Next generation tests for Latent TB

1.0 Background

TB Background

Development and evaluation of new assays which are able to differentiate LTBI from active infection, which can be used in patient groups for whom currently available IGRAs may not provide valid results, or which can quantify a patient's risk of developing active TB, are needed in order to more accurately identify patients who may benefit from chemoprophylaxis. The next generation Quantiferon Gold In-Tube aims to address these issues. It measures both CD4 and CD8 function and may therefore be useful in treatment monitoring (with repeated measurements of the ratio of these two). This study, which utilised processes, expertise and infrastructure established during the PREDICT study, will evaluate the performance of this new test for LTBI, including comparison with the currently available IGRAs and will evaluate other promising technologies such as whole blood transcriptional profile signatures. The study will also provide a foundation on which to build related studies using IGRAs.

2.0 Research questions

1) Sensitivity and specificity

Sensitivity of the new Quantiferon assay (in detecting LTBI) will be assessed in patients with active TB disease. Whilst the test is not intended for use in the diagnosis of TB disease, TB patients are known to be infected with *Mycobacterium tuberculosis* so the percentage of patients with a positive result will reflect the test's sensitivity.

Specificity of the new assay may be measured as the percentage of patients negative by existing IGRA who are also negative by the new assay.
[These would be not be "true" specificity estimates but would show whether the tests were comparable to the existing IGRAs.]

The distribution of quantitative results will be analysed to determine whether there are clear cut-points to define positive and negative samples.

Sensitivity and specificity will be assessed separately in HIV-positive and HIV-negative participants.

2) Risk of progression

By linking the data collected in this study to the UK Enhanced Tuberculosis Surveillance (ETS) dataset, the rates of development of active TB will be compared for individuals with different baseline LTBI status, with LTBI defined as a) positive result for Quantiferon; b) positive result for TSpot; c) positive result for next generation Quantiferon. The strength of association between baseline result and subsequent disease (adjusted for potential confounders) will be compared for the three tests.

Indirect evidence of potential associations with progression will be obtained by multivariate analyses of the association between baseline LTBI status according to the three definitions above and known risk factors for progression, e.g. prevalence in country of birth, co-morbidities.

3) Use of next generation Quantiferon in individuals with HIV

To evaluate the performance of the next generation Quantiferon in individuals with HIV, patients with active TB will also be recruited through TB/HIV clinics. Sensitivity and specificity of the new assay will then be assessed as above specifically in this group of HIV-positive individuals. For those with latent TB and HIV infection, the risk of progression to active TB will be assessed.

4) Treatment monitoring

The relationship between LTBI and active treatment and results of the next generation Quantiferon will be assessed by analysing the change in the assay result over time in participant groups defined by their self-reported level of treatment compliance (e.g. taking drugs correctly all, some, or none of the time).

5) IGRA conversion during Hajj & Umrah

Existing networks will be used to recruit Hajj and Umrah pilgrims in London prior to their journey to Saudi Arabia. In addition to the baseline questionnaire and blood samples (including those for the next generation Quantiferon), these participants will also provide a second set of blood samples on their return from the pilgrimage to assess the risk of conversion. Information on respiratory (non-tuberculosis) symptoms experienced during the pilgrimage will also be collected. A baseline nasal swab will be taken before travel and a second nasal swab collected on their return from the pilgrimage.

6) Utility of Next Generation Tests to enhance investigation of a TB incident at a London School

To evaluate the clinical utility of next generation tests, next generation Quantiferon and other promising technologies, for diagnosing latent TB infection and enhancing TB screening among a younger age group (range: 11 – 15 years) in the context of a rapidly evolving high yield TB outbreak. In addition, this substudy will explore associations between infection status and epidemiological markers of exposure to TB or transmission.

3.0 Method

3.1 Research questions 1) – 5)

3.1.1 Design

3.1.1.1 Setting, population and disease burden

This prospective cohort study will recruit individuals 11 years or older, who are close contacts of cases of active TB.

The work will take place across a network of hospitals in London and general practices in three Primary Care Trusts (PCTs) in North East (NE) London: City and Hackney, Newham and Tower Hamlets PCTs. Patients will be recruited from TB clinics and from the NE London TB Primary Care Network. These hospitals have been selected based on the high incidence of TB (located in areas with over 40 TB cases per 100,000 population including several, such as Newham and Brent, with rates comparable to developing countries). There is also a concentration of high levels of socioeconomic deprivation and ethnic diversity in these boroughs reflecting the population of TB cases nationally.[1]

3.1.1.2 Recruitment and inclusion criteria

1. Contacts of pulmonary and extra-pulmonary TB patients (whether smear-positive or smear-negative), attending participating TB clinics or primary care centres for screening will be invited to take part. Contacts will include all individuals with a cumulative duration of exposure of greater than eight hours to the relevant index case in a confined space during the period of infectiousness (prior to initiation of treatment). A study TB specialist or practice nurse will identify eligible persons and give them written information sheets (translated as appropriate). Written informed consent will be obtained (with the help of a translator where appropriate) from all patients willing to take part. The research nurse will complete a baseline assessment questionnaire including demographic and clinical information (see details of variables and data collection process below in section 3.2). As this is a pragmatic assessment of the use of these assays, the process is very similar to standard practice with the only differences being obtaining formal consent, systematic collection of data and offer of three IGRA tests at this stage. Details of our estimated recruitment rates are given in section 3.5.

2. To assess sensitivity of the IGRAs, we would recruit participants with active TB from the same clinics and primary care centres.

3. In addition, we would undertake a study to assess Hajj pilgrims before and after they travel from the UK to Saudi Arabia to determine the proportion of individuals that convert from a negative to a positive test. As well as Hajj pilgrims, who travel once per calendar year, there are groups of pilgrims attending Saudi Arabia for a religious observance known as Umrah throughout the year. Links will be formed with religious establishments and those helping to

arrange travel for them to recruit from this group also, which would have the same sampling and testing regimen as the Hajj pilgrims.

4. In addition, for eg school incidents where children age 11 and over have been exposed to tuberculosis, we will contact children and their parents or guardians during the incident investigation to inform them about the study and determine whether they would like to be involved with this.

3.1.1.3 Health technologies being assessed

Blood samples:

LTBI measures: Participants will be tested by the two currently available IGRA tests, Quantiferon-Gold In Tube (ELISA) and ELISpot assay (the same assay as in TSpotTB). We will also evaluate the 4th Generation Quantiferon test. All tests will be conducted using standardised protocols.[17-19] Residual blood will be kept to repeat the small number of indeterminate results expected. We will ensure that the study processes do not disrupt routine patient care and remain consistent with current practice. All clinics will undertake the assessment for latent infection among contacts at about six weeks after last known exposure, to ensure that nearly all participants have been given sufficient time to develop a cell mediated immune response detectable by IGRA. In addition, clinics in East London routinely undertake a baseline TST and Quantiferon Gold In Tube assay for all patients as part of routine care with a follow up visit at six weeks (this will be paid for by local services). We will use these data to assess conversions and reversions, which we expect to be minimal.

Samples and transportation: A 40 ml blood specimen will be collected. After IGRA testing, full blood count, and repetition of indeterminate assays, the residuum will be stored for future research (see Section 5). The samples will be collected by study nurses at TB clinics or general practices and stored in bottles for full blood count at the local laboratory. Samples will be transported using appropriately equipped courier services to a study testing centre (Royal Free Hospital).

Nasal swabs:

This section refers to only the Hajj recruits to the study.
When participants attend clinic for the repeat IGRA blood test, they will be asked for a nasopharyngeal swab (taken by a nurse) and a lower nasal swab (self administered), as detailed in the patient information. If a participant doesnot attend for the clinic visit, they will be contacted by phone and may take the lower nasal swab themselves, if they agree, which will be posted to them.

Swabs will be tested for the presence of various bacteria and viruses which can be present in the nasal flora, in particular *Streptococcus pneumoniae, Haemophilus influenza and Neiserria meningitidis,* with the addition of other microbes if routine surveillance suggests this would be useful.

Other blood tests:
This section refers to only the school incidents study.
When participants attend clinic for IGRA blood test as part of routine care, they will be asked for an extra blood sample of 10 mls to assess the 4th generation Quantiferon test (4 mls of blood) and a new proteomic test produced by Protein Logic (6 mls of blood). This will be the extra blood taken over and above that obtained for routine IGRA testing (generally less than 10mls), as detailed in the patient information and attached enhanced protocol.

3.1.1.4 Follow-up

All participants will be followed for an average of 24 months (between 18 and 36 months) from the date of IGRA testing using a number of approaches a) phone call to GP and/or patient at 24 months or at the end of follow up; b) search of national enhanced TB surveillance reports; c) search of national database of culture proven TB; d) Clinic records. The use of these data sources enables comprehensive national follow-up of participants. This should minimise loss due to transfer of care to other centres by patients or physicians. The follow up will be undertaken by the study coordinator,

administrator and database manager in collaboration with participating centres and coordinated from Public Health England.

The majority of newly infected patients who go on to develop disease will be expected to do so in the first five years, usually within 12 to 24 months[5;21]. Most participants will be followed up for 24 months, with those recruited at the beginning of the study followed up for 36 months and those at the end for 18 months. In this study, if we assume a loss to follow up of 20% (based on clinic data), an average of 24 months follow up will be adequate to ensure that we achieve a 5% progression rate for all participants.

3.1.2 Data Collection

Data will be collected by trained research nurses for the baseline study data and through the web-based national surveillance system, supplemented by clinic and primary care records, for the outcome data. The web based TB surveillance system already holds demographic, clinical and microbiological details of all TB cases in England and Wales and includes a contact investigation module which will be modified to collect data for this study. All data will be imported into a purposely built database maintained at Public Health England with anonymised records at UCL for analysis. This unit has considerable experience of data management and holds the national enhanced tuberculosis and mycobacterial laboratory databases.

Data items to be collected from participants include age, gender, country of birth and date of entry to the UK for non UK born persons, ethnicity, duration of contacts measured in hours and nature of contact (place and size of shared air space), duration of residence in the UK, current employment, the nature of contact with the source case, the time interval between the most recent suspected exposure date and the date of diagnosis in the source case, details of any previous contact tracing, history of previous TB including treatment, results of previous TST and chest radiographic findings, BCG vaccination status (scar and record), vitamin D status, associated medical diagnoses or use of immunosuppressive agents, drugs used for the treatment of latent infection and simple measures of compliance with, and adverse effects of, chemoprophylaxis (i.e. treatment of latent infection).

HIV status will be determined at the end of the follow-up period through pseudo-anonymised record linkage with the national HIV surveillance system, which has been demonstrated to be a reliable mechanism for identifying infected cases.[22] The assessment of the predictive value of IGRA in HIV infected populations was recommended by NICE as a research priority as they are an important sub-group with an extremely high rate of progression to active TB. Although the study is not powered to detect differences in this group (only secondary analyses are proposed), useful data will be obtained as the record linkage is undertaken routinely at Public Health England and does not add extra cost to the study.

DNA finger printing data, from the UK national TB strain typing database hosted by Public Health England, will be utilised to ascertain transmission between index cases and subsequent diagnoses among contacts. This will assist in confirming that any TB disease in secondary cases has arisen from the initial infection (i.e. that the test result relates to the outcome) and not due to subsequent exposure to another case.

3.1.3 Outcome measures and definitions

3.3.1 Primary Outcome:
a. Study 1: Development of active TB (Incident Rate Ratios). Prognostic values of tests quantified as incidence rate ratios among contacts. Cases of active tuberculosis will include a) culture confirmed cases with a microbiological diagnosis (isolation of *M. tuberculosis* in the presence of clinical disease) and b) clinically diagnosed cases (signs and symptoms of TB with radiological or histological evidence of tuberculosis). Cases will include all diagnosed individuals reported to the national surveillance system, national microbiological database or diagnosed at participating clinics. Every effort, as recommended by NICE guidelines,[15] will be made to achieve a microbiological diagnosis.

b. Study 2: Proportion of cases with active TB who have a positive IGRA test (separately for each type of IGRA including 4th Generation Quantiferon)

c. Study 3: Proportion converting from a negative to a positive IGRA test

3.3.2 Secondary Outcome: Side effects from chemoprophylaxis.

3.1.4 Analysis Plan

Study 1: The primary analysis will focus on estimating the ability of the three tests (individually and in combination) to predict the occurrence of progression to active TB in the cohort who do not receive prophylaxis, and to make comparisons between tests. Estimates of rates of progression to active TB will take into account the follow-up period for each individual, and for those under 35, the differential sampling proportions of test positives and test negatives will be adjusted for to correct for partial verification. The predictive performance of each test (ELISpot, ELISA and new assay) alone and in different combinations will be summarised as incidence rates of developing active TB in test positive and test negative groups, which will be expressed with 95% confidence intervals computed using Poisson exact methods. The discriminatory predictive value of the test will be based on the relative comparison of these rates (the relative incidence rates comparing test positives and test negatives) together with the prevalence of test positive results (which indicates the number who would be treated should the test be used for recommending chemoprophylaxis). Comparisons of the predictive value of pairs of tests or test strategies will be made using Generalised Estimating Equation Poisson regression exploiting within patient comparisons of tests.[7]

Study 2: We will assess sensitivity of quantiferon by calculating the proportion with a positive test.

Study 3: We will determine the proportion converting to a positive test and assess the risk factors for conversion.

3.1.5 Sample Size

The study size (and associated power) has been informed by simulating the study and its analysis 1000 times and observing the proportion of simulations yielding significant results across various scenarios. Conventional methods for sample size calculation would not account for the Poisson nature of the data and the within-patient comparisons of the tests, hence simulation was necessary. The simulations indicated that a cohort of 2,000 participants amongst whom 90 incident events were observed would have around 85% power to detect significant ($P<0.05$) differences in predictive performance that would arise from differences in sensitivity and specificity of 10% between tests. These differences correspond to increases in predictive performance (expressed as a ratio of relative rates between test positives) of 30%, which would be clinically useful.

We would therefore be requiring 4000 Quantiferon 4th generation kits which would be distributed to cover all study objectives.

For the secondary data analyses, based on the rule of thumb of requiring 10 events for every variable in a multiple regression model[23] the cohort should provide adequate events to fit regression models to estimate progression on treatment using regression models to adjust for test-dependent treatment decisions, although the power in particular subgroups may be limited.

3.2 Research question 6: Utility of Next Generation Tests to enhance investigation of a TB incident at a London School

3.2.1 Objectives

Objective 1: Enhanced epidemiological investigation

Sub-objective a: To use whole genome sequencing added to epidemiological methods to determine if transmission events within this outbreak arose from one or several sources, and explore variations in strain transmissibility.

Hypothesis and outcomes: We hypothesise that the source case arose within the family of the “index” school boy and that the other cases of active TB within the school result from recent infection with the index’s mycobacterial strain. However, the strain identified within the family may or may not be identical to strains currently circulating in the community. We futher hypothesise that genomic and/or

epidemiological factors that explain differences in transmissibility within or between strains can be identified.

Sub-objective b: To determine whether crowd contact rather than close contact predicts the risk of TB transmission and estimate the rate at which the index case(s) produced infectious quanta.

Hypothesis and outcomes: We hypothesise that crowd contact will better predict TB infection risk than close contact. Here, crowd contact is time spent in the same indoor space as another individual, whereas close contact is conversational contact and anything more intimate. These definitions do not include a minumum duration of contact. We also hypothesise that the rate at which the index case produced infectious quanta will be high relative to that measured in other settings. An infectious quanta is the number of infectious airborne particles required to infect.

Objective 2: Enhanced clinical utility for LTBI diagnosis and disease progression
Sub-objective a: To compare the yield of infection between NextGen tests with standard tests of infection (ELISPOT or TST) and against epidemiological predictors of infection risk at baseline.

Hypothesis and outcomes: We hypothesise that NextGen tests will have equivalent yield to standard IGRA and, due to greater specificity, accurately identify fewer individuals that should receive preventive therapy when compared to the TST.

Sub-objective b: To determine, among TST+ individuals in whom active disease has been excluded, the strength of association between baseline NextGen test results and subsequent active disease (adjusted for potential confounders). Key confounders include provision of isoniazid preventive therapy and BCG vaccination.

Hypothesis and outcomes: By linking the data collected in this study to the UK Enhanced Tuberculosis Surveillance (ETS) dataset, the rates of development of active TB will be compared for individuals with different baseline LTBI status.

A protocol describing methods for this objective is attached.

4.0 Ethics and Research Governance

Multi Centre Research Ethics Committee (MREC) approval will be sought. Informed consent will be obtained from all participants. No LTBI treatment will be offered to those over 35 yrs (following NICE guidelines). The rationale for this is that patients over the age of 35 are more likely to develop side-effects and therefore the potential benefits are outweighed by the risks.

All study data will be held in accordance with NHS data protection principles including the use of secure password protected systems. UCL will be the nominated sponsor.

An advisory panel will be recruited to help oversee the research and supervise the draft report to the NIHR HTA.

5.0 Bio-repository

Residual samples will be stored for up to 15 years at the UCL biobank located at the Royal Free Hospital, ensuring that white blood cells and plasma remain available for the evaluation of new markers that predict the development of active tuberculosis among latently infected persons in the future. It is essential to develop a repository to assess the predictive value of the next generation of assays for latent tuberculosis infection at a much lower cost. The initial priority will be for the repeat testing of the two LTBI assays. Storage of cellular and plasma fractions will be at –80°C, with the Royal Free Hospital providing freezer capacity and security of samples. A small number of plasma and cellular samples will be repeated for quality control evaluation of the assays and storage conditions. The biobank thus provides an effective link between clinically-relevant next-generation IGRA technologies and the unique statistical power of the proposed cohort. By enabling determination of the prognostic value of next-generation IGRAs, it provides important added value and makes this proposal future-proof ensuring its continued relevance for policy development several years into the future.

6.0 Expected output of the research

A comprehensive report will be prepared. In addition to a formal report to NIHR, the research will be disseminated through peer reviewed publications, conference presentations and engagement with policy makers (Department of Health), patients and the public (via local clinical networks in London, community-based programmes working with at-risk for TB populations and voluntary sector agencies such as TB Alert).

7.0 Timetable

Milestones	
Year 1	Ethics application
	Purchase of equipment and study material
	Staff recruitment
	Organisation of recruitment of participants
	Publication of study protocol
	Start of recruitment
	Start of follow up
Year 2	Interim analysis of event rates prior to closing recruitment
	End of recruitment
	End of baseline formal assays
Year 3	Interim analysis of event rates prior to closing follow-up
	End of follow up
	Start of data cleaning
	Matching with HIV data
	Statistical analysis
	Economic analysis
	Preparation of final report

8.0 Service User Input

Input from service users will be obtained through the user group of the NE London TB Network Primary Care Practices, TB Alert and through the user involvement mechanisms arranged for our NIHR funded TB Programme Grant. In addition, the advisory group will include two service users identified with the help of the national TB charity, TB alert. We will ensure that service users are adequately remunerated for their time and input; and that we adhere to the principles of good practice in active public involvement promoted by INVOLVE.

9.0 Complaints

In the event of complaint about the conduct of the study, the complaint should be reported immediately to the Joint Research Office research-incidents@ucl.ac.uk who will decide which complaints policy applies and who will be the lead organisation. The NHS complaints policy can only apply where the research subject is recruited through an NHS Trust. In other circumstances the UCL complaints policy will apply.

10.0 Insurance

University College London holds insurance against claims from participants for harm caused by their participation in this clinical study. Participants may be able to claim compensation if they can prove that UCL has been negligent. However, if this clinical study is being carried out in a hospital, the hospital continues to have a duty of care to the participant of the clinical study. University College London does not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees. This applies whether the hospital is an NHS Trust or otherwise.

11.0 Serious unexpected adverse events and incidents

All serious Unexpected Adverse Events (if applicable) to a research subject in the study must be reported immediately to the sponsor using the following email address research-incidents@ucl.ac.uk.

All incidents must be reported through the appropriate Trust incidents reporting system. Where no Trust is involved the incident should be reported by completing form at http://www.ucl.ac.uk/jro/postapproval. Where the study is being conducted at UCLH then the incidents should be reported through Datix.