NETSCC, HTA

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Prognostic value of Interferon Gamma Release Assays in predicting active tuberculosis among individuals with, or at risk of, latent tuberculosis infection

A. Summary of the project

The objective of this study is to assess the prognostic value of the current two Interferon Gamma Release Assays (IGRA) compared with the standard Mantoux Tuberculin Skin Test (TST) for predicting active tuberculosis among those at increased risk of tuberculosis; specifically, contacts of active tuberculosis cases and new entrants. It will also assess the cost effectiveness of a two step strategy (as recommended by NICE), as well as an alternative testing strategy. 10,000 participants will be recruited from 12 hospitals and a network of GP surgeries in London. All participants will have a TST, blood taken for both assays at baseline. Participants will then be followed up for an average of 24 months using the national register of clinical reports, a phone call and the national microbiological database. During follow-up there will be no additional diagnostic procedures unless symptoms occur, i.e. in line with current policy. The study end point is the development of active tuberculosis, or end of study period. A cost-effectiveness analysis will be performed to estimate the costs (£) and health effects (QALYs) of eight key screening strategies: no screening; each test on its own (TST, ELISA or ELISpot); TST followed by ELISA or ELISpot if positive (NICE recommendation); and ELISA or ELISpot followed by TST if positive.

B. Changes to the application since the outline proposal

In response to the comments received we have modified the proposal to prioritise the recruitment of individuals who are not treated for latent tuberculosis infection. We have provided justification for recruiting participants in the treated group to achieve a secondary objective at a relatively low cost. We have also provided justification for the provision of a bio-repository and outlined our detailed recruitment strategy.

C. Planned Investigation

1.0 Background

Tuberculosis (TB) is a major cause of disease burden worldwide. The UK has seen a resurgence of TB since the late 1980s and there are currently over 8000 new cases each year[1]. Most cases occur in major cities, particularly in London, and an increasing proportion occur in persons born abroad (currently two-thirds of TB cases and higher still in London) and other groups with specific risk factors. Control measures have traditionally relied on the prompt diagnosis of active TB cases and ensuring that patients complete their treatment.

The majority of TB cases in the UK arise due to reactivation of latent infection.[1] Among immigrant groups, the infection is likely to have been acquired abroad whereas among the elderly UK-born population, the infection is likely to have been acquired in earlier years when TB was highly prevalent in the UK. The policy of targeting active TB cases for treatment will not be sufficient alone to control and eventually eliminate TB in the UK. The identification and treatment of individuals with latent TB infection (LTBI) who are at high risk of developing active TB, may therefore be an essential additional measure provided that (1) true LTBI can be identified (and distinguished from prior BCG vaccination), (2) the probability of developing active TB in people with untreated LTBI can be determined, and (3) the intervention strategy available (treatment of latent infection) is effective and can be successfully implemented.

1.1 Identification of latent tuberculosis infection

It is estimated that about one-third of the world's population is latently infected with the tubercle bacterium and widely accepted that, among those with latent TB, there is an approximately 10% lifetime chance of progression to active TB. The tuberculin skin test (TST) remained the only tool for the diagnosis of latent TB over the last century. TST assesses the delayed type hypersensitivity response to a purified protein derivative (PPD - which contains antigens shared by several mycobacteria and *Mycobacterium bovis* BCG) by measuring the

size of the skin induration following the injection of the antigen. Limitations to the validity (sensitivity and specificity) of TST as a measure of latent infection have been recognised for many years. These are partly due to variability in the quality of administration and interpretation of the test; as well as the state of host immunity.

1.1.1 Interferon Gamma Release Assays

Interferon Gamma Release Assays (IGRA) are based on the detection of *Mycobacterium tuberculosis* complex specific region of difference (RD1) antigens such as Early Secretory Antigenic Target 6 (ESAT-6), Culture Filtrate Protein 10 (CFP-10) plus other TB specific antigens. Two commercial assays have been developed using these *M. tuberculosis* antigens, QuantiFERON TB Gold In Tube (Cellestis, Australia) – which uses an ELISA format and T Spot TB (Oxford) – which is an ELISpot-based assay. The T Spot TB assay was developed by Prof Lalvani.

1.1.2 Predictive value studies of IGRAs

Recent systematic reviews[2;3] of the role of IGRAs in the diagnosis of infection with *M. tuberculosis* concluded that they have several advantages over the TST including higher specificity, better correlation with exposure to *Mycobacterium tuberculosis* and less cross reactivity with BCG vaccine and environmental mycobacteria. In the absence of a gold standard for the diagnosis of latent TB infection, all the studies in these reviews have used other proxy measures to estimate the sensitivity and specificity of IGRAs for latent infection. These measures include "degree of contact with a case of TB" and the use of "active TB disease" as an indicator of those truly positive and "low prevalence of infection in the test population" to estimate true negative results.[4]

Very few published studies have examined the predictive value of an IGRA test in a low incidence country (table). The majority of the completed studies[5-10] are either small studies [5;10] and / or undertaken in high burden countries[6], and their results are conflicting. The two studies done in low incidence countries were both small with one concluding IGRAs are superior to TST among contacts[5] whilst the second did not find a significant difference among immigrants.[10] A further study was undertaken in Turkey, an intermediate burden country. This study had limited power despite a large sample size due to the extensive use of chemoprophylaxis in children. The studies in high burden countries inevitably include a higher proportion of cases arising due to re-infection. In addition to the published data listed in the table, a number of studies (summarised by Andersen et al[11]) are currently underway in high burden, low/intermediate income, countries including Godfrey-Fassett et al recruiting 8000 participants in Zambia and reporting in 2010, Hanekom et al recruiting 8000 participants in South Africa and reporting in 2009 and Vaz et al recruiting 7500 in India and reporting in 2010. Despite these ongoing studies, the draft European guidelines on contact tracing[12] concluded that data on the predictive value of IGRAs would remain lacking in low incidence settings. A similar conclusion was reached in a consensus document of European public health experts.[13]

Our study, if funded, will be the largest, and first adequately powered, study on the predictive value of IGRAs in a low incidence setting. It will influence tuberculosis control and elimination policy in Europe and North America.

Table: Published studies on the predictive value of Interferon Gamma Release Assays

Author	Country, population	Sample size/ Follow up duration	Predictive value	Limitations
Diel R et al 2008[5]~	Germany, contacts	601 contacts over 103 weeks	6/41 (14%) QFT positive developed TB versus 0/535 QFT negative. 5/219 with TST 5mm.	Limited power due to the small sample size. Does assess immigrant screening
Bakir M et al 2008[7] (Lalvani A)	Turkey, household contacts	908 children for 1201 person years	11/381 TSpot TB positive developed active TB (20.5/1000 person-years) versus 12/550 TST	A study in an intermediate burden country, however, 76% of children had

			positive developed TB (16.6/1000 person-years).	chemoprophylaxis.
Hill P et al 2008[6]	The Gambia, household contacts	2348 contacts over 2 years (4312 person-years)	11/649 (1.7%) ELISPot positive developed active TB (9.24/1000 person-years) versus 14/843 (1.7%) TST positive (9.02/1000 person-years). 10/1087 ELISpot negative developed TB vs 11/1387 TST negative participants.	Undertaken in a high burden country with insufficient power to address the research question. High risk of re-infection.
Kik S et al 2008[10]~	Netherlands, immigrants	339 immigrants for 2 years (499 person years)	7/184 (3.8%) were positive for TST (cut-off ≥15 mm), 5/178 (2.8%) for QFT and 6 of 181 (3.3%) for TSpot TB	Limited by small sample size.
Doherty PM et al 2002[8]	Ethiopia, household contacts	24 contacts over 2 years	6/7 (86%) with TB had positive ESAT-6 and all 17 (100%) with no TB had negative ESAT-6	Very small study in a high burden country
Higuchi K et al 2007[9]	Japan, school contacts	84 TST positive/IGRA negative students for 3.5 years	No cases reported 100% negative predictive value	Small study with no cases of active TB

~ low burden countries, ESAT6 - Early Secretory Antigenic Target 6, QFT – Quantiferon, TST – tuberculin skin test

1.2 National and International Guidelines and Policy Context

1.2.1 National Policy

The national policy for the control of tuberculosis is based on the action plan published in 2004 by the Chief Medical Officer, "Stopping TB in England"[14] with a major focus on the prompt identification of active TB cases and ensuring that they complete treatment.[14] A recent meeting (November 2008) of TB experts convened by the Department of Health in London to review progress on the TB Action Plan concluded that more widespread identification and treatment of latent infection should be prioritised.

1.3.2 Guidelines

The national guidelines for the clinical and public health management of TB, developed by a group which included several of the co-applicants, were published by the National Institute for Health and Clinical Excellence (NICE) in March 2006.[15] These guidelines include recommendations on the diagnosis and management of active and latent TB infection including the use of IGRA tests. Based on cost effectiveness analysis, undertaken in the absence of data on the predictive value of IGRAs, the recommended approach for screening consists of a two step process, a TST is administered, and if positive, followed by an IGRA test. This conflicts with guidelines from other countries such as the USA[16] where IGRA tests have been recommended to replace TST (although BCG vaccination is not routinely given in the USA).

2.0 Objectives

2.1 Primary objectives

1. To assess the prognostic value of the current two IGRA tests compared with the standard Mantoux TST for predicting active TB among untreated individuals at increased risk of TB, specifically:

a. Contacts of active TB cases.

b. New entrants from high TB burden countries.

2. To assess the cost effectiveness of various screening strategies (including the NICE recommended two step approach) using IGRA tests and/or TST in defined patient groups (contacts and new entrants stratified by age and risk).

2.2 Secondary objectives

1. To independently quantify and compare the predictive value of a whole blood ELISA based assay and an ELISpot based assay.
2. To assess the prognostic value of IGRAs compared with TST for predicting active disease in HIV infected individuals including those from high burden countries.
3. To estimate the prognostic value of IGRA tests for those on treatment for latent tuberculosis infection (LTBI).
4. To develop a collection of specimens (serum and white blood cells), with linked clinical and epidemiological information, to investigate the prognostic value of new biomarkers for TB infection and disease progression.

3.0 Method

3.1 Design

3.1.1 Setting, population and disease burden

This prospective cohort study will recruit individuals 16 years or older, who are: (a) close contacts of cases of active TB or (b) new entrants from high incidence countries (>40/100000) who have arrived in the UK within the past 2 years.

The work will take place across a network of hospitals in North East, North Central and North West London (see Appendix 2) 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. All study sites will be coordinated from the HPA Respiratory and Systemic Infections Department, Centre for Infections. 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 with rates comparable to developing countries such as Newham and Brent). 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]

3.1.2 Recruitment and inclusion criteria

Contacts of all active TB (pulmonary and extra-pulmonary) patients and new entrants from high incidence countries (>40/100000) 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). Although patients with sputum smear positive pulmonary TB cases are known to be more infectious, UK data suggest that the yield of contact investigation in the households of extra pulmonary cases is equally high.[15] 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 both TST and two IGRA tests at this stage. Details of our estimated recruitment rates are given in section 3.5.1.

3.1.3 Health technologies being assessed

LTBI measures: Participants will be tested by Mantoux TST and two IGRA tests Quantiferon-Gold In Tube (ELISA) and ELISpot assay (the same assay as in TSpotTB). 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, and for new entrants at least six weeks after arrival in the UK, to ensure that

nearly all participants have been given sufficient time to develop a cell mediated immune response detectable by TST and 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 40ml blood specimen will be collected. After IGRA testing, full blood count, vitamin D assay (see section D) and repetition of indeterminate assays, the residuum will be stored for future research (see bio-repository for details in section D). 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 to using appropriately equipped courier services to a study testing centre (the national Mycobacterium Reference Unit (MRU), HPA Centre for Infections, Whitechapel or the TB Immunology Group laboratories at St Mary's Campus. The Quantiferon Gold In Tube assay will be offered by the relevant local laboratory where available.

3.1.4 Test results and further action

Clinicians will be informed of all test results by the testing laboratory. Subsequent action after testing will follow existing NICE guidance:[15]
A) If negative by TST and IGRA, follow up only.
B) If positive by either TST or IGRA tests, active TB will be excluded. Those without active TB will be followed up irrespective of age.
C) If positive by TST and either one or both IGRA test(s): those 35 years or over, follow up only. For those 16-34 years, chemoprophylaxis will be offered after excluding active TB, with balanced advice about potential benefits/risks.

Discussion with patients will cover the need to refer for tests to exclude active disease, the recommendation of chemoprophylaxis if under 35 years and the need for observation in those 35 years and over. Where appropriate BCG will be offered in line with NICE guidelines.

We will prioritise the recruitment of patients who are not eligible for chemoprophylaxis (over 35 years) amongst whom we will be able to estimate and compare the ability of TST and IGRA tests to predict natural progression to active disease.

However, we also plan to test and collect data on those under 35 who may be offered chemophrophylaxis. From previous studies and experience in study centres we recognise that: a) many of those aged 16 to 34 years years will have negative TST and/or IGRA results and not be offered chemoprophylaxis, and b) not all of the participants offered chemoprophylaxis will accept it. Both of these groups will be eligible for inclusion in the natural progression analysis (although the proportion in b) will need to be adjusted for in the analysis). Also chemoprophylaxis may not always be effective so data from those taking chemoprophylaxis may still be useful in a preliminary comparison of the predictive value of these assays among treated individuals, and allow investigation of factors associated with treatment failure. Including those aged between 16 and 34 years will not extend the duration of the study, and can be done with minimal additional cost (the only additional cost is that of the tests). However it does require all tests to be undertaken on the whole cohort as the participants eligible for inclusion will only be evident at a later date.

As all participants will have all three assays done we will be able to estimate and compare the predictive value of each test individually, combinations of pairs of test results, and stepped sequences of tests including the NICE recommended two step approach based on only those with a positive TST being offered IGRAs. This proposal will therefore provide answers to both a “triage” two step approach and a “replacement” strategy where IGRAs replace TST.[20]

3.1.5 Follow-up

All participants will be followed for an average of 24 months (between 18 and 36 months) from the date of IGRA/TST testing using a number of approaches (see Appendix 1 – flow chart): 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 the HPA Centre for Infections.

The majority of newly infected patients will be expected to develop disease 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.

We propose also to continue the follow up of participants, beyond the study end point, through the national surveillance system and the mycobacterial surveillance database at no added cost to the NIHR to inform future research by identifying slow progressors (see further details below in section D).

3.2 Data Collection

Data will be collected using computer assisted interviewing by trained research nurses for the base line 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 the Respiratory and Systemic Infections Department of the HPA. 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 the Health Protection Agency and does not add extra cost to the study.

DNA finger printing data, from the UK national TB strain typing database hosted by the Centre for Infections, Health Protection Agency, 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.

3.3 Outcome measures and definitions

3.3.1 Primary Outcome:
a. Development of active TB (Incident Rate Ratios). Prognostic values of tests quantified as incidence rate ratios among contacts and new entrants. 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. Cost per QALY (see section 3.6 for details).

3.3.2 Secondary Outcome: Side effects from chemoprophylaxis.

3.4 Analysis Plan

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 (TST, ELISpot and ELISA) and of each test strategy (TST+ELISpot, TST+ELISA, and the two step strategies) 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]

Secondary analyses will investigate the degree to which progression to active disease, and the ability of tests and test combinations to predict progression, is influenced by patient characteristics and patient management. These analyses will be undertaken for the whole cohort. Poisson regression modelling will be used to investigate the impact of risk factors, including vitamin D levels, on progression to active TB. Including interaction terms between each test result and each risk factor will evaluate whether the predictive performance of any test is moderated by the risk factor. Results will be computed for the performance of the tests in key subgroups, such as HIV-infected persons and those treated with chemoprophylaxis.

3.5 Sample Size and Recruitment Rates (see appendix one for flow chart)

3.5.1 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 disease progression of simulated study participants data were created presuming a LTBI rate of 30%, 5% of LTBI participants progressing to active TB in 2 years if untreated, as observed in the German study[5] for contacts and the Dutch study for immigrants[10]. Test results were simulated for each participant using sensitivities and specificities ranging between 65% and 95%.

The simulations indicated that a cohort of 5,000 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.

50% of TB contacts and new entrants are aged 35 years and over, so a cohort of 10,000 would initially yield at least 5,000 for the primary analysis of progression without treatment amongst whom 90 evaluable events would occur. Given likely loss to follow-up (which based on clinic data is likely to be around 20%) and the possibility that progression to disease may be lower than 5%, the power of the study will be maintained by including in the cohort all participants aged 16 to 35 years who do not take chemoprophylaxis (estimated to be an additional 2500 – see below). A total of 90 incident events would still be observed should 7500 be recruited, 20% lost to follow-up and the progression to disease be only 4.2%.

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 explore a) comparing contacts and new entrants, b) estimating progression on treatment using regression models to adjust for test-dependent treatment decisions, although the power in particular subgroups may be limited.

3.5.2 Recruitment Rates

The study will recruit a total of 10,363 participants (5875 contacts and 2448 new entrants from TB clinics and 2040 new entrants from GP surgeries). To ensure that we have identified any potential difficulties, our GP network in East London has agreed to undertake a locally funded pilot study prior to the award of this grant. The North West London chest service is also currently undertaking a pilot of screening new entrants with TST and the Quantiferon Gold In Tube assay. These studies will inform our recruitment protocol.

We expect to be able to recruit 5875 contacts in 12 TB clinics at a rate of 7 per week. This is based on a survey of the number of contacts screened at nine of the 12 participating centres (average of 24 contacts per week) and assuming a participation rate of 30% (after excluding ineligible groups and refusals). Using surveillance data reported from each study centre (appendix 2), we can extrapolate that it would be possible to recruit at least 7 participants per clinic per week. We have similarly estimated that we can recruit 3 new entrants (of 10 normally seen i.e. participation rate of 30%) either directly in 12 TB clinics or through referring primary care services per week. Over a period of 68 weeks we will be able to recruit 2448 new entrants.

Our network of GP surgeries in East London consists of three PCTs (each with between 35 – 45 GP surgeries). In Hackney about 25 patients are registered per practice per week, of which 10-15 per practice will be new entrants and so eligible. Therefore, for each group of 6 practices in a PCT we have 50-75 eligible/week, so recruiting 10 per week should be possible. Numbers are similar in Tower Hamlets and Newham. We have calculated, based on this and data from a previous trial[24], that we will be able to recruit 2040 new entrants, i.e. 10 patients per PCT per week, over a 68 week period. For two practices in Newham that run a specialist immigrant service, we may be able to achieve a higher recruitment rate.

Those aged 16 to 34 years who adhere to the course of treatment for latent infection will therefore be at a substantially lower risk of developing TB (lost to the primary analysis). We estimate, based on data from two centres, that this will be about 49% of contacts in this age group; as approximately 70% would agree to, and around 70% comply with, chemoprophylaxis. In addition, only new entrants aged 16 to 34 years from countries with active TB incidence greater than 500/100,000 or from sub-Saharan African are recommended chemoprophylaxis. This age group will therefore contribute to meeting the minimum sample size and provide data for a secondary objective. It is important to include the 16 to 34 year age group as this is the group where chemoprophylaxis is recommended in national guidelines. This analysis may also provide valuable data on compliance with, and the effectiveness of, chemoprophylaxis in this age group – in turn informing future policy.

18 centres in high incidence areas have agreed to participate in the study. While our estimates for recruitment is based on 12 TB clinics, we will have the ability to recruit from a further 6 centres.

3.6 Economic Analysis

We will evaluate the cost-effectiveness of alternative screening strategies for patients with suspected latent TB infection. A decision model will be developed to estimate the costs (£) and health effects (QALYs) of eight key screening strategies: no screening; each test on its own (TST, ELISA or ELISpot); TST followed by ELISA or ELISpot if positive (NICE recommendation); and ELISA or ELISpot followed by TST if positive. Additional strategies can be modelled if desired. The strategies will be compared for different cohorts of patients: contacts and new entrants stratified by age and baseline risk (defined by level of exposure for contacts and incidence in country of origin for new entrants). If sufficient data are available, results will also be estimated for HIV positive subjects.

We anticipate that the model will comprise a decision tree (as illustrated in Appendix 3) linked to a dynamic state transition model (illustrated in Appendix 4). The decision tree will define the short-term pathways that members of a cohort may take under each of the strategies. This will allow for the possibility of loss to follow-up, for example if patients do not return to have their TST result read, as well as the various test results and treatment decisions. At the endpoints of the tree (the shaded boxes in Appendix 3), members of the cohort transfer to an appropriate starting point in the state transition model (Appendix 4). For example, without testing for latent infection, patients with active TB start at health state E and other patients at health state A. The state transition model then predicts long-term outcomes for the cohort. Over time, the patients with active disease may be detected and treated, and this treatment may or may not be successfully completed. Some patients who do not initially have active disease will develop it (the dotted arrows), or they may be diagnosed and treated for latent infection. By modelling the progress of the cohort over a defined time horizon, we will estimate the proportion of time that patients spend in the different states, and hence the associated screening, diagnosis and treatment costs and health outcomes (QALYs).

The model will use data from the cohort study to estimate the risk of developing active tuberculosis for individuals with different TST/IGRA results and baseline characteristics with and without prophylactic treatment (as estimated by the Poisson regression approach described above). Estimates of the effects of prophylaxis from the cohort study may, however, be subject to confounding. We will therefore also estimate the results based on treatment effects from published RCTs of chemoprophylaxis (updating the systematic review by Smieja et al).[15;25] The incidence of severe prophylaxis-related adverse effects will be estimated from the literature.[26] QALY losses due to active disease and treatment side effects will be estimated using utility estimates from the literature[27;28]. In addition, we have undertaken a study to determine the quality of life of TB patients in three London hospitals which provide local data for the generation of QALYs.[29] Background and TB-related mortality rates and other parameters will be estimated from national data (Government Actuaries Department and Health Protection Agency). Estimates of the costs of testing for latent infection, chemoprophylaxis and diagnosis and treatment of active TB will be obtained from the clinics and hospitals participating in the cohort study.

Benefits due to prevention of transmission will also be estimated, although we do not intend to develop a full population dynamic model. The HPA, in collaboration with the Imperial College and UCL, has developed a transmission dynamic model from a Department of Health funded study which will be available to this study. The probability of transmission is a function of the time that members of the cohort spend with untreated TB (health state E in Appendix 4). Using this information, and assumptions about the risks of transmission, we will estimate QALY losses for people infected by members of our cohort and the associated costs of contact tracing, testing and treatment. The plausibility of these assumptions will be tested against the results of the HPA model.

The methods of economic evaluation will follow the standard NICE reference case.[30] The analysis will be conducted from an NHS perspective with a 15 year time horizon. All costs and outcomes will be discounted at 3.5% per annum. Probabilistic sensitivity analysis will be used to investigate the impact of uncertainty over model parameters, and the value of collecting additional information. Deterministic sensitivity analysis will also be used to test the impact of structural uncertainty over modelling assumptions (e.g. the time horizon).

Once developed, the model could be quite easily adapted to estimate the cost-effectiveness of vitamin D testing and supplementation. If the cohort study indicates that vitamin D

deficiency is an important risk factor, and if time allows, we will conduct this additional analysis.

3.7 Impact of genetic variance in vitamin D processing

Some people are more likely to get TB than others. Risk of getting TB can depend on things like age, lifestyle, or diet, but genes that are passed through families can also affect the risk of getting TB, especially ones involved in how the body uses vitamin D. Understanding what these are might help develop better ways of preventing people getting TB. Analysis of the genes controlling vitamin D processing will be undertaken using the sample already collected for the main study i.e. no greater volume will be needed to achieve this assessment.

4.0 Justification for Study Design, Groups Included and Assay Choice

A prospective cohort design will be used to determine patient outcome as this allows the determination of incidence rate ratios comparing TST and IGRA tests and minimises bias. Sufficient numbers of participants will be recruited to determine the prognostic value of IGRAs among recent contacts of TB cases and among new entrants due to the high risk of TB and poor evidence base for subsequent action.

Every study that has compared the ELISpot and ELISA IGRA tests to each other has found a significant proportion of subjects with discordant test results,[31] the significance of which is unknown. Results from one test cannot therefore be extrapolated to the other test and it is important to independently determine the prognostic value of both assay platforms. Therefore all participants will have both whole blood ELISA (Quantiferon-TB Gold In Tube) and an ELISpot assay (on which the T-SPot.TB test is based) performed. The ELISA will be performed at each centre already running it routinely (approx 4-5). ELISpot will be performed at Professor Lalvani's laboratory at St Mary's Campus, Imperial College, London and the HPA Mycobacterium Reference Unit, London using an identical standardised protocol. These 2 centres will also perform ELISA for all centres with no Quantiferon service. Residual plasma and white blood cells will be stored in a biobank at these two centres.

Practical issues that may cause difficulties include obtaining consent from a multicultural group (to be done by trained nurses with translation facilities), taking 25 mls of blood (this should be possible with a single needle as all participants are adults), the frequency of indeterminate results (to be minimised by ensuring daily transportation of samples by study nurses and provision for retesting using residual blood).

5.0 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. The HPA will be the nominated sponsor.

D. Further activities at minimum cost which will add value to the study

We propose to undertake further work at minimal or no cost to the funders which will provide significant gain to the NHS. We have assembled a strong TB research network with potential access to a well-characterised cohort of individuals exposed to *M. tuberculosis* and therefore have a unique opportunity to undertake these additional tasks:

i. Bio-repository

A bio-bank of the residual samples will be created 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. The results of published studies to date suggest that IGRAs may not be vastly superior to TST testing. It is therefore 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 if necessary as well as serum 25(OH)D (vitamin D). Storage of cellular and plasma fractions will be at –80C, with the MRU and St Mary's providing freezer capacity and security of samples. A small number of plasma and cellular samples will be repeated at both sites 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.

ii. Assessing the role of vitamin D as a risk factor for progression to active disease

Vitamin D deficiency is very common in London TB contacts, and vitamin D supplementation enhances their immunity to mycobacterial infection.[32] Vitamin D was used to treat TB in the pre-antibiotic era,[33] and vitamin D metabolites enhance immunity to *M. tuberculosis* in vitro.[34] A number of case control studies have reported that vitamin D deficiency is associated with an increased risk of active tuberculosis.[35] Interpretation of this finding is limited by the retrospective nature of these studies – they cannot determine whether vitamin D deficiency is the cause or the consequence of active TB. A prospective study is needed to resolve this important question.

By measuring serum 25(OH)D (the accepted best measure of vitamin D status)[36] and performing IGRA in study participants at baseline, and then determining whether they subsequently develop active TB, we will be able to investigate whether vitamin D deficiency predisposes to reactivation of LTBI. No previous cohort study has addressed this question, and we are in a unique position to do so, given the very large size of our cohort, the very high prevalence of vitamin D deficiency in London TB contacts, and our use of state-of-the-art LTBI diagnostics (IGRAs) which have potentially superior sensitivity and specificity for the diagnosis of LTBI in comparison with the TST.[7]

Demonstrating that vitamin D deficiency predisposes to reactivation of LTBI would provide a rationale for conducting a large randomised trial to determine whether vitamin D supplementation prevents TB in contacts. The results of such a trial could be of very significant public health importance, as vitamin D supplementation is safe, inexpensive and easy to administer in intermittent bolus doses.

iii. Longer term follow up through routine surveillance

This will allow subsequent identification of cases of TB in the cohort thereby further strengthening the ability to study further predictors of active TB as well as improving our understanding to latency. We propose that at no extra cost, with appropriate ethical permission, we will under take follow up for 10 years using national surveillance and microbiological data.

iv. Obtaining valuable data to inform mathematical models which can in turn assist national policy decisions

A number of interventions, for example new entrant screening and the use of a mobile x ray unit for active case finding, have been evaluated on behalf of the Department of Health using mathematical models. Often, these are based on data that are not directly applicable to the UK setting. This study will provide UK-specific, relevant outcome measures which will improve our ability to assess interventions; and thus better refine models that deal with national needs and priorities.

E. Expected output of the research

A comprehensive report will be prepared. This will include recommendations to the NIHR HTA regarding evidence for the negative and positive predictive value of IGRAs among contacts, new entrants and HIV infected persons, as well as an economic analysis of the cost effectiveness of various testing strategies. The study will also demonstrate whether vitamin D deficiency predisposes to reactivation of LTBI. The report will summarise findings particularly

relevant to UK tuberculosis control policy. We will also develop a bio-repository which should allow the evaluation, in a cost efficient manner, of the next generation of assays for LTBI. In addition to a formal report to the HTA, the research will be disseminated through peer reviewed publications, conference presentations and engagement with policy makers (Department of Health and the Health Protection Agency), 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).

F. Project time table including milestones

10363 participants will be recruited in 68 weeks by 9 nurses in 12 TB clinics and 3 groups of primary care centres in high incidence areas.

Period	Pre study	Year one			Year two			Year three			Year four		
Month	-4	4	8	12	16	20	24	28	32	36	40	44	48
PRE STUDY PROCESSES													
• Ethics application	★												
• Purchase of equipment/materials for study	★												
• Staff recruitment	★												
• Organisation of recruitment of participants	★												
• Organisation of databases and data collection													
• Publication of study protocol	★												
RECRUITMENT AND FOLLOW UP													
• Recruitment		★											
• Laboratory assays		★											
• Follow up											★		
DATA CLEANING AND ANALYSES													
• Interim event rate analyses													
• Data cleaning											★		
• Matching with HIV data												★	
• Statistical analysis												★	
• Economic analysis												★	
REPORTING													
• Preparation of final report for HTA and paper writing													★

Milestones (marked ★)

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
Year 4	Statistical analysis
	Economic analysis
	Preparation of final report

G. 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.

H. Expertise in the team

We have constituted a multidisciplinary team with a wide and appropriate range of expertise in tuberculosis, study design, statistics and IGRA tests. The team includes authors of key primary studies evaluating IGRA tests, including the inventor of the TSpot TB assay and the health economist who undertook the cost effectiveness analysis for the NICE guidelines.

The team has expertise in all methodologies proposed, a strong record of research funding, and extensive health service research publications. It includes TB researchers of international and national reputation (AL, FD, AH, JW, IA, ML, CG) with highly experienced respiratory physicians (ML, OMK, GB) as well as clinical immunologists (AL, RC) and microbiologists (AL, RC, FD). The group has proven experience in health economics (JL) and statistics and the methodology of test evaluation (JD). FD, IA, JW, ML influence TB policy at national and international levels. Included in the team but not listed as co-applicants are other clinicians in London.

Ibrahim Abubakar is a Consultant Epidemiologist and heads the TB Section at the HPA Centre for Infections. He is an Honorary Senior Lecturer at the London School of Hygiene and Tropical Medicine and at University College London. Ibrahim runs the national enhanced TB surveillance system and a national clinical/public health advisory service on TB incident/outbreak management. He is a member of the WHO Global Task Force on TB Impact Measurement, acting chair of the National Knowledge Service TB and has published over 40 peer reviewed papers including several using IGRAs to investigate TB outbreaks. He has previously received funding from NIHR HTA. More recently he co-wrote the HPA issued position statement on the use of IGRAs.

Francis Drobniewski is Professor of Tuberculosis and Mycobacterial Diseases and Director of the HPA national Mycobacterium Reference Unit. He is Chairman of the WHO Euro Region Laboratory Task Force and a member of the WHO Strategic and Technical Advisory Group, the main global policy group. He has planned/implemented new nationally-available diagnostic services for LTBI, and active TB from patient specimens and reference cultures; and leads one of 25 WHO global Supranational Reference Laboratories. He drafted guidelines for laboratory standards and diagnosis for the WHO and the EU. He co-authored the HPA position statement on the use of IGRA tests, the European Policy statement on the public health use of IGRA assays and the HTA systematic review

of diagnostic methods for TB including IGRA. He brings an international reputation in TB diagnosis and treatment.

Ajit Lalvani is Chair of Infectious Disease and Co-Director of Respiratory Infection at Imperial College London. He invented, developed and validated the ELISpot-based IGRA platform and has studied over 7,000 participants at risk of TB infection in 10 countries. He has published 30 original research articles and 9 invited reviews/editorials on T cell-based diagnosis of TB which have had a major impact on TB control policy and guidelines internationally. He leads a world-leading multidisciplinary research group whose expertise spans the spectrum from bench through bedside to epidemiology and public health. The group's focus is to improve our understanding of the natural history of TB infection and to develop and validate 2nd and 3rd generation immune-based tests and biomarkers of TB infection, prognosis, disease and treatment response. He has worked with several of the hospitals in this consortium for 5-10 years and has a 7-year long collaboration with Prof J Deeks.

Marc Lipman is a Consultant in Respiratory and HIV Medicine at the Royal Free Hospital, London. He is Medical Director of the North Central London TB clinical network, Chair of the British Thoracic Society TB Specialist Advisory Group, a member of HPA TB Programme Board and Expert Advisor to the Department of Health and the National Knowledge Service. He has published widely in tuberculosis, and recently co-authored the HPA position statement on the use of IGRA. He will provide clinical advice to the study.

John M Watson is the Deputy Director of the Respiratory and Systemic Infections Department at the HPA Centre for Infections. He is also a Professor at the University College London and the London School of Hygiene and Tropical Medicine, chairs the European Center for Disease Control TB advisory group and is a member of the BCG sub group of the Joint Committee on Immunisation and Vaccination. He will contribute tuberculosis epidemiology expertise to the study and chair the advisory panel.

Onn Min Kon is a Consultant Respiratory Physician and Honorary Senior Lecturer at Imperial College. He is the lead clinician for TB in the North West London TB sector and also lead clinician for Imperial College Healthcare NHS Trust TB services. He will provide clinical input to the study.

Andrew Hayward is Senior Lecturer in Infectious Disease Epidemiology at the Research Department of Infections and Population Health. Andrew runs a programme of research in respiratory and other infections. He was a member of the NICE guidelines development group and is the Scientific Director of the Infectious Disease Research Network. He will provide methodological expertise to the study.

Chris Griffiths is Professor of Primary Care and General Practitioner in East London. He was a member of the NICE TB guideline development group and will lead the primary care component of this study. He has recently published a randomised controlled trial of evaluating approaches to screening for tuberculosis in primary care in the Lancet.

Graham Bothamley is a Consultant Respiratory Physician at the Homerton Hospital, Honorary Senior Lecturer at the Queen Mary University of London Medical School and the TB lead for the East London TB Network. He has published widely on IGRAs and will provide clinical input to the study.

Joanne Lord is a Reader in Health Economics at Brunel University. Formerly, at NICE, she carried out the economic analysis that informed the recommendations for IGRA testing in the NICE TB guidelines.

Ronnie Chee is a Consultant Immunologist at the Royal Free Hospital and will supervise Quantiferon testing. He will also contribute immunological expertise to the study.

Sarah Anderson is a Consultant in Communicable Disease Control at the North West London Health Protection Unit and has conducted studies on IGRA in the North West London TB Sector including the largest head to head observational study in London to date.

Jon Deeks is Professor of Health Statistics at the University of Birmingham and leads an NIHR funded research team focusing on the evaluation and systematic review of diagnostic tests. He has undertaken, in collaboration with Prof Lalvani, several studies on IGRAs including the recent papers

on the predictive value of IGRAs in Turkey. He will provide statistical and design expertise to the study, and supervise the study statistician and all analyses.

Centre Leads and collaborators:

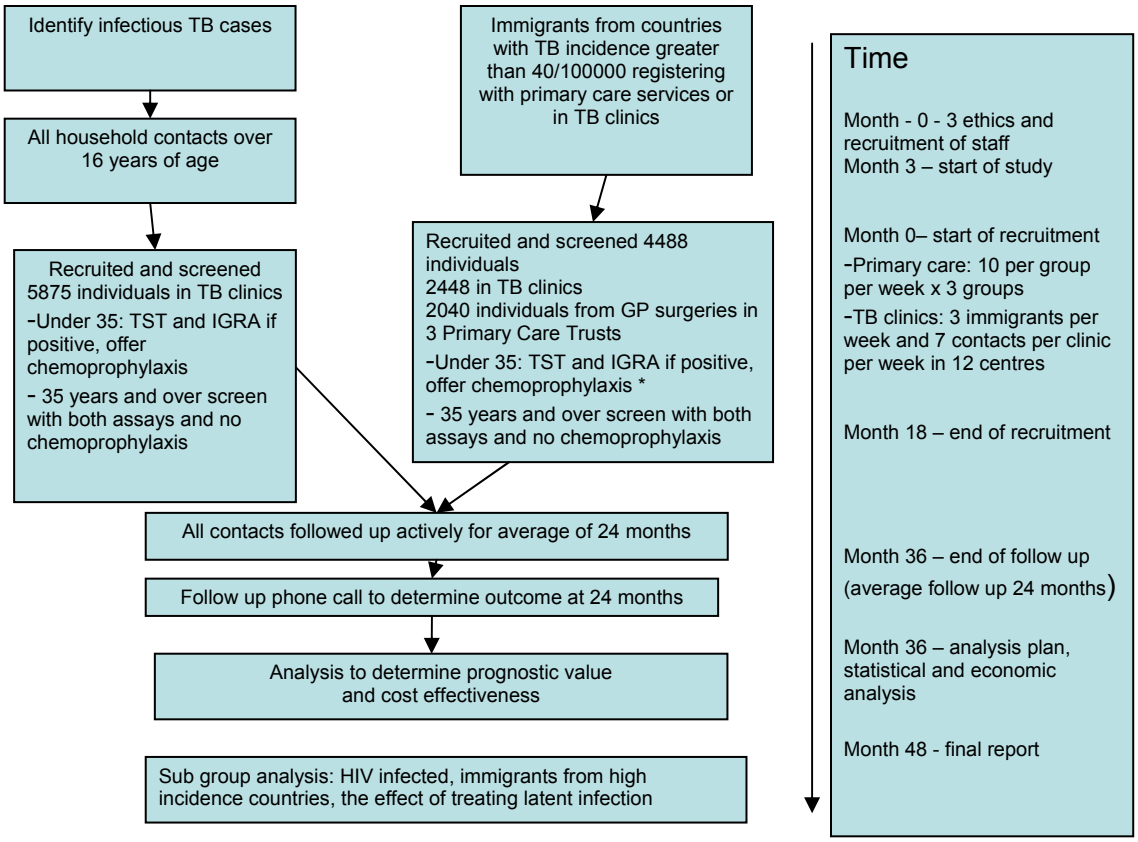
- Adrian Martineau, Queen Mary University of London
- Sudy Anaraki, Consultant in Communicable Disease Control, North East London HPU
- East London Primary Care Network - Prof Chris Griffiths
- Royal Free Hospital – Dr Marc Lipman
- North Middlesex Hospital – Dr Stefan Lozewicz
- Whittington Hospital – Dr Norman Johnson
- Barnet and Edgware Hospital – Dr Deen Creer
- University College London Hospital – Dr Helen Booth
- St Marys Hospital and Hammersmith Hospital – Dr Onn Min Kon
- Central Middlesex Hospital – Dr David Adeboyeku
- Homerton Hospital – Dr Graham Bothamley
- Barts and the London/London Chest – Prof John Moore-Gillon
- Charing Cross Hospital – Dr Frances Sanderson
- Northwick Park Hospital – Dr RN Davidson
- Willesden Chest Hospital - Dr David Adeboyeku
- Ealing Hospital – Drs Willian Lynn, Michael Rudolf and Derek Williams.
- St George's Hospital – Dr Felix Chua
- Whipps Cross University Hospital – Dr Mathina Darmalingam
- Newham General Hospital – Dr Geoff Packe
- Network of Primary Care Practices in North East London - Prof Chris Griffiths
- Peter White, Unit Head, Modelling and Economics Unit, HPA
- Helen Maguire, Regional Epidemiologist, HPA London
- Alistair Story, TB Nurse, Department of Health TB Find and Treat Project

I. The study advisory group, monitoring recruitment and event rates

An advisory panel will be recruited to help oversee the research and supervise the draft report to the NIHR HTA. The panel will be chaired by Professor John M Watson at the Centre for Infections in Colindale and include the co-applicants/collaborators and two patient representatives (recruited through TB alert). The group will meet 3 times a year and routinely review data on recruitment and follow-up rates.

Two interim analyses will be undertaken, one prior to closing recruitment (around month 20) and a second prior to closing follow-up (around month 36). The analyses will be undertaken by the statistical co-applicant (JD) who will prepare data on the number and observed incidence of events (blinded to all test results). If unexpectedly low incidence rates are observed that threaten the value of the study, options for extending recruitment and follow-up will be reviewed by the PI in consultation with the NIHR HTA.

Appendix 1: Flow Diagram

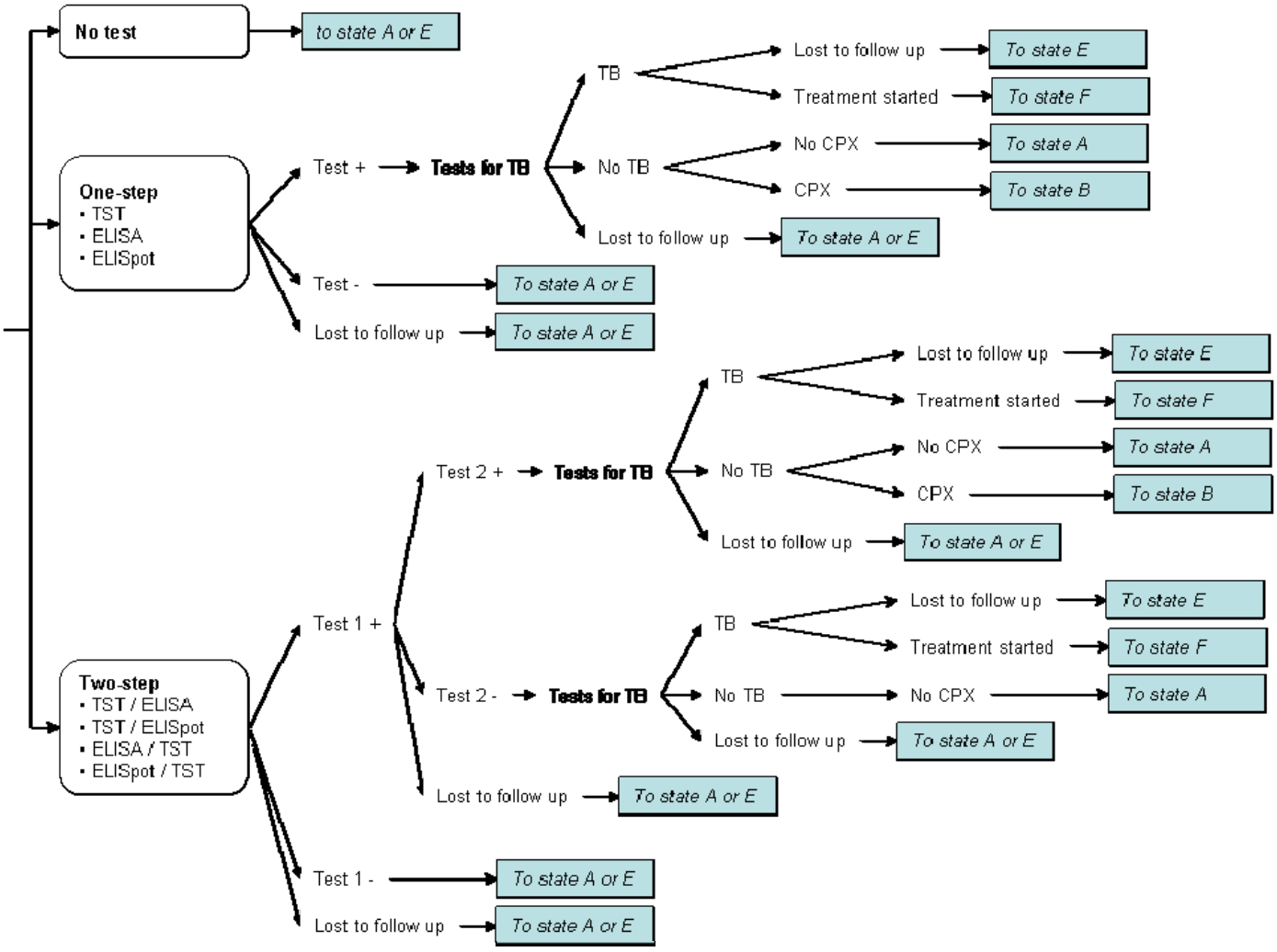


* Screening latent infection is only recommended for new entrants from countries with TB incidence over 500 per 100,000 or from sub-Saharan Africa.

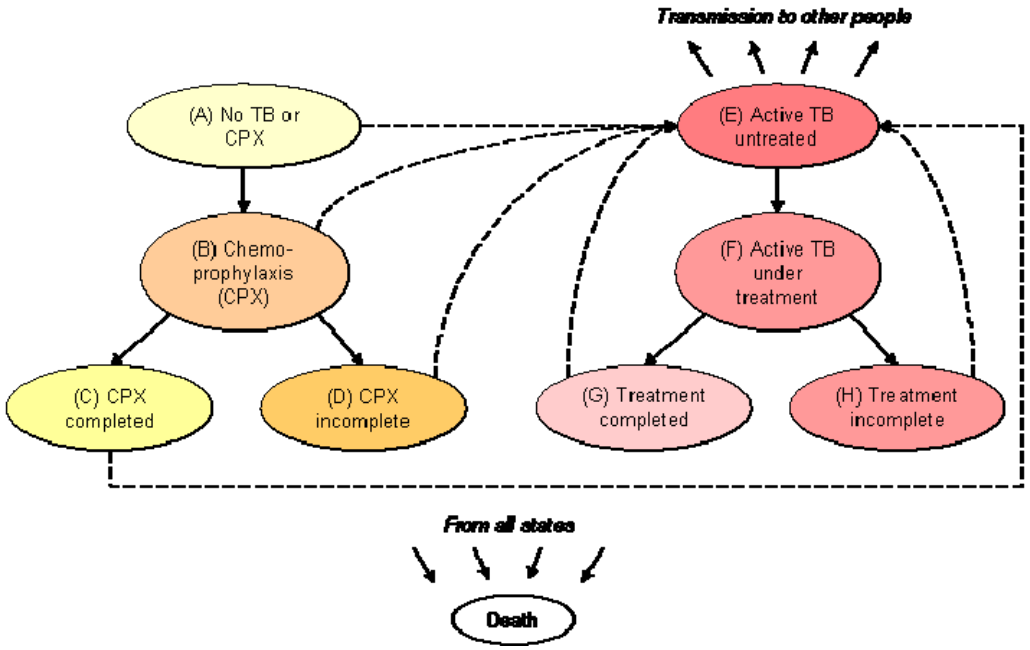
Appendix 2. Number of Active TB cases by age in participating hospitals in 2007

Chest Clinic/ Hospital	Total	0-16	17-35	36+	Sector
Edgware and Barnet TB Clinic	75	6	33	36	NC
North Middlesex Hospital	118	7	58	53	NC
Royal Free	85	4	39	42	NC
UCLH TB Service	118	11	53	54	NC
Homerton	134	17	58	59	NE
King George Hospital	123	4	66	53	NE
London Chest Hospital	231	16	121	94	NE
Newham Chest Clinic	223	10	136	77	NE
Whipps Cross University Hospital	99	7	54	38	NE
Charing Cross Hospital	48		24	24	NW
Chelsea & Westminster	51	4	26	21	NW
Ealing Hospital	194	23	71	100	NW
Hammersmith Hospital (ICH NHS Trust)	74	11	27	36	NW
Hillingdon Hospital	97	8	48	41	NW
Northwick Park Hospital	231	16	109	106	NW
St Mary's (ICH NHS Trust)	145	22	53	70	NW
West Middlesex University Hospital	106	2	59	45	NW
St George's Hospital	176	10	84	82	SW

Appendix 3. Illustration of decision tree



Appendix 4. Illustration of state transition model



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