic and probabilistic sensitivity analysis, will be critical appraised epidemiologically and against the broader available evidence base. Additionally we will search systematically for epidemiological models of outcomes (including quality of life) following treatment for acute diarrhoea, which might be linked to the evidence for the immediate effects of rapid testing.

6.2 Development of a health economic model

It is likely that there will be no published models available to address the decision problem, thus we will develop a *de novo* model. Estimates of the cost per QALY gained of interventions will be developed if evidence permits adequate robust modelling. Model development will follow NICE reference case recommendations (NICE 2011) where possible.

Initial scoping suggests a paucity of evidence upon which to extrapolate benefits of multiplex testing beyond the immediate index hospitalization. The only published decision model offering an extrapolation to one year, took a public health perspective, looking at reduction in cross infection as the source of benefit (Abubakar et al. 2007). The EAG note that the manufacturers have made neither claim nor present evidence for extended benefits or improved clinical outcomes. This is consistent with Public Health England advice that no infection-specific treatment is warranted in most patients. A final decision on the modelling approach will be made in the light of the clinical effectiveness findings. The time horizon of the model will be determined by evidence for differential morbidity associated with the interventions or comparator.

The EAG may draw on a range of modelling techniques and will finalise its methods when the evidence review is complete. Models will reflect net changes in intermediate, clinical and patient outcomes and costs as evidence allows. Modelling will use published utility values for conditions if available. If necessary published evidence will be augmented with elicitation of expert opinion from specialist advisors and/or the assessment sub-group (ASG) to characterise and value the consequences of multiplex testing.

Models of costing will include an NHS and Personal Social Services perspective, taking unit prices from routine NHS sources (NHS reference costs, Personal Social Services Research Unit (PSSRU) and the British National Formulary (BNF)).

NHS services and manufacturers (through NICE) may be approached to refine understanding and estimation of acquisition, maintenance, service configuration, training and running costs of interventions and the comparator.

Analyses will include an NHS and personal social services perspective. Models will be constructed in Excel. If cost/QALY modelling is not possible it may be necessary to summarise

value by presenting a cost and consequences analysis. Using this approach we will summarise the net costs of intervention and comparator approaches, exploring a range of parameters such as test performance, timed saved, scale of use, laboratory configuration, using deterministic and probabilistic sensitivity analyses and sub-group analyses as evidence allows.

7. Handling information from the companies

All data submitted by stakeholders will be considered if received by the EAG no later than 23rd March 2016. Data arriving after this date will not be considered. If the data meet the inclusion criteria for the review they will be extracted and quality assessed in accordance with the procedures outlined in this protocol.

Commercial in confidence data provided and specified as such will be highlighted in blue and underlined in the assessment report, followed by an indication of the relevant company name in brackets.

8. Competing interests of authors

The project team have no competing interests in connection with this assessment.

9. Timetable/milestones

Milestone	Date to be completed
Final protocol to NICE	22 December 2015
Progress report to NICE and NETSCC	23 March 2016
Draft assessment report to NICE	23 May 2016
Final assessment report to NICE	21 June 2016
1st Diagnostics Advisory Committee meeting:	20 July 2016
2nd Diagnostics Advisory Committee meeting:	21 September 2016

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11. Appendices

Appendices should be provided as appropriate for the nature of the assessment to be performed. These may include search strategies, extraction forms, ratings instruments, parameter lists, more detailed model structures, etc.

Appendix 1: Clinical effectiveness studies.

All databases will be searched from 1990 to the current date; there will be no language restrictions. Some studies not in English may be translated if they are assessed as useful, and if translation is available. Weekly auto-alerts will be run in Medline and Embase to identify evidence emerging during course of the review. Research in progress will be identified through ClinicalTrials.gov, UK Clinical Research Network Study Portfolio and the WHO (World Health Organization) Clinical Trials Search Portal. Inclusion and exclusion criteria will be refined for each search strategy following the scoping workshop.

1. exp Gastroenteritis/
2. exp *Diarrhea/
3. exp *Feces/
4. exp *Gastroenteritis/
5. exp *Gastrointestinal Diseases/
6. (gastrointestin* or stool* or enteric* or feces or faeces).tw.
7. 2 or 3 or 4 or 5 or 6
8. exp Molecular Diagnostic Techniques/
9. Multiplex Polymerase Chain Reaction/
10. (xtag or Luminex or Filmarray or biofire).tw.
11. ("Faecal Pathogens B" or "Faecal Panel B" or ausdiagnostics).tw.
12. (multiplex* adj4 (PCR or polymerase chain reaction or assay* or panel* or test*)).tw.
13. (gastrointestinal pathogen panel or gastrointestinal infection panel or diarrh?ea).tw.
14. 8 or 9 or 10 or 11 or 12 or 13
15. 7 and 14

Appendix 2: Epidemiological and interventional studies

The search strategy will include a search for acute diarrhea, identifying studies informing its natural history and treatment. Also for each pathogen where there are specific recommendations, a targeted search for trials of antibiotic treatment for that pathogen will be undertaken. The search strategy below will be used and adapted for specific pathogens.

1. exp *Diarrhea/
2. exp Disease Progression/
3. exp Epidemiology/
4. epidemiology.tw.
5. (natural history or (disease adj course) or clinical course or progression or (disease adj2 progress*)).tw.
6. 2 or 3 or 4 or 5
7. 1 and 6

8. randomized controlled trial.pt.
9. 1 and 8
10. (acute adj4 diarrh?ea).tw.
11. 9 and 10
12. 7 or 11

Appendix 3: Economic and quality-of-life studies

We will search systematically for extant economic and quality-of-life studies relating to the decision problem, using strategies and methods similar to the search for test performance.

16. exp Economics/
17. exp "Costs and Cost Analysis"/
18. Health Status/
19. exp "Quality of Life"/
20. exp Quality-Adjusted Life Years/
21. (pharmacoeconomic* or pharmaco-economic* or economic* or cost*).tw.
22. (health state* or health status).tw.
23. (qaly* or ICER* or utilit* or EQ5D or EQ-5D or euroqol or euro-qol or SF-36 or SF36 or SF-6D or SF-6D or SF6D or HUI).tw.
24. (markov or time trade off or TTO or standard gamble or hrql or hrqol or disabilit* or disutilit*).tw.
25. (quality adj2 life).tw.
26. (decision adj2 model).tw.
27. (visual analog* scale* or discrete choice experiment* or health* year* equivalen* or (willing* adj2 pay)).tw.
28. ("resource use" or resource utili?ation).tw.
29. (utility* adj2 (value* or index* or health or measure* or estimate*)).tw.
30. 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29
31. 15 and 30