Validation of New Technologies for Diagnostic Evaluation of Tuberculosis (VANTDET)

RESEARCH PROTOCOL

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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 Validation of New Technologies for Diagnostic Evaluation of Tuberculosis (VANTDET)

DESIGN VANTDET will utilise the biobank of clinical samples collected in the IDEA (IGRA in Diagnostic Evaluation of Active TB) study, a NIHR HTA-funded prospective cohort study assessing the role of IGRAs and conventional testing in the diagnostic evaluation of suspected active TB. Patients with suspected TB are recruited and followed up for 6 months to determine final diagnoses.

AIMS 1. To validate novel biomarkers and tests for rapid diagnosis of active TB in routine NHS practice, and determine optimum testing strategies and algorithms.

2. To carry out comprehensive health economic analyses to determine the cost effectiveness of the novel tests (and their use within proposed algorithms) in routine NHS practice.

POPULATION 1012 Adults presenting with *suspected active TB,* aged 16 years or over (recruited in the IDEA study).

DURATION 3 Years and 10 months.

2. GLOSSARY OF ABBREVIATIONS

BCG	Bacillus Calmette–Guérin
EPTB	Extra-PulmonaryTuberculosis
HPA	Health Protection Agency
ICER	Incremental Cost Effectiveness Ratio
IFN-y	Interferon Gamma
IGRA	Interferon Gamma Release Assay
IL-2	Interleukin 2
LTBI	Latent Tuberculosis Infection
MS	Mass Spectrometry
Mtb	Mycobacterium Tuberculosis
NHS	National Health Service
NICE	National Institute for Health and Clinical Excellence
PBMC	Peripheral Blood Mononuclear Cell
PPD	Purified Protein Derivative
PTB	Pulmonary Tuberculosis
RT PCR	Reverse Transcription Polymerase Chain Reaction
SOP	Standard Operating Procedure
TB	Tuberculosis
TNF-a	Tumour Necrosis Factor Alpha
TST	Tuberculin Skin Test
WHO	World Health Organisation

3. KEYWORDS

Tuberculosis (TB); Active TB (ATB); Latent TB Infection (LTBI); Biomarker; Diagnostics

4. BACKGROUND

Tuberculosis (TB) is a devastating disease worldwide, resulting in 8 million cases annually (1). Britain has among the highest rates of TB in Western Europe and the national burden has risen progressively over the last twenty years, reaching almost 9,000 cases in 2011 (2). The majority of TB cases occur in large cities (especially London, Birmingham and Leicester) and the disease disproportionately affects immigrants and socially marginalised and socioeconomically disadvantaged groups.

Prompt and accurate diagnosis and treatment of TB are essential to improve individual patient outcomes, as well as to prevent onward transmission in the community with its attendant health-care associated and social costs. However, the currently available diagnostic toolkit lacks the ability to provide results with clinically acceptable speed, sensitivity and specificity. Reversing the vast TB burden and progressing towards elimination are nationally and internationally agreed public health priorities at the level of the United Kingdom (UK), European Union (EU) and World Health Organisation (WHO) (1, 3). However, internationally agreed targets for improved global TB control are not achievable with existing tools. New, innovative methods for TB prevention, diagnosis and treatment are urgently needed and the WHO's *Global Plan* calls for research to deliver an improved, fit-for-purpose anti-TB toolkit, with major emphasis on the need for new diagnostic tests (3). Improved diagnostic tests would moreover enable the National Health Service (NHS) to turn the tide on the sustained 20 year increase in TB in the UK.

Currently Available Tests for Diagnosing Active TB:

Currently available tests recommended by the National Institute for Health and Clinical Excellence (NICE) for diagnosis of active TB comprise smear microscopy, *Mycobacterium tuberculosis* (*Mtb*) culture and radiography (4). Smear microscopy is rapid but lacks sensitivity. *Mtb* culture is the current gold standard, but is limited by its lack of speed (requiring 2-6 weeks to generate a result) and is only moderately sensitive. Importantly, both smear microscopy and *Mtb* culture suffer from reduced sensitivity in a number of key 'hard-to-diagnose' patient populations, including those with extra-pulmonary TB (EPTB) or HIV co-infection. Radiography lacks specificity.

The new GeneXpert MTB/RIF test (Cepheid Inc, CA, USA) has higher sensitivity than smear microscopy and provides a result on the same day. GeneXpert is a substantial improvement on conventional microbiological tests, but the sensitivity is slightly lower than culture, and sensitivity amongst culture-negative cases is very low. This means that culture-negative TB cases (another key 'hard-to-diagnose' patient subgroup) are mostly falsely-negative when tested by GeneXpert. As with microscopy and culture, sensitivity of GeneXpert is also reduced in EPTB and HIV co-infection. Furthermore, GeneXpert is expensive, and its cost effectiveness within routine NHS practice is unknown.

The Tuberculin Skin Test (TST) and Interferon Gamma Release Assay (IGRA) are immunological tests recommended by NICE for diagnosis of latent TB infection (LTBI) (4). However, neither test is able to discriminate between LTBI and active TB disease, and both tests lack sufficient diagnostic sensitivity to exclude a suspected diagnosis of TB (5).

The Unmet Clinical Need:

The lack of a rapid, accurate diagnostic blood test for TB is a major roadblock to improved management of TB patients and prevention of TB transmission. Rapid, sensitive and specific diagnosis of active TB would transform management of the disease by enabling prompt treatment initiation, thus improving patient outcomes, lessening morbidity and mortality and curtailing spread of infection. A rapid test of high specificity (i.e. the ability to distinguish

active TB from non-TB illnesses or latent TB infection) but with better sensitivity than culture and GeneXpert would be a major clinical advance.

In parallel, a test of ultra-high sensitivity for TB infection, even without distinguishing active from latent TB infection, would be clinically very useful by providing a rapid 'rule-out' test (since TB infection is a prerequisite for active TB disease). This would enable swift triage of patients with suspected active TB by rapidly excluding this potential diagnosis.

The public health need for better TB diagnosis is thus large, growing and acute. New, more accurate rapid diagnostic tests for TB would not only enable improved TB control in the UK but would provide a major new weapon in the global fight against TB. Development and validation of such innovative new tests would position the UK as an innovation-leader in this field and could engender substantial economic benefits as deployment of new diagnostic tests is scaled up internationally.

4.1 Existing Research towards Improved Diagnosis of TB

'Rule-In' of Active TB:

There has recently been an explosion in discovery of biomarkers for TB based on measurement of a wide range of host responses with very promising results obtained from: genome-wide gene-expression microarrays, proteomics and characterisation of functional T cell subsets by flow-cytometry (Table 1). The resulting gene expression (6, 7) (*Kafarou et al. PLoS Med. 2013*), proteomic (8) (Levin et al. Manuscript in preparation) and cellular immunologic (9, 10)(11)(*Pollock et al., J Infect Dis. 2013*) signatures can distinguish active TB disease from other non-TB diseases and from latent infection. Technological feasibility and clinical proof-of-principle of these novel approaches for diagnosis of TB has now been established. Their high diagnostic sensitivities and specificities could revolutionise TB diagnosis. *Given the large clinical and public health unmet need, it is therefore now an urgent priority to rigorously validate these promising biomarkers in a large, adequately powered prospective cohort study in routine clinical practice.*

<u>Whole Blood Gene Expression Signatures</u>
Genome-wide gene expression microarrays are able to define whole-blood transcriptomic signatures that can be correlated with clinical diagnoses. Such gene expression signatures have shown promise in TB by using a molecular snapshot to identify active TB in cross-sectional case-control studies (6, 7) (*Kafarou et al. PLoS Med. 2013*). Using controls with non-TB illnesses and healthy controls with latent infection, multi-gene expression signatures have been derived that can distinguish active TB from these other conditions with a high level of accuracy. In one study, the most differentially expressed genes were further examined by reverse transcriptase polymerase chain reaction (RT PCR) to provide technical confirmation of the signatures and quantify gene-expression (7).

These three studies are the only ones carried out to date of signatures that discriminate active TB from LTBI and/or other inflammatory/infectious diseases in reasonable numbers of patients, i.e. using more than a handful of subjects. Furthermore, in all three studies, the signatures were validated in at least two independent sets of subjects. (Maertzdorf *et al* (7) validated a previously identified signature reported in Jacobsen et al., 2007 (12).) Other gene expression studies reported in the literature are not described in this proposal as patients with active TB were compared only with healthy control subjects (13), or because small subjects numbers were used (14).

Sensitivity and specificity of these gene expression signatures for detection of active TB were high in each of these 'proof-of-principle', case-control studies (Table 2). They therefore

now need *validation in a prospective cohort study to quantify and evaluate their diagnostic performance and clinical utility in routine practice in patients presenting with suspected TB.*

Table 1. Novel signatures and tests requiring validation in the routine diagnostic work up of active TB

	Signature/Test	Method(s)	Source	References
Rule-in test for active TB	Whole-blood gene expression signature	Microarray RT PCR	Published signatures and collaboration	Berry et al., *Nature. 2010* Maertzdorf et al. *Genes Immun. 2011* Kaforou et al.(*PLoS Med, 2013*)
	Plasma-derived proteomic signature	Mass spectrometry	Published signatures and collaboration	Sandhu et al. PLoS One. 2012 Levin et al (*manuscript in preparation*)
	Cellular immunological signature	Flow cytometry	Developed by applicant	Pollock et al. *J Infect Dis.* 2013
Rule-out test of infection	Stimulated whole blood chemokine assay	RT PCR	Developed by applicant	Kasprowicz et al. *PLoS One. 2011* Millington et al. *PNAS. 2011* Connell et al *Thorax 2012 (BTS abst.)*

Table 2. Previously identified gene expression signatures of active TB

Study	Population	Signature	Sensitivity*	Specificity*
Berry et al. Nature. 2010	Training set: 13 PTB, 17 LTBI, 12 NID; Test set: 21 PTB, 21 LTBI, 12 NID (UK); Validation set: 20 PTB, 31 LTBI (South Africa)	393 genes distinguish TB v LTBI; 86 distinguish TB v OD	62-94% TB v LTBI 90-92% TB v OD	94-97% TB v LTBI; 83% TB v OD
Maertzdorf et al. Genes Immun. 2011	33 PTB, 34 LTBI, 9 NID (South Africa)	927 genes differentiate TB v LTBI/NID; 5 differentiate TB in RT PCR	94%	97%
Kaforou et al. (*PLoS Med, 2013)*	Training set: 157 TB, 128 LTBI, 140 OD; Test set: 42 TB, 39 LTBI, 34 OD (Malawi and South Africa)	27 genes discriminate TB v LTBI; 44 discriminate TB v OD	95% TB v LTBI 93% TB v OD	90% TB v LTBI 88% TB v OD

*Sensitivity and specificity of gene signature for diagnosis of active TB. PTB: pulmonary TB; LTBI: latent TB infection; NID: non-infected donors; HC: healthy controls; OD: other disease; rTB: recurrent TB; cTB: cured TB

<u>Proteomic Signatures</u>

Recent technological advances have resulted in proteomic techniques able to rapidly identify unique patterns of circulating proteins and protein fragments that have great potential as biomarkers. These have been exploited in recent years to define serum-derived proteomic signatures specific to active TB. A previous study described a distinctive proteomic fingerprint (based on mass spectral peaks) that distinguished patients with active TB from those with other respiratory illness, a proportion of whom had underlying LTBI (8). Subsequently, our collaborator Prof Levin has identified a 20 protein signature, also

discriminating active TB from other diseases and LTBI (*Levin et al. manuscript in preparation*). Sensitivity and specificity of these signatures were very high (Table 3) and they therefore warrant systematic prospective validation to determine *their diagnostic performance and clinical utility* in the routine diagnostic assessment of suspected TB.

Table 3. Previously identified proteomic signatures of active TB

Study	Population	Signature	Sensitivity*	Specificity*
Sandhu et al. PLoS One. 2012	151 ATB, 97 symptomatic controls (53 LTBI), Peru	3-peak mass spectra fingerprint	89% ATB v LTBI 90% ATB v OD	82% ATB v LTBI 92% ATB v OD
Levin et al (*manuscript in preparation)*	Training set: 157 TB, 128 LTBI, 140 OD; Test set: 42 TB, 39 LTBI, 34 OD (Malawi and South Africa)	20 proteins	90% ATB v LTBI 85% ATB v OD	85% ATB v LTBI 90% ATB v OD

ATB: active TB; LTBI: latent TB infection; OD: other disease

Cellular Immunological Signatures

Functional characterisation of antigen-specific T cells has been exploited as a means of distinguishing different stages of *Mtb* infection. A predominance of *Mtb*-specific T cells secreting IFNγ-only or TNFα-only reflects breakdown of immune control and higher antigen and bacterial burden. Notably, the frequency of TNFα-only-secreting *Mtb*-specific T cells was reported to distinguish patients with active disease from those with LTBI with 92% specificity, albeit with low sensitivity (67%) (10). TNFα-only-secreting *Mtb*-specific T cells furthermore correlate with mycobacterial burden in TB (9).

Our recent work has exploited the potential diagnostic value of TNFα-only-secreting *Mtb*-specific T cells whilst simultaneously improving diagnostic accuracy by combining measurement of T cell memory/differentiation phenotype (based on cell-surface markers) in parallel. Indeed, measurement of the proportion of TNFα-only secreting PPD-specific CD3+CD4+ cells that were CD45RA-CCR7-CD127- (i.e. highly-differentiated late-effector T cells) was found to be highly discriminatory between ATB and LTBI (Fig 1(a)). The signature had 100% sensitivity and 93% specificity in a test cohort of 34 patients (13 ATB, 21 LTBI), of whom 50% were HIV-positive (Fig 1(b)) (11) (*Pollock et al., J Infect Dis. 2013*). Since then, additional cellular immune signatures have been identified which also show promise in their ability to discriminate between active TB and LTBI. These included those which measure the proportion of Mtb-specific CD4+ IFNγ+ cells with either an activation phenotype (15) (*Adekambi et al., JCI. 2015)* or the levels of CD27 as a ratio or in combination with CD45RA experssion (16, 17) (*Portevin et al., Lancet. 2014; Petruccioli et al., J Infect. 2015*). Flow cytometry is already established in many NHS diagnostic laboratories throughout the UK and is suitable for routine diagnostic use (18). These signatures therefore warrants prospective validation in a large-scale independent cohort in routine practice.

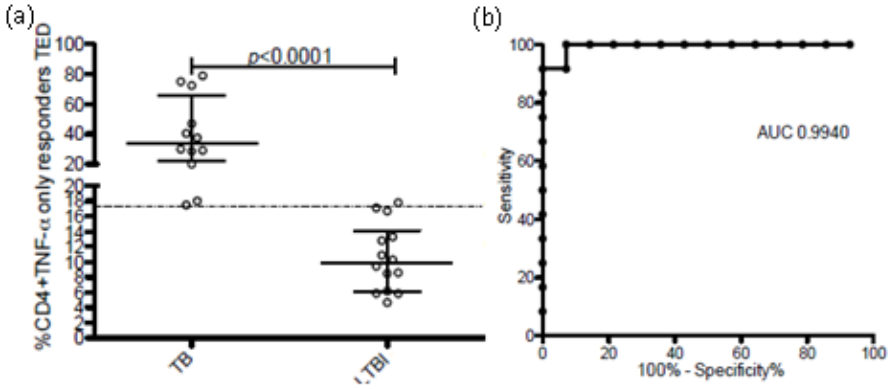

Fig 1. Cellular immunological signature of active TB. Graphs show (a) the % of Mtb-specific, TNFα-only secreting cells that are CD45RA-CCR7-CD127- in TB and LTBI, and (b) a ROC curve of the sensitivity and specificity of the signature for ATB.

'Rule-out' of Active TB:

IGRAs have been evaluated for their potential as 'rule-out' tests in the routine diagnostic work-up of active TB, but lack sufficient sensitivity for this important clinical indication (5). One powerful and pragmatic approach to enhance test sensitivity is to include measurement of additional immune mediators whose secretion is promoted by IFNγ (19). Use of such downstream mediators can provide an amplified signal of antigen-specific IFNγ. Use of *Mtb*-specific antigens for stimulation ensures that, although different secreted immune mediators are being meaured than in IGRA (ie chemokines instead of IFNγ), diagnostic specificity is not compromised.

We have developed a highly sensitive whole blood molecular test with high potential to reliably rule out the diagnosis of TB based on measurement of IFNγ-dependent chemokine release in response to *Mtb*-specific antigens (20). The assay involves overnight stimulation of blood with ESAT-6 and CFP-10, and subsequent measurement of CXCL9 and CXCL10 by RT PCR.

More recently, we incorporated a third highly immunodominant and *Mtb*-specific antigen, EspC which we discovered (21). The assay was 100% sensitive for active TB in a case-control study (n=39) when combining pairs of antigens, regardless of the pairing (ie any 2 of ESAT-6, CFP-10 and EspC (also known as Rv3615c)) (*Connell et al. Thorax. 2012* (BTS Abstract)). Furthermore, the recently completed IDEA study identified that a combination of four Mtb antigens in the T-SPOT TB assay provided the highest sensitivity for active TB (*Tawkoingi et al, HTA Report. Unpublished, 2016*). Use of all four of these antigens together provides potential to maintain 100% sensitivity should diagnostic sensitivity transpire to be <100% in large prospective studies. Specificity was also high in the initial study (94%), with only 1 of 16 IGRA-negative BCG-vaccinated healthy controls scoring positive in the assay. These compelling data suggest potential for a test with sufficiently high diagnostic sensitivity to be used to rule out TB infection (and therefore active TB disease) at first presentation. The promising results with this assay to date now merit validation in a large scale prospective cohort study of patients undergoing routine diagnostic work up for suspected active TB.

4.2 Risks and Benefits

Left untreated, each person with active TB will infect between 10 and 15 people (on average) per year (1). Without treatment, the mortality rates for active TB are 70% for pulmonary disease, and higher for disseminated disease, reaching 99% for military TB.

Therefore, consequences of delayed diagnosis of active TB include prolonged morbidity and increased risk of mortality for the individual patient concerned, as well as further spread of infection across the community. This is exacerbated by the fact that, within the UK, TB disproportionately affects disadvantaged, underprivileged, and marginalised population groups (including homeless, prisoners etc) (2).

Rapid identification and treatment prevents further spread of TB by rendering patients non-infectious, thus reducing risk to the community as well as NHS costs incurred in contact investigations and outbreak control. Rapid, accurate exclusion (rule-out) of a diagnosis of TB also saves substantial costs since a large part (approx. 80%) of TB healthcare-associated costs are incurred during hospital admission when extensive inpatient investigations are often required. Conversely, missed diagnoses due to false-negative results from tests of poor diagnostic sensitivity incur large costs due to individual patient morbidity and secondary transmission in the community.

4.3 Rationale for Current Study

As described, existing microbiological tests lack diagnostic sensitivity and speed, while current immunodiagnostic tests are unable to distinguish active TB disease from latent infection. In the proposed study we aim to clinically validate 4 new classes of diagnostic tests and to define their clinical role and cost-effectiveness in real-life NHS practice. Each new class of test measures facets of the host response that are not currently routinely measured in clinical practice but each uses technological platforms that have recently been introduced to large diagnostic service laboratories in the NHS and are being increasingly widely deployed. Hence, after clinical validation in this proposal, the novel tests could readily be widely adopted in the NHS. Based on the published and soon-to-be-published evidence to date (from our own lab, our collaborators and other research groups), these tests are very likely to have higher diagnostic accuracy than currently used tests and will be rapid, providing results on the same day or the next morning. *These advantages, if validated by our proposal, could deliver a step-change in diagnostic assessment of TB in routine practice.*

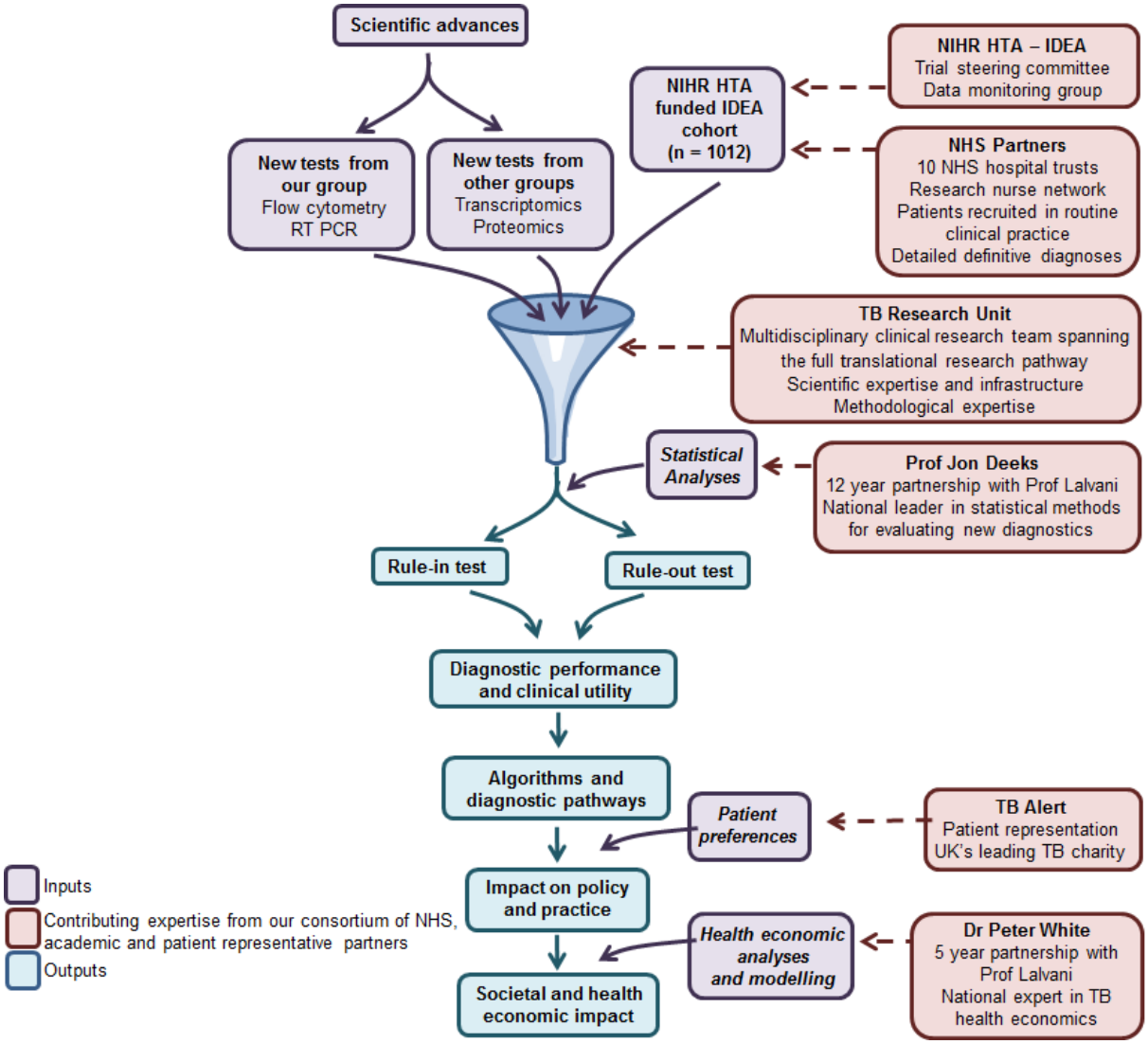

Figure 2. Translational research pathway for VANTDET. Inputs and outputs of the proposed study are illustrated. The TB Research Unit represents the pipeline through which recent scientific advances are translated into tangible improvements in clinical practice with input from our NHS, statistical, health economic and patient representative partners.

We plan to carry out the proposed research on a biobank of clinical samples stored as a part of a study that we are currently running, 'Interferon Gamma Release Assays (IGRAs) in Diagnostic Evaluation of Active TB' (IDEA). IDEA is an NIHR HTA-funded, prospective, multi-centre UK cohort study of over 1,000 adults presenting with suspected active tuberculosis at NHS out- or in-patient services at 10 participating NHS hospitals in London, Birmingham and Leicester. Patients with *suspected* TB are recruited at the point of initial diagnostic work-up in secondary care in routine clinical practice, before a confirmed diagnosis has been determined. Blood is collected for IGRA, and patients are followed up at two and six months to establish a final diagnosis. A core objective of IDEA is the creation of the large, high quality biobank of clinical samples that forms the platform for the current proposal.

The IDEA biobank, from a prospectively recruited cohort in routine clinical practice, is a uniquely valuable resource. It provides the ideal opportunity to validate promising tests previously identified from 'proof-of-principle' case-control studies in a large-scale, longitudinal prospective cohort in routine NHS clinical practice. With many of the key aspects of a study to evaluate diagnostic tests already established in IDEA, this constitutes an ideal

platform on which to carry out the proposed research and provides enormous added value, as the costs of recruiting the patients and transporting and storing/biobanking the clinical samples are already covered. Hence, the proposed study benefits from substantial synergies and represents excellent value for money.

With the progressive and almost continuous two decade rise in TB incidence in the UK, there is an urgent public health imperative to deliver the translational outputs of this research as soon as practicably possible.

5. RESEARCH OBJECTIVES

The overall objectives of the current study are three-fold. First, we aim to validate promising new biomarkers and diagnostic assays for rapid diagnosis of active TB in routine NHS practice. Second, we will determine optimum testing strategies and clinical algorithms incorporating the new test into the diagnostic work-up for patients with suspected TB. Third, we will perform a comprehensive health economic analyses to determine the cost effectiveness of the novel tests (and their use within proposed algorithms) in routine NHS practice. Our specific aims are as follows:

1. To validate the role of previously identified whole blood gene-expression signatures in diagnostic evaluation of active TB using host transcriptomic microarray;

2. To validate the role of previously identified serum proteomic signatures in diagnostic evaluation of active TB using mass spectrometry (MS);

3. To validate our previously established cellular immunological signature, and those identified by others, in diagnostic evaluation of active TB using flow cytometry;

4. To validate our previously described stimulated whole blood chemokine-based RT PCR assay for rapid patient triage by excluding the diagnosis of active TB;

5. To define optimal diagnostic algorithms that delineate the role of these new tests in the routine diagnostic assessment of patients with suspected active TB in the NHS;

6. To assess the cost effectiveness of the novel tests validated in this proposal in the diagnostic work up of TB patients in routine NHS practice.

6. RESEARCH DESIGN AND METHODOLOGY

The proposed study will be carried out on the comprehensive, high quality biobank of prospectively-collected clinical samples in routine practice generated by IDEA. The research design is illustrated in Figure 3.

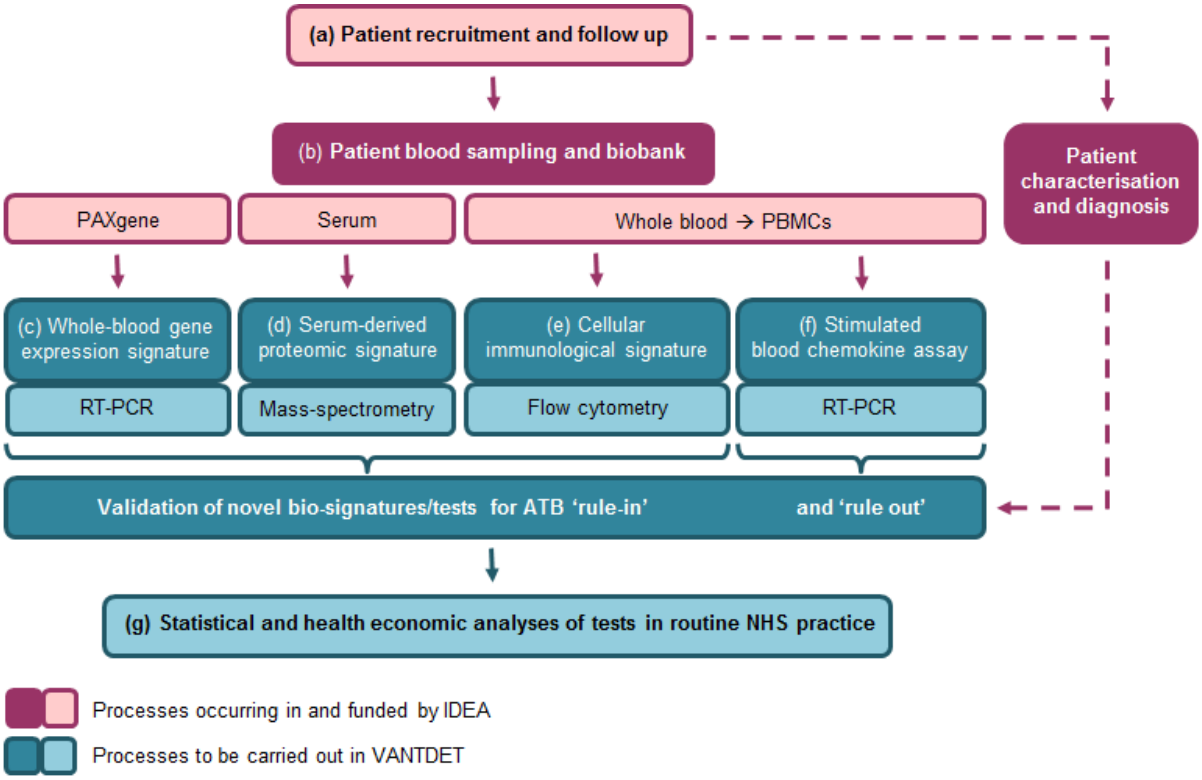

Figure 3. Research design and work plan for the proposed study. Processes (a) and (b) will be complete by the time VANTDET commences.

7. STUDY POPULATION AND SAMPLE SIZES

IDEA has recruited >1,012 patients with suspected active tuberculosis in inpatient or outpatient clinical settings from NHS 10 hospitals across London, Birmingham, Oxford and Leicester. The study population is truly representative of the national TB burden, with a high proportion of foreign-born immigrant patients and a wide ethnic mix.

The IDEA population reflects the real-life spectrum of cases presenting with suspected active TB in the NHS, including substantial proportions of patients with traditionally harder-to-diagnose clinical presentations (Table 4), including extra-pulmonary TB and culture-negative TB. In addition, IDEA is the largest prospective UK study (>1000 individuals) of active TB diagnosis and has been powered to detect clinically meaningful differences in diagnostic test performance in a number of key patient subgroups who are predisposed to active TB, including 200 patients with HIV co-infection. *These key features will ensure that results of both IDEA and VANTDET will be generalisable to UK practice in general; the study population is thus a major strength of this proposal.*

Table 4. IDEA cohort inclusion characteristics and exclusion criteria

IDEA Cohort
Inclusion characteristics
Adults with suspected ATB • routine NHS practice
Range of presentations • PTB, EPTB, C+TB, C-TB
Key patient subgroups • HIV+, diabetic, renal failure
Diverse ethnic mix
Exclusion criteria
Children, aged <16 years
Unable to give informed consent

Proposed Sample Sizes for each study

In routine clinical practice, the imperative of a diagnostic test is to deliver sensitive and specific results with narrow confidence intervals (CIs). Sample sizes have therefore been calculated to maximise precision with which sensitivity and specificity can be estimated whilst maintaining high power to detect a difference in sensitivity between new tests and the current gold standard.

Sample Size for Transcriptomic Signature Validation: The 'TB versus other disease (OD)' signatures are likely to have the greatest clinical impact. Use of the whole IDEA cohort would result in 95% CIs 3% either side of the estimated 85% sensitivity of the transcriptomic and proteomic TB vs OD signatures amongst all TB cases; and 5% either side amongst subgroups of pulmonary/extra-pulmonary TB. CIs wider than these would be unacceptable in clinical practice. For transcriptomic study, we therefore plan to use all available samples from IDEA subjects in whom active TB was diagnosed (n= 292) or excluded (n=320) and RNA samples were collected for validation of these signatures. The TB versus LTBI signatures (expected sensitivity: 85%; specificity: 85%) will be validated in all IGRA-positive patients in whom active TB was excluded and an age-, gender- and ethnicity-matched group of IDEA patients with active TB.

Sensitivity of the transcriptomic and proteomic signatures are conservatively estimated at 85%, based on a 5-10% reduction in this validation cohort compared to original publications. Using the whole IDEA cohort we would be able to detect differences in sensitivities between the signatures and *Mtb* culture with extremely high power, and at the 1% significance level to account for evaluation of several biomarkers. An observed sensitivity of 85% of the signatures would be a 25% increase compared to culture amongst all TB cases, and a 15% or 35% increase in PTB or EPTB, respectively. This would constitute a large improvement. However, any increase in sensitivity of >15% compared to culture would be clinically meaningful, and we still have very high power to detect this difference using the whole IDEA cohort.

Sample Size for Proteomic Signature Validation: For the proteomics experiments samples will be chosen at random from the IDEA cohort (HIV-), the 1st 90 will be compared using SELDI-TOFF and LC-MS techniques and the sensitivity and specificity compared to the landmark studies (above). The Levin et al.,(unpublished SELDI signature) is currently being adapted into an ELISA based test, if this SELDI signature is validated in the IDEA cohort (n=90, Sensitivity >85%), this ELISA test will be used as validation of the entire IDEA (HIV-/+) cohort to provide CI <5% for ATB versus OD. For LC-MS a total of 200 samples (HIV-/+) will be analysed and this dataset used to explore the diagnostic performance of both previously identified and novel protein markers.

Sample Size for Cellular Immunological Signature Validation: The sensitivity of our cellular immunological signature amongst the test cohort was very high (100%, 95% CIs 74-100%); and the cohort included PTB and EPTB, and HIV+ and HIV- individuals (11). The sample size required to validate the immunological signature is thus much lower than for the other signatures. Based on a conservative estimated sensitivity of 80% (20% lower than sensitivity determined in the test cohort, but accounting for the wide CIs), 324 subjects (130 ATB) would be required to detect a 20% increase in sensitivity compared to culture with 80% power and at the 1% significance level (to account for evaluation of multiple tests). However, we are limited by the number of patients with LTBI and other diseases, who have available PBMCs, within the final IDEA cohort (n=82). Using a nested case control approach, 82 active TB patients were randomly selected (but enriched for those with HIV infection), to add to the 82 LTBI patients, to provide a final cohort of 164 individuals. Whilst smaller than the ideal sample size, this will still be more than sufficient to provide enough power to demonstrate 95% sensitivity and specificity.

Sample Size for Molecular Rule Out Test Validation: In order for the molecular test to be used to rule out TB, it must have extremely high sensitivity, and therefore must provide a positive result for those individuals who are infected with Mtb but who fail to test positive in the most sensitive of the currently-available tests for Mtb infection (the TSPOT-TB). We have therefore selected an initial cohort of 100 individuals recruited into IDEA, selected at random, but conforming to the following criteria (25 x individuals with active TB and a positive TSPOT-TB; 25 x individuals with active TB and a negative TSPOT-TB result; 25 x individuals with other diseases with a positive TSPOT-TB result; 25 x individuals with a negative TSPOT-TB result). Once this initial cohort has been tested using the molecular rule out test, the results will be analysed to test the performance of the assay in ruling out active TB. If the results are promising (i.e. > 95% sensitivity is demonstrated), we will continue to assay the rest of the available PBMC samples from the IDEA cohort, in order to maximise the confidence of our findings.

8. OUTCOME MEASURES

The proposed outputs of this study will be 4 new tests to improve the diagnostic work up of suspected active TB in routine NHS practice (Table 5). The outcome measures are three-fold:

(1) Validation of the bio-signatures/tests, defined in terms of their ability to serve as
- 'Rule in' tests of active TB (transcriptomic signature, proteomic signature, flow cytometry)
- 'Rule-out' test of *Mtb* infection and disease (ultra-sensitive RT-PCR chemokine assay)

(2) The place of the new tests in diagnostic algorithms and patient care pathways alongside (or instead of) currently available diagnostic tests, including immunodiagnostics and microbiology.

(3) The cost effectiveness of the proposed diagnostic testing strategies in routine NHS practice and the overall health economic impact from an NHS perspective.

9. ASSESSMENT AND FOLLOW UP

Patients are followed up at 2 and 6 months post recruitment (as per IDEA study protocol) to allow for full and robust diagnostic assessment of patients recruited, as described in Section 3. No interventions or changes to the routine patient care pathway are introduced by IDEA (which is purely observational) and none will be introduced by the current proposal. Since neither IDEA nor the proposed research involve any intervention, and patient care pathways are not impacted, follow up for efficacy and safety purposes is not required. Procedures are in place to appropriately deal with adverse events (AEs) in IDEA.

10. ETHICAL ARRANGEMENTS

Existing *granted* ethics approval: Ethical approval was obtained for IDEA from the North West London REC1 ethics committee (REC ref: 11/H0722/8) on 14th March 2011, and local NHS R&D approval was obtained at all participating NHS trusts. The *Tuberculosis Research Unit* manages a TB Research Tissue Bank which was granted ethical approval by St. Mary's Research Ethics Committee on 14th September 2007 (REC reference: 07/H0712/85) enabling use of the IDEA samples for this research. This has since been renewed (REC reference: 07/H0712/85+5). All samples stored for future research are registered under the Imperial College London Human Tissue Act (HTA) research licence (licence number 12275).

A recent inspection in relation to Human Tissue Licence requirements confirmed that the procedures, patient confidentiality and governance are all 100% compliant for storage of such research material.

Consent: Patients provide broad consent for use of samples in future unspecified research including genetic analysis (as ethically approved in IDEA).

Confidentiality: The Chief Investigator and all members of the research team respect confidentiality of study participants and abide by the Data Protection Act 1998. Participants are allocated a unique anonymised identification code on recruitment, and no personal identifiers are recorded on any sample. Case record forms are anonymised and stored in a locked filing cabinet in an entry pass-code protected room for the sole use of clinical research nurses and senior members of the study co-ordination team.

Risks and anticipated benefits for trial participants: Neither IDEA nor the proposed research will directly benefit or cause risk to participants.

11. RESEARCH GOVERNANCE

The study will be conducted in accordance with the Research Governance Framework for Health and Social Care (2001 and 2005 revision) and the Declaration of Helsinki 1964 (and later revisions).

Sponsor: Imperial College London is the sponsor for IDEA and for the proposed study. Imperial College London holds applicable negligent and non-negligent harm insurance policies, which have been arranged through its Joint Research Office.

Trial Steering and Data Monitoring Committees: A Trial Steering Committee (TSC) and Data Monitoring Group (DMG) are in place for IDEA. The members and roles of each committee are detailed in the IDEA SOP (and in brief in the Appendix).

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 the Research Governance Framework for Health and Social Care (2nd edition).

Retention of relevant trial documentation: In accordance with Imperial College London Joint Research Office standard operating procedures ensuring regulatory compliance of primary research studies, data and documentation for IDEA and the current study will be archived for 10 years following study completion.

12. SERVICE USERS

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 IDEA. In addition, the advisory group will include 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.

14. FLOW DIAGRAM

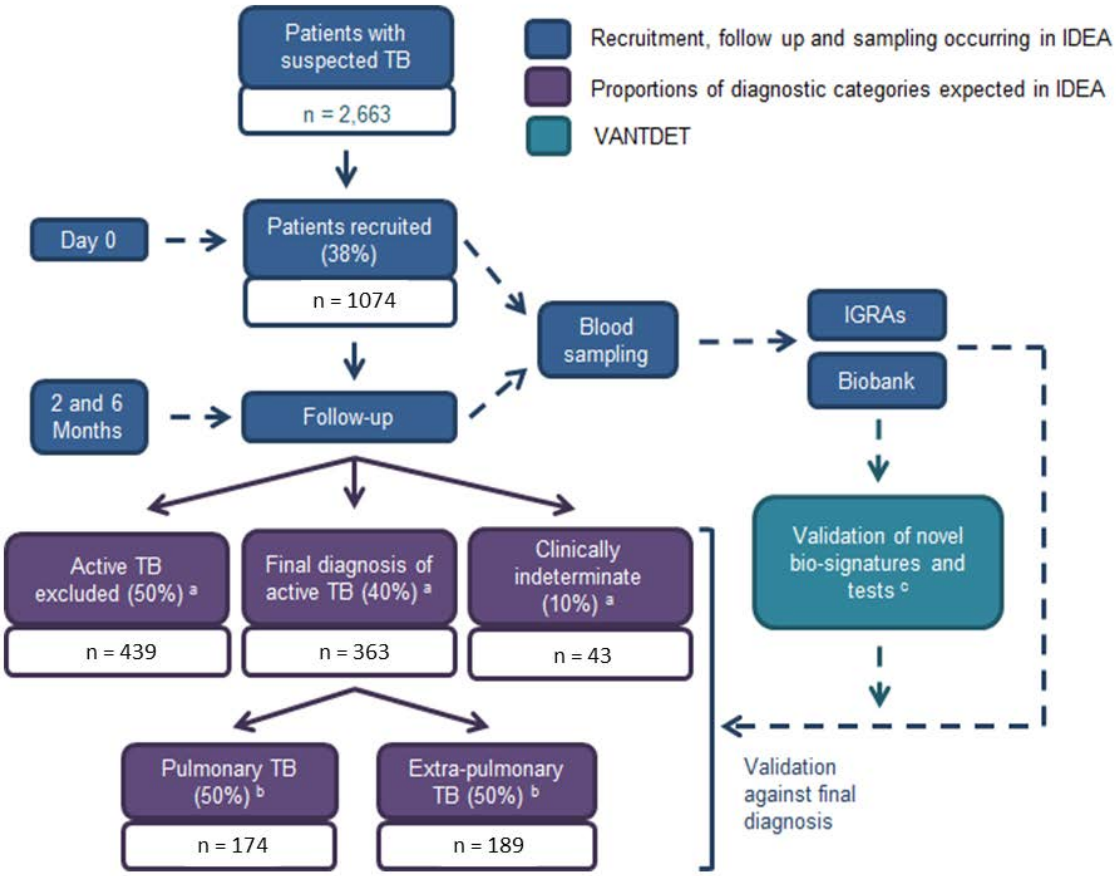

Figure 6. Flow chart for IDEA and VANTDET.

[a] Proportions of patients who had active TB excluded, have a final diagnosis of active TB, or to be clinically indeterminate in the IDEA cohort.
[b] Proportions of patients with a final diagnosis of pulmonary or extra-pulmonary TB.
[c] Validation of the transcriptomic and proteomic signatures and molecular rule out test will be carried out using all IDEA samples (except those from the clinically indeterminate group). Validation of the cellular immunological signature will be performed on a subset of 164 samples, of which 82 will be from patients with a final diagnosis of active TB.

15. REFERENCES

1. World Health Organization. Global tuberculosis control: WHO report 2011. Geneva: World Health Organization; 2011. viii, 246 p. p.
2. Health Protection Agency. Tuberculosis in the UK: Annual report on tuberculosis surveillance in the UK, 2012. 2012.
3. Department of Health. Stopping Tuberculosis in England. An Action Plan from the Chief Medical Officer. 2004.
4. NICE. Clinical diagnosis and management of tuberculosis, and measures for its prevention and control: National Institute of Clinical Excellence; 2011 [updated March 2011. CG117:[Available from: http://www.nice.org.uk/guidance/index.jsp?action=byID&o=13422.
5. Dosanjh DP, Hinks TS, Innes JA, Deeks JJ, Pasvol G, Hackforth S, et al. Improved diagnostic evaluation of suspected tuberculosis. Ann Intern Med. 2008;148(5):325-36.
6. Berry MP, Graham CM, McNab FW, Xu Z, Bloch SA, Oni T, et al. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. Nature. 2010;466(7309):973-7.
7. Maertzdorf J, Repsilber D, Parida SK, Stanley K, Roberts T, Black G, et al. Human gene expression profiles of susceptibility and resistance in tuberculosis. Genes Immun. 2011;12(1):15-22.
8. Sandhu G, Battaglia F, Ely BK, Athanasakis D, Montoya R, Valencia T, et al. Discriminating active from latent tuberculosis in patients presenting to community clinics. PloS one. 2012;7(5):e38080.
9. Day CL, Abrahams DA, Lerumo L, Janse van Rensburg E, Stone L, O'Rie T, et al. Functional capacity of Mycobacterium tuberculosis-specific T cell responses in humans is associated with mycobacterial load. Journal of immunology (Baltimore, Md : 1950). 2011;187(5):2222-32.
10. Harari A, Rozot V, Enders FB, Perreau M, Stalder JM, Nicod LP, et al. Dominant TNF-alpha(+) Mycobacterium tuberculosis-specific CD4(+) T cell responses discriminate between latent infection and active disease. Nat Med. 2011;17(3):372-6.
11. Pollock KM, Whitworth HS, Montamat-Sicotte DJ, Grass L, Cooke GS, Kapembwa MS, et al. T-cell immunophenotyping distinguishes active from latent tuberculosis. J Infect Dis. 2013;208(6):952-68.
12. Jacobsen M, Repsilber D, Gutschmidt A, Neher A, Feldmann K, Mollenkopf HJ, et al. Candidate biomarkers for discrimination between infection and disease caused by Mycobacterium tuberculosis. Journal of molecular medicine (Berlin, Germany). 2007;85(6):613-21.
13. Ottenhoff TH, Dass RH, Yang N, Zhang MM, Wong HE, Sahiratmadja E, et al. Genome-wide expression profiling identifies type 1 interferon response pathways in active tuberculosis. PloS one. 2012;7(9):e45839.
14. Mistry R, Cliff JM, Clayton CL, Beyers N, Mohamed YS, Wilson PA, et al. Gene-expression patterns in whole blood identify subjects at risk for recurrent tuberculosis. The Journal of infectious diseases. 2007;195(3):357-65.
15. Adekambi T, Ibegbu CC, Cagle S, Kalokhe AS, Wang YF, Hu Y, et al. Biomarkers on patient T cells diagnose active tuberculosis and monitor treatment response. The Journal of Clinical Investigation. 2015;125(5):1827-38.
16. Portevin D, Moukambi F, Clowes P, Bauer A, Chachage M, Ntinginya NE, et al. Assessment of the novel T-cell activation marker–tuberculosis assay for diagnosis of active tuberculosis in children: a prospective proof-of-concept study. The Lancet Infectious Diseases.14(10):931-8.
17. Petruccioli E, Petrone L, Vanini V, Cuzzi G, Navarra A, Gualano G, et al. Assessment of CD27 expression as a tool for active and latent tuberculosis diagnosis. Journal of Infection. 2015;71(5):526-33.

18. Sester U, Fousse M, Dirks J, Mack U, Prasse A, Singh M, et al. Whole-blood flow-cytometric analysis of antigen-specific CD4 T-cell cytokine profiles distinguishes active tuberculosis from non-active states. PloS one. 2011;6(3):e17813.
19. Lalvani A, Millington KA. T Cells and Tuberculosis: Beyond Interferon-gamma. The Journal of infectious diseases. 2008;197(7):941-3.
20. Kasprowicz VO, Mitchell JE, Chetty S, Govender P, Huang KH, Fletcher HA, et al. A molecular assay for sensitive detection of pathogen-specific T-cells. PloS one. 2011;6(8):e20606.
21. Millington KA, Fortune SM, Low J, Garces A, Hingley-Wilson SM, Wickremasinghe M, et al. Rv3615c is a highly immunodominant RD1 (Region of Difference 1)-dependent secreted antigen specific for Mycobacterium tuberculosis infection.