NETSCC, HTA

15th September 2011

IGRA in Diagnostic Evaluation of Active TB ('IDEA')

RESEARCH PROTOCOL

Version *two* – 19th April 2011

MAIN SPONSOR: Imperial College London
FUNDER: **NIHR Health Technology Assessment (HTA)**
STUDY COORDINATION CENTRE: Imperial College London, St Mary's Campus

REC reference: 11/H0722/8 (North West London REC1)

Study Management Group

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Dr Peter Kelleher (Chelsea & Westminster Hospital)
Dr John Wiggins (Wexham Park Hospital)
Dr Martin Wiselka (Leicester Hospital)

Study Sites

The study will take place at the following eight (8) hospital NHS Trust sites:

- Chelsea & Westminster Hospital NHS Foundation Trust
  - PI: Dr Anton Pozniak
- Heatherwood & Wexham Park Hospitals NHS Foundation Trust
  - PI: Dr Sarah Menzies
- Imperial College Healthcare NHS Trust
  - PI: Dr Onn Min Kon
- Northwest London Hospitals NHS Trust
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- Oxford Radcliffe Hospitals NHS Trust
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- Royal Free Hampstead NHS Trust
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  - PI: Professor Derek Macallan
- University Hospitals of Leicester NHS Trust
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- Heart of England NHS Foundation Trust (covers Heartlands Hospital and Birmingham Chest Clinic)
  - PI: Dr Martin Dedicoat
- Sandwell and West Birmingham Hospitals NHS Trust (covering Birmingham City Hospital)
  - PI: Dr Naz Nathani

Study Coordination Centre

For general queries, supply of study documentation, and collection of data, please contact:

Study Coordinator: ****TBC**** (as this is a multi-centre investigation)
Address: ****TBC****

Position: ****TBC****

Tel: ****TBC**** E-mail: ****TBC****

Clinical Queries

Clinical queries should be directed to (****TBC****) who will answer or direct the query to the appropriate person.

Sponsor

Imperial College London is the research sponsor for this study. For further information regarding the sponsorship conditions, please contact the Research Governance Manager (Lucy Parker) at:

Joint Research Office, Room G02,
Sir Alexander Fleming Building,
Imperial College
Exhibition Road
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www.imperial.ac.uk/clinicalresearchoffice

Funder

The *NIHR HTA* has provided Project Grant funding for this study.

This protocol describes the above study and provides information about the procedures for entering participants. Every reasonable care has been taken in the drafting of this protocol. However, corrections or amendments may be necessary; any changes will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

This study will adhere to the principles outlined in the NHS Research Governance Framework for Health and Social Care (2nd edition). It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate, including Good Clinical Practice (GCP).

CONTENTS

1. STUDY SUMMARY

TITLE IGRA in Diagnostic Evaluation of Active TB ('IDEA')

DESIGN Prospective cohort study, analysing testing regimes for 1,012 *suspected active TB* volunteers.

AIMS The overall aim of this study is to compare the diagnostic accuracy of the *ELISpot* based (T-SPOT.TB) and *ELISA* (QunatiFERON-TB GOLD in-tube) based IGRAs, with conventional diagnosis of *suspected active TB*, in routine NHS clinical practice.

POPULATION Adults presenting with *suspected active TB* aged 16 years-of-age, or over.

ELIGIBILITY Adults (male and female) presenting with *suspected active TB* (aged 16 years-of-age, or over) without a history of TB medication.

DURATION 3 Years.

2. GLOSSARY OF ABBREVIATIONS

AE	Adverse Event
ATB	Active TuBerculosis
BAL	BronchoAlveolar Lavage
BCG	Bacille Calmette-Guérin
CD4	Cluster of Differentiation IV
CI	Confidence Interval
DNA	Deoxyribonucleic acid
DMC	Data Monitoring Committee
ELISA	Enzyme-Linked Immunoabsorbent Assay
GCP	Good Clinical Practice
HIV	Human Immunodeficiency Virus
HTA	Health Technology Assessment
IGRA	Interferon-Gamma Release Assay
LTBI	Latent TuBerculosis Infection
NHLI	National Heart and Lung Institute
NHS	National Health Service
NICE	National Institute for Health and Clinical Excellence
NIHR	National Institute of Health Research
M. tuberculosis	Mycobacterium tuberculosis
PBMC*s*	Peripheral Blood Mononuclear Cells
ROC	Receiver Operating Characteristic
SAE	Serious Adverse Event
SSA	Site Specific Assessment
TB	TuBerculosis
TSC	Trial Steering Committee
TST	Tuberculin Sensitivity Test
UK	United Kingdom
WHO	World Health Organisation

KEYWORDS

Active TB (ATB), Assay, BCG, Diagnostic Test, ELISA, ELISpot, IGRA, TuBerculosis (TB), TST.

3. INTRODUCTION

3.1 Research Aims

The aim of this study is to compare the accuracy of diagnostic strategies, including a comparison between existing interferon-gamma release assays (IGRAs) and conventional diagnostic testing. The role of IGRAs for patients (termed *'participants'* from here on) who have been diagnosed as having suspected active tuberculosis (ATB) will also be defined.

The study will determine sensitivity, specificity, positive and negative predictive values and likelihood ratios of the ELISpot-based IGRA and the ELISA-based IGRA for diagnosis of active tuberculosis, in routine clinical practice. The study will be measured against a validated pre-defined composite reference standard for active tuberculosis[1]. Next-generation IGRAs will be evaluated in parallel. The accuracy of these different testing strategies will be compared and an optimal testing algorithm defining the precise role of these technologies in the diagnostic work-up of suspected active tuberculosis in the NHS (National Health Service) will be developed. Health economic analysis, from an NHS perspective, will determine the cost-effectiveness of each testing strategy, following NICE (National Institute for Health & Clinical Excellence) guidelines.

3.2 Background to research

Globally, in 2007, an estimated 9.27 million incident cases of Tuberculosis (TB) indicated a strong and continued increasing trend in the burden of this infectious disease[2]. Over 8,000 cases of TB were reported in the UK in 2007 (incidence rate of 13.8 per 100,000 population) with the main burden of disease concentrated in specific urban areas; 39% of all cases were in London with the incidence in Leicester City at 67 per 100,000 population[3]. The majority of cases occurred in two distinct categories: in young adults and among those born outside of the United Kingdom (UK)[3]. Diagnosis of active TB disease is central to controlling the TB epidemic[1]. However, the poor speed and sensitivity of existing diagnostic tools delays diagnosis and treatment of active TB[3].

The risk of developing TB infection can be dramatically increased when a person is HIV (human immunodeficiency virus) infected[4, 5]. Infection with TB and HIV is commonly termed 'TB and HIV co-infection'. The severe nature of HIV in the presence of TB has led some to consider it "the cursed duet", mainly because of a combined duel effect upon the disease process, which can cause rapid and progressive health deterioration[6]. It has been estimated that in 2003, 8.3% of TB cases in England and Wales were co-infected with HIV[7] and in 2007, an estimated 1.37 million (15%) of incident TB cases globally were HIV-positive[2].

TB is caused by an active infection with *Mycobacterium tuberculosis* (*M. tuberculosis*). Reliable determination of infection status, whether active or latent, could accelerate diagnostic assessment by enabling rapid exclusion of TB. Current conventional methods of diagnosing active TB primarily rely upon the identification of bacilli through microscopy, nucleic acid amplification and culture which may take several weeks to confirm a diagnosis. While highly specific for *M. tuberculosis* these techniques have poor sensitivity. Clinical symptoms and radiological features indicative of TB, histology (where appropriate) and on occasion the use of the tuberculin skin test (TST) can also be used to determine infection status. However, the sensitivity of TST, which measures the delayed-type hypersensitivity response to intradermal inoculation of a crude mixture of *M. tuberculosis* antigens, is insufficient for reliable exclusion of TB from the differential diagnosis. Poor sensitivity, especially in HIV-infected

patients[8-14], often confounded by prior BCG (Bacille Calmette-Guérin) vaccination, can produce false-positive results.[15]

There is an urgent need to develop alternative diagnostic modalities, able to rapidly and reliably exclude the diagnosis of TB; improved diagnosis is essential to contain and reverse the rising global burden of this disease[3]. New blood tests, called interferon gamma release assays (IGRAs) have recently been developed to diagnose *M. tuberculosis* infection. IGRAs detect interferon gamma release from T cells (lymphocytes that play a key role in cell-mediated immunity) in response to *M. tuberculosis*-specific antigens, which are absent from the BCG vaccine and most environmental mycobacteria[16]. Test results are not confounded by previous BCG vaccination, conferring higher specificity than TST[17-20]. An important component to effective and efficient clinical diagnosis, results are available within 24 hours and do not require the patient to make a return visit. IGRA has been recommended by UK, European and North American guidelines to diagnose latent tuberculosis infection (LTBI) although its role in active TB remains unclear.

The sensitivity and specificity of IGRAs compared to TST in active TB have been compared in a large number of studies[21]. IGRAs are more specific than the TST for diagnosing *M. tuberculosis* infection and ELISpot is more sensitive than TST for diagnosing TB. Unfortunately, IGRAs, in common with TST, are unable to distinguish active from latent infection and the causal relationship produced is (currently) one of poor specificity for active TB. However, with an appropriately high sensitivity in active TB disease, IGRAs could potentially allow the rapid exclusion of active TB disease within 24 hours; notably, weeks before culture results would (typically) become available. The two types of commercially available IGRAs are the whole-blood enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immunospot assay (ELISpot) both utilising peripheral blood mononuclear cells (PBMCs). The whole-blood ELISA is available commercially as QuantiFERON-TB Gold In-tube (QFT-GIT, Cellestis, Carnegie, Australia) and the ELISpot is available commercially as T-SPOT.*TB* (Oxford Immunotec, Abingdon, United Kingdom).

Diagnostic accuracy of existing ELISpot-based IGRA and ELISA-based IGRA, have not been compared directly 'head-to-head' in suspected active TB in the UK, nor comprehensively assessed in immunosuppressed patients. There is uncertainty in the role and clinical utility of IGRAs in the diagnostic work up of suspected TB and the cost-effectiveness in this particular setting.

Key issues specific to this research study include:

A) The first study to compare the diagnostic accuracy of both types of IGRA with conventional diagnostic testing for active TB in the UK;

B) The largest cohort globally to-date for evaluation of IGRAs; and

C) The largest study evaluating IGRA in HIV-TB co-infection, globally. The results of this study will influence tuberculosis control and elimination policy here in the UK, in Europe and North America.

3.3 NIHR HTA report detailing outputs of the research

A comprehensive report for the NIHR HTA will be produced. This will include recommendations based upon the evidence generated in this study, on the accuracy and clinical utility of diagnostic strategies that include IGRAs, and how they compare to conventional diagnostic testing for the work-up of patients suspected of active TB. An evidence-based optimal testing algorithm that defines the role of IGRAs in the diagnostic work-up of suspected active TB in the NHS will be presented and the cost-effectiveness (from an NHS perspective) of each testing strategy will be quantified and compared. Recommendations will also be provided for TB patients co-infected with HIV.

The report will summarize findings particularly relevant to UK TB control policy, including health and health economic effects on TB transmission in the UK. It is intended that the research will also be

disseminated through peer-reviewed publications, conference presentations and engagement with policy makers (Department of Health and Health Protection Agency), patients and the public via local clinical networks in London and voluntary sector agencies such as *TB Alert*.

4. STUDY OBJECTIVES

4.1 Primary Objectives:

- To compare the sensitivity, specificity, positive and negative predictive values of the ELISpot-based IGRA (T-SPOT.TB), the ELISA-based IGRA (QuantiFERON-TB Gold in-tube) and conventional diagnostic testing for the diagnosis of active pulmonary and extrapulmonary TB, in routine clinical practice;

- To develop an evidence-based optimal testing algorithm that defines the role of IGRAs in the diagnostic work-up of suspected active TB;

- To deliver the objectives above for a key subgroup: HIV co-infected patients (the highest risk subgroup of TB); and

- To quantify and compare the cost-effectiveness of a range of possible testing strategies against the present testing regime. Importantly, the benefit to individual patients and prevention of transmission to others (i.e. infections averted) will also be considered.

4.2 Secondary Objectives:

- To quantify sensitivity, specificity, positive and negative predictive values of both IGRAs and conventional diagnostic testing in a number of key patient 'sub-groups'. For example, it is expected that a range of participant sub-groups meeting the study inclusion criteria will include patients with pre-existing diabetes, end-stage renal failure and iatrogenic immunosuppression;

- To quantify the use of next-generation IGRAs (that have high diagnostic sensitivity) compared to existing commercially-available assays;

- To analyse samples and thereby determine whether there is a genetic component which might determine severity of disease progression, via basic DNA analysis; and

- To determine the sensitivity, specificity, positive and negative predictive values of the two IGRAs as applied to bronchoalveolar lavage samples in patients with suspected pulmonary TB who are sputum smear-negative.

5. STUDY DESIGN

5.1 Patient recruitment

A prospective multi-centre cohort study will be conducted in the UK (in routine clinical practice) of 1,012 adults (≥ 16 years old) presenting with suspected active tuberculosis at either NHS out-patient or in-patient services within participating hospitals in London, Leicester, Birmingham and Oxford. Please see appendix A, for further clarification.

Patients will be recruited at the point of diagnostic work-up in secondary care, before a confirmed diagnosis has been determined. Thus, potential participants for the study will be indentified at this point. The study population is representative of the national tuberculosis burden in terms of ethnic mix and range of co-morbidities and will include sufficient numbers of HIV-infected patients to reliably

determine the role of IGRAs in this key population. The participating hospitals chosen do not routinely use IGRAs in their diagnostic work-up of suspected TB, and it is expected that this will assist in minimising the influence of IGRA results on the attending clinicians' diagnoses which could otherwise bias the evaluation of IGRA accuracy.

6. PARTICIPANT ENTRY

6.1. Pre-registration evaluations

Potential participants (patients presenting at participating NHS centres) will be referred to a TB Research Nurse by the attending clinician (see table below, detailing TB diagnostic criteria). A second participant screening process will then be carried out by the TB Research Nurse, to ensure that participants meet the set inclusion criteria. To be included in the study, participants will need to be aged 16 years-of-age or over and present with suspected active TB (pulmonary or extra pulmonary), at either an inpatients or outpatients department.

Table 1.0 Pre-defined composite reference standard for active tuberculosis, validated in a prospective UK study in routine practice[1].

Diagnostic Category	Criteria
1: Culture-confirmed tuberculosis	Microbiological culture of *Mycobacterium tuberculosis* and suggestive clinical and radiologic findings
2: Highly probable tuberculosis	*M. tuberculosis* culture negative but clinical and radiologic features highly suggestive of tuberculosis and unlikely to be caused by other disease *and* supportive histology and/or cytology if available *and* a decision to treat has been made and appropriate response to therapy
3: Clinically indeterminate	A final diagnosis of tuberculosis was neither highly probable nor reliably excluded with no alternative diagnosis and the patient was not subsequently diagnosed with TB within 6 months
4: Active tuberculosis excluded	All microbiological samples smear and culture negative and a definite alternative diagnosis identified and the patient was not subsequently diagnosed with TB within 6 months

During the second participant screening process, the TB Research Nurse will also determine whether to exclude participants from the study. The exclusion criterion includes a participant being aged less than 16 years-of-age, any previous treatment for TB, or an inability to provide informed consent.

If the TB status (or any other clinically relevant details) of each potential participant is seen to be adherent to the study inclusion/exclusion criteria, then he or she will be asked by the TB Research Nurse to ascertain whether or not they are willing to take part in the study, in accordance with good clinical practice (GCP). Each participant will be provided with an information sheet and a verbal description of the research study, why they have been approached and informed that participation is voluntary. When a participant has been given adequate time to reflect upon and consider participation in this study and only when they have agreed to take part, will they be asked to read, sign and date a consent form. The form will be signed in the presence of a TB Research Nurse, who will countersign the consent form. Three copies of the consent form will be made; a copy will be kept in the research file, a copy given to the patient and a copy put into the participant's medical notes.

6.2 Withdrawal criteria

Participants may withdraw from the study at any time, if they so desire. Assurance will be provided by the TB Research Nurse that any such refusal will not affect the care they are provided with, in any way. In accordance with GCP, participants who withdraw will not be required (nor asked) for a reason why they have decided to do so. Participants can also be removed from the study if a research investigator (or other member of the research team) considers that this is an appropriate course of action.

7. STUDY PROCEDURES

Blood taking (venepuncture) procedures will be carried out during the study period. The frequency and timing of blood taking procedures will be at three set time intervals: the first visit, time nought (T0); 2 months (2M) after T0, and finally 6 months (6M). Follow-ups, in addition to the set time intervals, may be carried out, if clinically indicated. A 35ml blood sample will be taken at each time point – this will be used for IGRA testing (ELISpot and ELISA based) and for storage (PBMCs and serum) in the bio-repository; please see figure 1.0 (page 12) for further clarification.

7.1. Blood samples

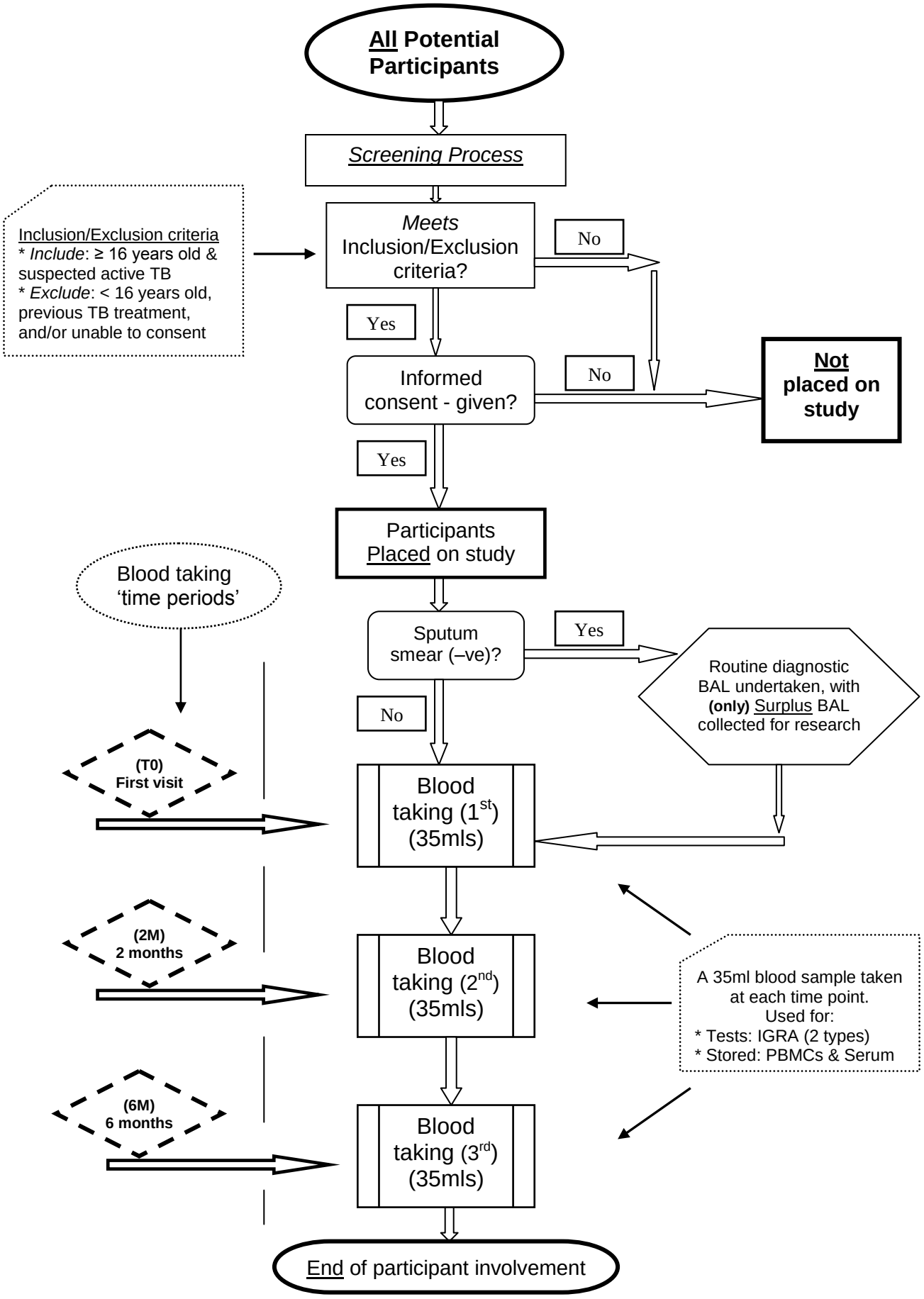
A 35ml quantity specimen of blood will be collected from all consenting participants for IGRA testing; residual sample will be stored to repeat the small number of indeterminate results expected and for future research. PBMCs will be stored in a liquid nitrogen tank and serum and plasma will be stored in a -80^{o}C freezer at the Tuberculosis Research Unit (led by Professor Ajit Lalvani) at Imperial College London. On the same day as sample collection, blood samples will be transported by either the Research Nurses or by courier in appropriate UN-type approved packaging to the Clinical Immunology Laboratory at Imperial College London (TB Research Unit) at St. Mary's Hospital, for testing.

Blood samples will be taken at the same time as a participant's routine clinic appointment. Quantity of taken bloods, per tube, will comprise of the following: QFN (3ml), PBMCs (28ml) and Serum (3ml) with PaxGene tube (1ml) all stored within the Clinical Immunology Laboratory.

7.2. <u>Diagnostic</u> Bronchoscopy

In patients with sputum smear negative pulmonary TB, diagnostic bronchoscopes are performed and collection of BAL (BronchoAlveolar Lavarge) is obtained as part of routine clinical care. The bronchoscopic procedure, along with collection of BAL, will occur in the endoscopy suite at St. Mary's Hospital, in accordance with set clinical practice guidelines. In cases where an amount of BAL is left over, after the procedure, an aliquot from the sample will be cryopreserved and stored in the research bio-repository for subsequent testing by IGRA. No additional BAL will be taken for this research study, and BAL samples <u>will only</u> be used for research if there is any left over after the procedure. The BAL sample consent is covered under the consent form, as "tissue samples". However, patients will still be informed if a surplus BAL sample is to be taken for this research study.

Figure 1.0 Participant time-line diagram:



7.3 Sample analysis

Each participant's sample will be tested with the two existing commercially-available IGRA tests: the ELISA-based Quantiferon-TB Gold in-tube (Cellestis Inc, Carnegie, Australia) and the ELISpot-based T-SPOT.TB (Oxford Immunotec Ltd, Abingdon, UK). All tests will be conducted and interpreted using manufacturers' instructions for processing the samples and scoring of assays.[1, 22, 23]

ELISpot^{PLUS} [1], which has enhanced sensitivity (described in reference 1), will also be carried out on blood samples from all participants in real-time and in parallel with the commercial assays, with results reported as part of the study. Bronchoalveolar lavage (BAL) will be carried out as part of the routine work-up of patients with suspected pulmonary TB with negative sputum smear results (estimated at approximately 200 BAL samples from our overall study population, based on HPA data on prevalence of sputum-smear-negative pulmonary TB [HPA 2008]); an aliquot from these samples will be cryo-preserved in the Research Tissue (TB) biobank and the ELISpot-based IGRA will subsequently be performed on thawed mononuclear cells in the category 3 biosafety containment facilities at Imperial College London.

A bio-repository of residual plasma and mononuclear cells will also be created to enable evaluation of next-generation IGRAs. These assays will be funded, carried out and reported separately from this research study. ELISpot incorporating promising new *M. tuberculosis* antigens that enhance diagnostic sensitivity without compromising specificity will be carried out on cryo-preserved mononuclear cells. ELISA to measure a novel chemokine, IP-10 (and potentially other cytokines) will be carried out on stored stimulated supernatants from the Quantiferon Gold in-tube assays[24]. The large study sample size and stringent design will allow calculation of robust estimates of the sensitivity, specificity and negative and positive predictive values of these next-generation assays on blood and BAL samples. See appendix A, which shows a diagram of the logistical flow chart for sample collection.

8. ADVERSE EVENTS

8.1. Definitions

Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.

Serious Adverse Event (SAE): any untoward and unexpected medical occurrence or effect that:

- **Results in death**
- **Is life-threatening** – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*
- **Requires hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

8.2. Blood venepuncture risks

The potential risk, as with any other blood sample venepuncture procedure, is a small chance of some bruising and tenderness around the area. When bruising does occur, the tenderness (typically) lasts for between two and four days after completing the procedure.

8.3 Reporting procedures

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

8.3.1 Non serious AEs

All such events, whether expected or not, should be recorded.

8.3.2 Serious AEs

An SAE form should be completed and faxed to the Chief Investigator and the Sponsor within 24 hours. All SAEs should be reported to the North West London REC1 Research Ethics Committee where in the opinion of the Chief Investigator, the event was:

- 'Related', ie resulted from the administration of any of the research procedures; and
- 'Unexpected', ie an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted to ethics, the sponsor and the R&D office within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies.

Contact details for reporting SAEs
Fax: 020 7262 8913 for the attention of Professor Ajit Lalvani
Please send SAE forms to: Respiratory Infections, Wright Fleming Institute, NHLI, Imperial College, St Mary's Campus, London, W2 1PG
Tel: 020 7594 3854 (Monday to Friday: 09.00 to 17.00)

9. ASSESSMENT AND FOLLOW-UP

9.1 Study participants

Study participants will have the opportunity to be seen beyond the set time periods (see section 7.0) if either they request this or a clinician decides that there is a clinical reason to do so. In this way participants can be assessed regularly by the investigating team, with any potential or actual adverse events detected rapidly. Participants meeting the criteria for a serious adverse event (see section 8.0) will be offered prompt treatment as appropriate.

Standard hospital follow-up will be in accordance with local hospital procedures. When the study is completed, study participants will not be routinely followed-up by the research study team; participants will return to the care of their GP (or other healthcare professional, as appropriate) upon completion of the study.

9.2 Test Results and Further Action

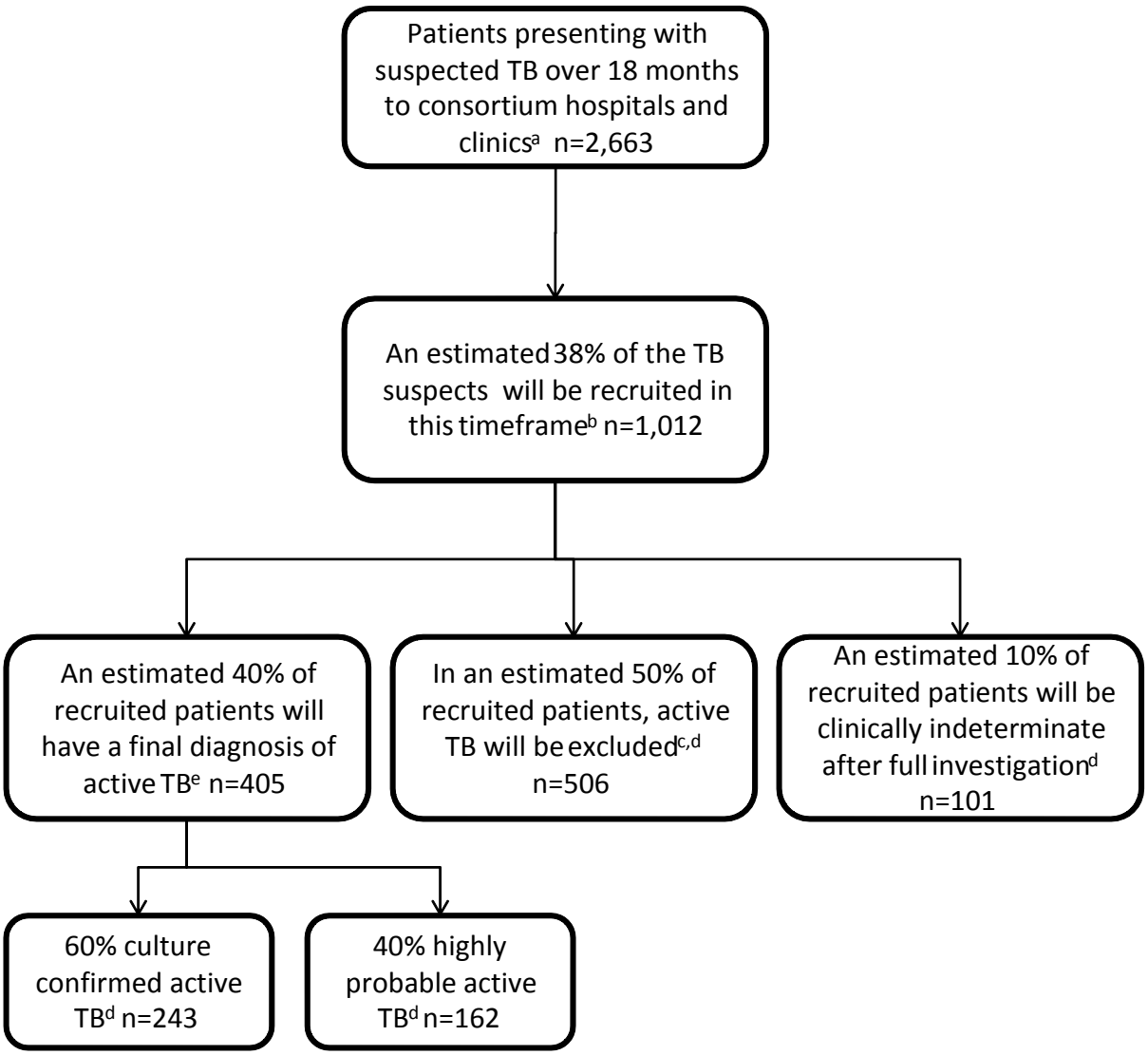
IGRA results of blood samples taken for the study from patients suspected of TB will be entered into the database by the Research Assistants for analysis. These results are for this specific study only and will not be made available for clinical use. Although IGRA testing is not common practice for HIV-negative patients suspected of TB in the hospitals of our consortium and is not currently recommended for HIV-positive patients suspected of TB,[15] any case-by-case IGRA testing requested by the attending clinician will run in parallel and these results will not be recorded or analysed in this study. IGRAs are not currently recommended in HIV-infected patients with suspected TB by current guidelines because of limited data, which highlights the importance of this study.

10. STATISTICS AND DATA ANALYSIS

10.1 The study diagram

A study diagram has been produced to show participant/patient numbers (estimated) for the study. Please see figure 2.0 below, which should be read in conjunction with the figure in appendix A.

Figure 2.0 Participant numbers - *estimates*



a. Estimated 200 HIV co-infected patients with suspected ATB to be recruited during study arise from the initial overall population of TB suspects presenting at Consortium hospitals. The corresponding study flow diagram for the subgroup would be the same as this study flow diagram, with proportionally smaller numbers. The only difference being that 62% of HIV TB suspects will be recruited and 50% of HIV TB suspects are expected to have a final diagnosis of ATB.
b. Proportion of suspects recruited determined by the number of days per week that TB Research Nurses attend participating hospitals – roughly 3 to 4 days at each Unit – enabling a recruitment rate of 34% of TB suspects without ascertainment bias.
c. Exclusion of ATB requires definite alternative diagnosis/absence of TB over 6 months follow-up.
d. Estimates based on average culture positive rates.
e. Estimates based on the study refereed to as Dosenjah *et al* in Annals of Internal Medicine.

Source: Detailed Project Description (Part II), page 18.

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10.2 Sample size calculation

The primary clinical utility of IGRA results in assessment of suspected active TB is in their negative predictive value, which enables clinicians to reliably rule-out TB from the differential diagnosis. This in turn depends on the diagnostic sensitivity and the prevalence of TB in the tested population. A recent meta-analysis computed diagnostic sensitivity of T-SPOT.TB and QFT-in tube in active TB as 90% and 70% respectively[21]. However, these estimates are based on pooling together several small datasets. The two largest studies to-date (n=194 TB cases diagnosed from n=389 TB suspects[1]; n=216 TB cases diagnosed from n=413 TB suspects[25]) gave more robust estimates of 85.1% (95% CI 79.2, 89.9) and 85.2% (95% CI 76.1, 91.9) for T.SPOT.TB for the respective studies and 78.1% (95% CI 70.7, 84.3) for QuantiFERON-Gold in-tube.

This study has been powered to detect a conservatively estimated 10% difference in diagnostic sensitivity between the tests, assuming a sensitivity of 85% for T-SPOT.TB and 75% for QFT-in tube and skin testing. To detect this difference at the 5% significance level (2-tailed) with 90% power, 855 patients are required (each receiving both tests), assuming a 40% prevalence of active TB in the study population. The number of patients recruited will be 1,012 to allow for missing data, indeterminate results, withdrawal of consent and possible logistical errors. These calculations have been made using methods which account for the paired nature of the data (based on McNemar's test) assuming independence of test errors[26].

In the HIV-infected subgroup, published evidence indicates that diagnostic sensitivity of QFT-in tube is reduced while that of T-SPOT.TB is unaffected. It is estimated that diagnostic sensitivities will be of 85% and 65% for T.SPOT.TB and QFT-in tube respectively, with this study powered to detect such a difference, at the 5% significance level, assuming a 50% prevalence of active TB in the HIV-infected subgroup; 156 patients are required to give 80% power and we expect to recruit 200 patients.

The previous diagram (figure 2.0) detailed how the numbers of patients in each of the final diagnostic classifications will arise from the initial overall population of patients presenting with suspected TB, in routine clinical practice. Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including follow-up period, according to Imperial College London policy.

10.3 Data Analysis

Sensitivity, specificity, positive and negative predictive values and likelihood ratios for each test and combination of tests, will be calculated to determine diagnostic accuracy and clinical utility for the representative cohort as a whole and within the appropriate subgroup of HIV co-infected patients. Participants to be included with reference to standard results from categories 1 (culture-confirmed), 2 (highly probable), and 4 (active tuberculosis excluded) for the primary analyses. Categories are described in table 1.0. The proportion of participants in Category 3 (clinically indeterminate) will be reported, and their impact on estimates ascertained by including them in sensitivity analyses. Similarly, sensitivity analyses will be carried out to investigate the impact of including Category 2 participants, particularly regarding test sensitivity. Full disclosure of all results will be reported, including numbers of indeterminate ELISpot and ELISA results.

Comparisons between ELISA and ELISpot IGRA tests will be provided to exploit the paired nature of data, using repeated measures and marginal logistic regression models, utilising the reference standard as an outcome. In the subset of patients presenting with a TST as part of their clinical diagnostic work-up, the performance of this particular test will be compared with IGRA results. Variation in test performance with HIV status and other characteristics will be estimated by including covariates in the models.[27]

The performance of combinations of two or more tests using logistic regression models constructed in the same form as Bayesian updating (post-test odds = pre-test odds x likelihood ratio) by including the log of the pre-test odds of prevalence (a constant term of known value) as an offset in the model, will be assessed. A linear predictor will then estimate log likelihood ratios, rather than log odds ratios. However, bootstrap methods will be required to obtain valid CIs (confidence intervals)[28]. Model parameterizations from Knottnerus[29] to compute likelihood ratios for the additional diagnostic value of each test in a testing sequence, will be used. Non-parametric, bias-adjusted CIs for parameter estimates from 1,000 bootstrap samples will be computed.

The primary clinical utility of immune-based testing is to exclude TB. Thus, when interpreting analysis and conclusions, focus will be made primarily on sensitivities, negative likelihood ratios and negative predictive values. This analytical plan is similar to that recently developed by Professor Ajit Lalvani and Jon Deeks[1].

The magnitude of the interferon-gamma response in both ELISpot and ELISA will be recorded, with cut-off thresholds recommended by the manufacturers of these commercial tests, applied to determine dichotomous positive and negative test results. The continuous data (magnitude of interferon-gamma response) will also be recorded and analysed to characterize the size of response contributes to the diagnostic accuracy of these tests through ROC (Receiver Operating Characteristic) curve analysis. Two co-applicants, Professor Ajit Lalvani and Dr Peter Kelleher[30], along with others [31], have previously shown that combining the size of the interferon-gamma ELISpot response with CD4 (Cluster of Differentiation IV) lymphocyte counts (as a ratio of interferon-gamma spot-forming cells to CD4 count) in HIV-infected TB patients can to some extent distinguish active from latent TB. The large study size of the HIV-coinfected TB subgroup (the largest such cohort to date for IGRA evaluation) will enable determination of real clinical utility (or otherwise) of this ratio analysis of IGRA and CD4 counts.

11. REGULATORY ISSUES

11.1 Existing (*granted*) ethics approval – Research Tissue Biobank

Ethical approval has been granted to the Tuberculosis Research Unit for a Research Tissue Biobank by St. Mary's Research Ethics Committee on 14th September 2007 (REC reference: 07/H0712/85) which provides the existing ethical framework for this new study that is to be undertaken. Ethics approval was granted for 5 years and so will cover the duration of this study and a recent inspection in relation to Human Tissue Licence Requirements confirmed that the study procedures, patient confidentiality and governance are all 100% compliant. Newly recruited Research Nurses will have Research Passports to enable them to work across the different Hospitals, using this research system.

11.2 Ethics approval for this study

This study has received ethical approval from the North West London REC1 ethics committee (REC ref: 11/H0722/8). The research study will be submitted for Site Specific Assessment (SSA) at each participating NHS Trust. The Chief Investigator will require a copy of the R&D approval letter before accepting participants into the study. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Declaration of Helsinki 1964 and later revisions.

11.3 Consent

Consent to enter the study will be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent will be obtained. The right of the participant to refuse to participate without giving reasons will be respected. After the participant has been entered on to the study, the clinician remains free to give any treatment that he or she considers might be required, or to refer onto an appropriate healthcare

professional, at any stage if it is felt to be in the participant's best interest; with reasons for doing so, recorded. In such cases the participants will remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the study without giving reasons and without prejudicing further treatment.

11.4 Confidentiality

The Chief Investigator and all members of the research team will preserve the confidentiality of participants taking part in the study and abide by the Data Protection Act. Participants will be allocated a unique indentifying code (anonymised) on recruitment, with no personal identifiers recorded on any samples.

11.5 Indemnity

Imperial College, London as sponsor of this study holds negligent and non-negligent harm insurance policies which apply to this study. These have been arranged through the Joint Research Office.

11.6 Sponsor

Imperial College London will act as the main sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

11.7 Funding

Following a successful project grant application, the HTA is funding this study. They are acting as sole funders and this agreement is in place. The investigators will not receive any additional payment above their normal salaries.

11.8 Audits and inspections

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd edition).

12. STUDY MANAGEMENT

12.1 Day-to-day study management

The day-to-day management of the study will be co-ordinated through the trial co-ordinator *****tbc*****. Close support will be provided by Professor Ajit Lalvani of Imperial College Healthcare.

12.2 Trial steering committee

A Trial Steering Committee (TSC) will be established. This committee will include an independent chair, at least two other independent members and a Patient and Public Involvement representative (recruited through *TB Alert*) along with key members of the co-lead applicants and co-applicants.

12.3 Data monitoring committee

A Data Monitoring Committee (DMC) will also be established. This committee will be independent of the applicants and of the TSC. The group will meet once a year and routinely review data on recruitment, and the prevalence of TB and HIV in the study cohort. The DMC will report to the HTA

programme via the TSC. Recruitment will be monitored by the study co-ordinator every 3 months. If recruitment is unexpectedly low, recruiting HIV-negative patients from a large pool of patients will be begin from Consortium hospitals in which currently only HIV-positive patients are recruited (starting with the St Mary's Hospital site).

13. PUBLICATION POLICY

13.1 Dissemination of study findings

The expectation is that after analysis, data from this study will be widely distributed within the medical and scientific community. Facilitated by presentations at local, national and international meetings, findings will be published widely in the medical literature. There is an excellent media department at Imperial College which has considerable experience of publishing high quality research. No identifying participant information will be published.

14. REFERENCES

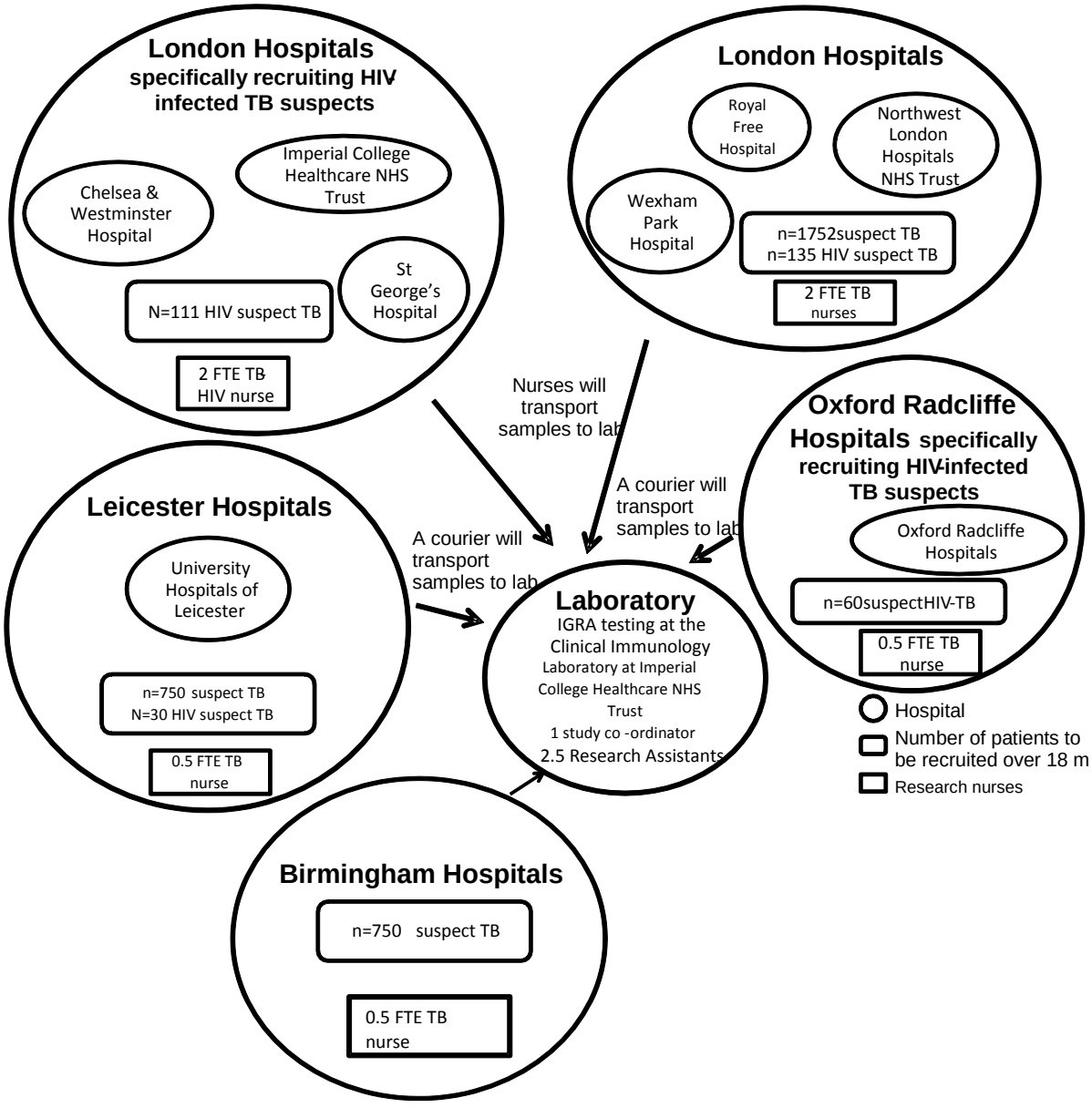
1. Dosanjh DP, Hinks TS, Innes JA, *et al.* (2008) Improved diagnostic evaluation of suspected tuberculosis. Annals of Internal Medicine. 148(5):325-36.
2. World Health Organization (2009) Global tuberculosis control: epidemiology, strategy, financing. Geneva, Switzerland. WHO/HTM/TB/2009.411.
3. Health Protection Agency Centre for Infections (2008) Tuberculosis in the UK: Annual report on tuberculosis surveillance in the UK. London.
4. Selwyn PA, Hartel D, Lewis VA, *et al*. (1989) A prospective study of the risk of tuberculosis among intravenous drug users with human immunodeficiency virus infection. The New England journal of medicine. 320(9):545-50.
5. Sonnenberg P, Glynn JR, Fielding K, Murray J, Godfrey-Faussett P, Shearer S. (2005) How soon after infection with HIV does the risk of tuberculosis start to increase? A retrospective cohort study in South African gold miners. The Journal of Infectious Diseases. 191(2):150-8.
6. Pratt R, Grange, J Williams, V (2005) Tuberculosis: A foundation for Nursing and Healthcare Practice. Hodder Arnold.
7. Ahmed AB, Abubakar I, Delpech V, *et al*. (2007) The growing impact of HIV infection on the epidemiology of tuberculosis in England and Wales: 1999, 2003. Thorax. 62(8):672-6.
8. Holden M, Dubin MR, Diamond PH. (1971) Frequency of negative intermediate-strength tuberculin sensitivity in patients with active tuberculosis. The New England Journal of Medicine. 285(27):1506-9.
9. Graham NM, Nelson KE, Solomon L, *et al*. (1992) Prevalence of tuberculin positivity and skin test anergy in HIV-1-seropositive and -seronegative intravenous drug users. Jama. 267(3):369-73.
10. Markowitz N, Hansen NI, Wilcosky TC, *et al*. (1993) Tuberculin and anergy testing in HIV-seropositive and HIV-seronegative persons. Pulmonary Complications of HIV Infection Study Group. Annals of Internal Medicine. 119(3):185-93.
11. Huebner RE, Schein MF, Hall CA, Barnes SA. (1994) Delayed-type hypersensitivity anergy in human immunodeficiency virus-infected persons screened for infection with Mycobacterium tuberculosis. Clinical Infectious Diseases. 19(1):26-32.
12. Johnson MP, Coberly JS, Clermont HC, *et al.* (1992) Tuberculin skin test reactivity among adults infected with human immunodeficiency virus. The Journal of Infectious Diseases. 166(1):194-8.
13. Yanai H, Uthaivoravit W, Mastro TD, *et al.* (1997) Utility of tuberculin and anergy skin testing in predicting tuberculosis infection in human immunodeficiency virus-infected persons in Thailand. International Journal of Tuberculosis Lung Disease. 1(5):427-34.
14. Moreno S, Baraia-Etxaburu J, Bouza E, *et al*. (1993) Risk for developing tuberculosis among anergic patients infected with HIV. Annals of Internal Medicine. 119(3):194-8.
15. Pozniak AL, Collins S, Coyne KM, Freedman AR JM, Lipman MCI, Lucas SB, Miller RF and Ormerod LP (2009) On behalf of the BHIVA Guidelines, Committee W. British HIV Association guidelines for the treatment of TB/ HIV co-infection. Guideline awaiting publication.

16. Behr MA, Wilson MA, Gill WP, *et al*. (1999) Comparative genomics of BCG vaccines by whole-genome DNA microarray. Science. 284(5419):1520-3.
17. Menzies D, Pai M, Comstock G. (2007) Meta-analysis: new tests for the diagnosis of latent tuberculosis infection: areas of uncertainty and recommendations for research. Annals of Internal Medicine. 146(5):340-54.
18. Lalvani A. (2007) Diagnosing tuberculosis infection in the 21st century: new tools to tackle an old enemy. Chest. 131(6):1898-906.
19. Pai M, Riley LW, Colford JM, Jr. (2004) Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. The Lancet Infectious Diseases. 4(12):761-76.
20. Richeldi L. (2006) An update on the diagnosis of tuberculosis infection. American Journal of Respiratory and Critical Care Medicine. 174(7):736-42.
21. Pai M, Zwerling A, Menzies D. (2008) Systematic Review: T-Cell-Based Assays for the Diagnosis of Latent Tuberculosis Infection: An Update. Annals of Internal Medicine. 149(3):177-84.
22. Cellestis. (2009) QuantiFERON-TB Gold In-Tube Method.
http://wwwcellestiscom/IRM/Company/ShowPageaspx?CPID=1170
23. Department of Health (2005) The Mantoux Test: Administration, Reading and Interpretation.
http://wwwimmunisationnhsuk/Publications/mantouxtestpdf
24. Ruhwald M, Bodmer T, Maier C, *et al*. (2008) Evaluating the potential of IP-10 and MCP-2 as biomarkers for the diagnosis of TB. European Respiratory Journal. 32:1607-15.
25. Goletti D, Stefania C, Butera O, *et al*. (2008) Accuracy of immunodiagnostic tests for active tuberculosis using single and combined results: a multicenter TBNET-Study. PLoS ONE. 3(10):e3417.
26. Alonzo TA, Pepe MS, Moskowitz CS. (2002) Sample size calculations for comparative studies of medical tests for detecting presence of disease. Statistics in Medicine. 21(6):835-52.
27. Leisenring W, Pepe MS, Longton G. (1997) A marginal regression modelling framework for evaluating medical diagnostic tests. Statistics in medicine. 16(11):1263-81.
28. Chan SF, Deeks JJ, Macaskill P, Irwig L. (2008) Three methods to construct predictive models using logistic regression and likelihood ratios to facilitate adjustment for pretest probability give similar results. Journal of Clinical Epidemiology. 61(1):52-63.
29. Knottnerus JA. (1992) Application of logistic regression to the analysis of diagnostic data: exact modeling of a probability tree of multiple binary variables. Medical Decision Making. 12(2):93-108.
30. Clark SA, Martin SL, Pozniak A, *et al*. (2007) Tuberculosis antigen-specific immune responses can be detected using enzyme-linked immunospot technology in human immunodeficiency virus (HIV)-1 patients with advanced disease. Clinical and Experimental Immunology. 150(2):238-44.
31. Rangaka MX, Diwakar L, Seldon R, *et al*. (2007) Clinical, immunological, and epidemiological importance of antituberculosis T cell responses in HIV-infected Africans. Clinical Infectious Diseases. 44(12):1639-46.

Appendices

Appendix A.

Figure 3.0 Logistical flow chart of sample collection - deployment shown across consortium hospitals.



Please note: the figures for this appendix come to 3,588 and refer to total numbers before final determination of the study's 1,102 participants, as shown on page 16. This appendix has been included for clarification only.