**Systematic review on the diagnostic performance of novel skin-based *in vivo* tests for TB infection compared to blood-based *in vitro* interferon-gamma release assays**

**Study Protocol**

**Investigators:**

Maria Krutikov1, \*Lena Faust2, Vladyslav Nikolayevskyy3, Yohhei Hamada1, Rishi K. Gupta1, Daniela Cirillo4, Alberto Mateelli5, Alexei Korobitsyn6, Claudia Denkinger 7,9, Molebogeng X. Rangaka 1,8,10

Affiliations

1. Institute for Global Health, University College London
2. McGill International TB Centre, and Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Canada
3. UK National Mycobacterium Reference Service, Public Health England, UK, and Imperial College London, UK
4. IRCCS San Raffaele Scientific Institute, Milan, Italy
5. Collaborating Centre for TB/HIV Co-infection and TB Elimination, Dept of Infectious and Tropical Diseases, University of Brescia, Brescia, Italy
6. Global TB Programme, World Health Organisation, Geneva, Switzerland
7. Centre of Infectious Disease, University of Heidelberg
8. Medical Research Council Clinical Trials Unit, UK
9. FIND, Geneva, Switzerland
10. School of Public Health, and CIDRI-AFRICA, University of Cape Town, South Africa

**Introduction**

**Background**

Approximately 25-27% of the world’s population is estimated to have latent TB infection (LTBI) 1,2 with a lifetime risk of progression to active infection of 5-10% which is higher in those with pre-disposing factors or in the first 18 months after acquisition of infection3,4 . These are important populations to target for screening and treatment of LTBI in order to prevent reactivation and subsequent transmission. Currently available tests for LTBI are imperfect, as they cannot accurately distinguish between active TB disease and latent infection, nor are they useful predictors of progression to active disease 5. Given the recognition of the identification and management of LTBI as an essential element of the End TB Strategy, research into more accurate diagnostic tests is critical to the achievement of these milestones 6,7 .

The diagnostic tests in current use are the tuberculin skin test (TST) and interferon gamma release assay (IGRA). The TST is commonly performed using the Mantoux technique, which consists of intradermal placement of 2 tuberculin units (TU) of RT-23 or 5TU of PPD-S which causes a delayed hypersensitivity reaction after 48-72 hours. The result is reported as *mm* of induration in the transverse diameter, read after 2 days 8. However, the PPD TST has relatively low specificity (false positives in those with previous recent BCG vaccination) 9, lacks sensitivity in immunosuppressed individuals (e.g. HIV infected), requires two clinic visits (one to administer the test and one to read the result), and failure to attend the clinic for evaluation of reaction within 48-72 hours renders the results invalid. Despite its limitations, due to its low cost and wide availability it remains the most commonly used test for LTBI globally, however current shortages of PPD threaten its continued use.

The IGRA measures T-cell release of Interferon-gamma (IFNɣ) following stimulation by ESAT-6 and CFP-10 antigens that are specific to the *Mycobacterium tuberculosis* (MTB) complex and are encoded in the RD1 locus of the MTB genome 10. There are two types of IGRA: the enzyme-linked immunosorbent assay (ELISA)-based whole-blood method, and the enzyme-linked immunosorbent spot (ELISPOT) assay. The ELISA whole-blood test uses ESAT-6 and CFP-10 as well as peptides from one additional antigen (TB7.7 [Rv2654c]), which is not an RD1 antigen, in an in-tube format. The result is reported as quantification of IFNɣ in international units (IU) per millilitre. The ELISPOT assay is performed on separated and counted peripheral blood mononuclear cells (PBMCs) that are incubated with ESAT-6 and CFP-10 peptides. The result is reported as the number of IFNɣ-producing T cells (spot-forming cells). Unlike the TST, IGRAs are not affected by prior BCG vaccination as the RD1 locus is specific to the MTB genome and therefore these antigens are not present in *Mycobactrium Bovis*, used in BCG vaccines, or other non-tuberculous mycobacteria 11. Moreover, compared to the TST, some IGRAs remain relatively unimpaired in HIV and other immunosuppressive conditions 12. Thus these are useful for evaluation of LTBI in BCG-vaccinated individuals and with high specificity, particularly in countries where BCG vaccination is administered after infancy or repeated vaccinations are given. However, the IGRA platforms are more expensive to run, requiring specialised kits, a qualified technician and an accredited laboratory in order to ensure test results are reproducible, as well as a phlebotomist to obtain blood samples 13. Furthermore, large variability has been observed even if pre-analytical steps were performed within the recommendations of the manufacturer limiting the reproducibility of the tests14..

Over the last decade, newer skin-based tests have been developed that aim to maximize the advantages of the currently available implementation platforms. Examples of these are the C-Tb(Staten Serum Institut), Diaskin Test (Generium) and ESAT6-CFP10 test (Anhui Zhifei Longcom), all of which contain recombinant ESAT-6 (dimer) and CFP10 (monomer) antigens derived from MTB thatmay provide diagnostic performance improvements over the standard PPD TST (particularly in respect to specificity). In comparison with IGRA, these could increase the price pressure in the market.All tests use intradermal injection of antigen and, like the PPD TST, are read after 48-72 hours as induration in mm 15,16. Emerging evidence suggests that compared to IGRAs, these tests may have similar specificity17 and provide more reliable results in children and in HIV-infected cohorts, with the C-Tb, for example, having shown similar sensitivity in HIV-infected and uninfected individuals (although lower sensitivity was found among HIV+ individuals with CD4 counts below 100) 18.

We plan to conduct a systematic review to synthesise current evidence on the diagnostic performance of novel *in vivo* tests for LTBI compared to that of currently available *in vitro* IGRA tests and the PPD-TST. This will provide clinicians and policy-makers with the evidence required to inform decisions regarding implementation of these tests in the place of IGRAs, and in addition will serve to identify future research priorities in this field.

**Hierarchy of reference standards**

The study of the diagnostic performance of tests for LTBI is hampered by a lack of adequate reference standard. Existing tests for LTBI measure the cell-mediated immune response (memory T cell response) to exposure to TB antigens and are thus proxies for infection. As the diagnostic accuracy for LTBI cannot be directly assessed, we will utilise a hierarchy of *a priori* agreed reference standards that also reflect diagnostic accuracy study designs previously used in the evaluation of IGRA 19 (Figure 1).

 

Figure 1: Hierarchy of reference standards (adapted from 19)

**Aims and Objectives**

**Aim**

To evaluate the performance of novel *in vivo* skin tests for detection of TB infection in at risk populations compared to currently available *in vitro* IGRA tests or the PPD-TST.

**Objectives**

**Primary objectives**: To evaluate the performance of novel recombinant antigen skin tests against recognised reference standards:

1. To evaluate concordance and discordance with commercial IGRAs and the TST when using crude and BCG-stratified TST measurements (standard 5)
2. To assess sensitivity and specificity for diagnosis of active TB (standard 4)
3. To assess the association between test result and proximity of exposure among TB case contacts (standard 3)
4. To assess the predictive value of novel recombinant skin tests for incident TB among risk-stratified populations (standard 2)
5. To investigate efficacy of preventive therapy based on test result (standard 1)

**Secondary objectives**: To assess patient and operational outcomes of novel recombinant antigen skin tests

1. Evaluate cost-effectiveness of novel recombinant skin test when compared with TST or IGRA
2. Evaluate the operational feasibility of implementing novel recombinant skin test using indicators of robustness, patient and provider value and implementation
3. Assess reproducibility of novel recombinant skin test result by assessing inter-rater reliability or agreement when comparing with TST or IGRA
4. Evaluate safety of novel recombinant skin tests by assessing side effects reported in studies

**Methods**

**Inclusion criteria**

All cross-sectional or case-control (using authors definitions of case and control which will be further characterised at analyses) and longitudinal (prospective or retrospective) studies, original research studies evaluating the index tests (C-Tb or DiaskinTest or ESAT6-CFP10) alone or with a recognised comparator tests (QFT, T-SPOT, PPD-TST) in humans will be reviewed, with no language restrictions. Detailed inclusion criteria, by study objective, are presented in Table 1.

Index tests:

* C-TB (Staten Serum Institut)
* Diaskin Test (Generium)
* ESAT6-CFP10 (Anhui Zhifei Longcom)

Comparator tests:

* QFT-gold or plus (Qiagen)
* T-SPOT TB test (Oxford Immunotec)
* PPD-TST
* DPPD

**Exclusions:**

Letters without original data; 2) case reports; 3) reviews; 4) studies reporting insufficient data to determine diagnostic accuracy measures; 5) studies evaluating non-commercial TST or IGRA as comparator;6) mathematical modelling or case-base studies; 6) animal studies.

Table 1: Inclusion criteria according to objective

|  |  |  |
| --- | --- | --- |
| Objective | Study design | Inclusion criteria |
| 1a | Studies reporting results of comparator test alongside index test in any population | Must report comparator test and index test result and cut-off measurement used. Desirable to report order of test administration (eg in case of QFT and possible boosting effect of skin test) |
| 1b | Studies that report index test result in participants with clinical suspicion of or confirmed active TB | Defined gold standard for active TB (see case definitions) |
| 1c | Studies reporting index test result in contacts of active TB cases | Must stratify contacts according to proximity to TB cases |
| 1d | Longitudinal studies reporting development of incident TB in population tested with index test during study period | Must be free of active TB at baseline; Must report method of TB diagnosis (microbiological or clinical) |
| 1e | Longitudinal studies that report index test result in population eligible for LTBI screening, preventive therapy given and cases of incident TB during study period | Must be free of active disease at baseline; Must report method of TB diagnosis (microbiological or clinical) |
| 2 | Studies that have met inclusion criteria for primary objectives or that have specified, patient and operational outcomes regarding implementation of index test in LTBI screening programme, as their primary objective |

**Study Screening and Data Collection Process:**

The search strategy will be devised with the help of specialised library services. Titles and abstracts will be reviewed by 2 independent reviewers, and study eligibility determined as per the criteria. Discrepancies in inclusion/exclusion between the 2 reviewers will be resolved by discussion between the 2 reviewers or if needed with a senior person. Bibliographies of studies included in the review will be hand-searched for additional relevant studies. The systematic review management platform Rayyan20 will be used for study screening and tracking of exclusion reasons. Data extraction will be carried out using specific data extraction sheet in in Microsoft Excel.

**Case definitions:**

**Incident TB disease:** any new case of TB (new or relapse) diagnosed subsequent to initial screening test

**Prevalent TB:** any case of known TB disease at the time of the diagnostic test

**Active TB**: Hierarchy of reference standards (as per19):

1. Culture or smear-confirmed (if high TB incidence setting of ≥50/100,000),

2. Sputum smear-positive without culture confirmation in low to intermediate burden setting (<50/100,000)

3. Clinical diagnosis based on presenting symptoms, radiology and / or response to TB treatment without microbiological confirmation

**Adverse events grading (Toxicity grading tables)** 21**:**

Based on published literature, we expect most frequent adverse reactions to be injection site reactions, although systemic reactions like headache, fever and even lymphadenitis have also been reported16,22.

Grading of adverse reactions will be according to the following widely-accepted DAIDS classification:

Grade 1: mild event

Grade 2: moderate event

Grade 3: severe event

Grade 4: potentially life-threatening event

Grade 5: death

**Search strategy:**

The systematic review protocol and search strategy will be registered on PROSPERO and will follow PRISMA guidelines. The initial search will be carried out in Medline and Embase by a medical librarian for all studies published until present with no language restrictions. In order to include as many studies as possible, the test manufacturers will be contacted for supplementary studies and abstracts. As Generium is a Russian company and most studies evaluating Diaskintest performance have been carried out in the ex-Soviet bloc, we will be searching e-library (www.e-library.ru) to look for additional Russian language studies not already included in the search. The same will not be done for ESAT6-CFP10 (Anhui Zhifei Longcom) as this has not progressed beyond the development phase. However, the company will be contacted for a full database of known studies.field regarding most suitable databases. A search will be performed in Chinese Biomedical Literature Database and the China National Knowledge Infrastructure databases. The detailed search strategy and search terms are provided in Appendix 1. 2.

**Data variables:**

Table 2: Variables of interest

|  |  |
| --- | --- |
| **Category** | **Variables** |
| Study design | Study design, country, setting, period of recruitment, sample size |
| Population summary measures | Age, gender, history of immunosuppression, HIV status, BCG vaccination history, TB contact history (method of diagnosis and DST of case, proximity to case), migration history, homelessness, imprisonment  |
| Index test | Recombinant antigen skin test used, cut-off point used, cost  |
| Comparator | IGRA assay and cut-off used, TST dose and cut-off used, cost |
| Outcome | Intervention test results, comparator test results, preventive therapy given, numbers progressing to active TB and method of diagnosis  |

Table 2 details the principal variables of interest. Data will be mapped to a data extraction sheet. Although not all studies will include all of these data, the minimum data for inclusion are stated in the inclusion criteria. A log will be maintained by reviewers in order to address the secondary objectives, as well as issues raised when mapping to data extraction sheet that will subsequently be clarified with contributing authors.

**Quality assessment (risk of bias):**

The quality of each included study will be formally evaluated using a quality assessment tool appropriate to the study design. Studies will be stratified by study design to explore the bias. For all diagnostic accuracy studies, study quality will be assessed using a modified version of the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool23.This assesses risk of bias and concerns regarding applicability in four domains: patient selection; index test; reference standard; and flow & timing. An additional domain pertaining to the involvement of commercial test manufacturers in study design, conduct or analysis and related risk of bias will be added to the QUADAS-2 tool to assess the impact of possible conflicts of interest.

Non-randomised longitudinal cohort and case-control studies will be assessed using the Newcastle-Ottawa tool 24. This allows assessment of bias in 3 main domains: selection (intervention cohort, non-intervention cohort, intervention); comparability of cohorts; outcome (assessment, duration and adequacy of follow up).

The GRADE framework 25 will be used to systematically assess the quality of evidence and strength of recommendations regarding the use of novel skin tests (classified as high, moderate, low or very low) according to these criteria:

1. Study design
2. Risk of bias (as per QUADAS-2 tool or Newcastle-Ottawa scale)
3. Directness
4. Inconsistency
5. Imprecision
6. Publication / reporting bias

For the purposes of objective 1b, studies that evaluate test performance in patients with presumed active TB will be judged as the highest quality evidence, whereas those focussing on known active TB cases, lower quality.

**Data Analysis:**

Study characteristics will be represented descriptively in tables. Where possible, outcome measures will be stratified by: type of test; HIV status; children under 5; BCG status; TB contacts and other immunocompromised states.

Outcome and effect measures of interest will be evaluated separately for each objective as described in Table 3. Where possible and to minimise heterogeneity, analysis will be performed according to risk-stratified populations: age under five; HIV infected; history of BCG vaccination.

For each objective, heterogeneity will be visually assessed using forest plots and heterogeneity characterised using the I-squared statistic and statistically tested using the chi squared test for each objective. Univariate and bivariate random-effects models will be used where appropriate to determine pooled estimates in the meta-analysis, in order to account for heterogeneity in the study populations.

Table 3: Effect measures according to objective

|  |  |
| --- | --- |
| **Objective**  | **Effect measure** |
| **Primary objectives:** evaluating performance of novel recombinant antigen skin tests against recognised reference standards |
| 1a. To evaluate concordance and discordance of index test with comparators when using crude and BCG-stratified TST measurements | **\*% Concordance/Discordance,** total and by test pairs. **Concordance:** will be defined by summary comparison in proportion test positivity between index and comparator**Discordance:** will be measured by difference in proportions of test negativity for index and comparator test. |
| 1b. To assess sensitivity and specificity for diagnosis of active  | **Sensitivity** = proportion of people with positive skin test among those with microbiologically confirmed TB (groups 1 and 2 in case definitions) **Specificity** **for active TB**= proportion of people with negative skin test among those who have had active TB ruled out.  |
| 1c. To assess the association between test result and proximity of exposure among TB case contacts | **Odds Ratio** according to contact proximity for each study**Concordance** and **discordance** between index and comparator test result according to proximity among contacts |
| 1d. To assess the predictive value of novel recombinant skin tests for incident TB among risk-stratified populations | **Incidence rates** for disease progression in risk-stratified populations**Incidence Rate Ratios****Negative and Positive Predictive Values** for progression with confidence intervals |
| 1e. To investigate efficacy of preventive therapy based on test result  | **Incidence rates** for disease progression stratified by test result **Incidence Rate Ratios****Negative and Positive Predictive Values** for disease progression |
| **Secondary objectives** |
| 2a. Evaluate cost-effectiveness of novel recombinant skin test when compared with TST or IGRA | **Difference in cost****Cost ratio****Change in QALYs** |
| 2b. Evaluate the operational feasibility of implementing novel recombinant skin test within TB control programme | **Indicators of robustness:** Indeterminate rate, sensitivity and specificity (as proxies for patient relevant outcomes)**Patient and provider value:** satisfaction, perceived appropriateness, intention to continue use **Implementation:** time to carry out test, ease of method, cost of materials, need for cold chain, time required to train providers in test techniqueWhere available, qualitative synthesis and evaluation of quantitative objective measures will be performed  |
| 2c. Assess reproducibility of novel recombinant skin test result | **\*Inter-rater variability or agreement**  |
| 2d. Evaluate safety of novel recombinant skin tests by assessing side effects reported in studies | **Risk:** proportion with adverse events outout of total tested (injection site related or systemic) |

\*Measures of interrater agreement or variability (e.g McNemar’s, kappa) will be evaluated as appropriate for dichotomous data. As Kappa does not account for magnitude in difference and cannot differentiate between positive and negative findings; weighted kappa will be used where appropriate.

**Dissemination plans:**

Once completed, a full report will be shared with researchers and policy makers including the WHO. In addition, data will be published in peer-reviewed journals in order to disseminate findings widely amongst the scientific community and the public.

**Timeline:**

|  |  |
| --- | --- |
| **Milestone** | **Target date** |
| Completion and registration of protocol 1st draft | May 2019 |
| Completion of study screening and data extraction | November 2019 |
| Completion of analysis | February 2020 |
| Write-up and dissemination | June 2020 |

PRISMA Diagram 26:

Additional records identified through other sources

 (n = 314)

Studies included in quantitative synthesis (meta-analysis)
(n = )

Studies included in qualitative synthesis
(n = )

Full-text articles excluded, with reasons
(n = )

Full-text articles assessed for eligibility
(n = )

Records excluded
(n = )

Records screened
(n = )

Records after duplicates removed
(n = )

## Identification

## Eligibility

## Included

## Screening

Records identified through database searching
(n = 1,512)

**References:**

1. Houben RMGJ, Dodd PJ. The Global Burden of Latent Tuberculosis Infection: A Re-estimation Using Mathematical Modelling. *PLoS Med*. 2016;13(10). doi:10.1371/journal.pmed.1002152

2. Abajobir AA, Abate KH, Abbafati C, et al. Global, regional, and national disability-adjusted life-years (DALYs) for 333 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet*. 2017;390(10100):1260-1344. doi:http://dx.doi.org/10.1016/S0140-6736%2817%2932130-X

3. Comstock GW, Livesay VT, Woolpert SF. The prognosis of a positive tuberculin reaction in childhood and adolescence. *Am J Epidemiol*. 1974;99(2):131-138. doi:10.1093/oxfordjournals.aje.a121593

4. Small PM, Fujiwara PI. Management of tuberculosis in the United States. *N Engl J Med*. 2001;345(3):189-200. doi:10.1056/NEJM200107193450307

5. Pai M, Behr M. Latent Mycobacterium tuberculosis Infection and Interferon-Gamma Release Assays. *Microbiol Spectr*. 2016;4(5). doi:https://dx.doi.org/10.1128/microbiolspec.TBTB2-0023-2016

6. World Health Organization. Global Strategy and Targets for Tuberculosis Prevention, Care and Control after 2015. *Geneva World Heal Organ*. 2014.

7. Dye C, Glaziou P, Floyd K, Raviglione M. Prospects for tuberculosis elimination. *Annu Rev Public Health*. 2013;34:271-286. doi:http://dx.doi.org/10.1146/annurev-publhealth-031912-114431

8. Deck F, Guld J. The WHO tuberculin test. *Bull Int Union Tuberc*. 1964;34(1):53-70.

9. Farhat M, Greenaway C, Pai M, Menzies D. False-positive tuberculin skin tests: What is the absolute effect of BCG and non-tuberculous mycobacteria? *Int J Tuberc Lung Dis*. 2006;10(11):1192-1204.

10. Sorensen AL, Nagai S, Houen G, Andersen P, Andersen AB. Purification and characterization of a low-molecular-mass T-cell antigen secreted by Mycobacterium tuberculosis. *Infect Immun*. 1995.

11. Andersen P, Munk ME, Pollock JM, Doherty TM. Specific immune-based diagnosis of tuberculosis. *Lancet*. 2000;356(9235):1099-1104. doi:10.1016/S0140-6736(00)02742-2

12. Rangaka MX, Diwakar L, Seldon R, et al. Clinical, Immunological, and Epidemiological Importance of Antituberculosis T Cell Responses in HIV-Infected Africans. *Clin Infect Dis*. 2007;44(12):1639-1646. doi:10.1086/518234

13. Pai M, Denkinger CM, Kik S V, et al. Gamma interferon release assays for detection of Mycobacterium tuberculosis infection. *Clin Microbiol Rev*. 2014;27(1):3-20. doi:https://dx.doi.org/10.1128/CMR.00034-13

14. Tagmouti S, Slater M, Benedetti A, et al. Reproducibility of interferon gamma (IFN-γ) release assays a systematic review. *Ann Am Thorac Soc*. 2014;11(8):1267-1276. doi:10.1513/AnnalsATS.201405-188OC

15. Aggerbeck H, Giemza R, Joshi P, et al. Randomised Clinical Trial Investigating the Specificity of a Novel Skin Test (C-Tb) for Diagnosis of M. tuberculosis Infection. *PLoS One*. 2013;8(5). doi:10.1371/journal.pone.0064215

16. Kiselev VI, Baranovskii PM, Rudykh I V, et al. [Clinical trials of the new skin test Diaskintest for the diagnosis of tuberculosis]. *Probl Tuberk Bolezn Legk*. 2009;(2):11-16. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=med6&NEWS=N&AN=19382637.

17. Ruhwald M, Cayla J, Aggerbaek H, Dheda K, Andersen PL. Diagnostic accuracy of the novel C-Tb skin test for LTBI, results from two phase III trials. In: ; 2016:OA3037. doi:10.1183/13993003.congress-2016.oa3037

18. Hoff ST, Peter JG, Theron G, et al. Sensitivity of C-Tb: A novel RD-1-specific skin test for the diagnosis of tuberculosis infection. *Eur Respir J*. 2016;47(3):919-928. doi:10.1183/13993003.01464-2015

19. World Health Organization. Use of tuberculosis release assays (IGRAs) in low and middle-income countries. *World Heal Organ Policy Statement*. 2011. doi:WHO/HTM/TB/2011.18

20. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan-a web and mobile app for systematic reviews. *Syst Rev*. 2016;5(1). doi:10.1186/s13643-016-0384-4

21. U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases D of A. Division of AIDS table for grading the severity of adult and pediatric adverse events Corrected Version 2.1. [July 2017]. *… Allergy Infect Dis Div AIDS*. 2017. doi:10.7326/0003-4819-157-11-201212040-00003

22. Aggerbeck H, Ruhwald M, Hoff ST, et al. C-Tb skin test to diagnose Mycobacterium tuberculosis infection in children and HIV-infected adults: A phase 3 trial. *PLoS One*. 2018;13(9). doi:10.1371/journal.pone.0204554

23. Whiting PF, Rutjes AWS, Westwood ME, et al. Quadas-2: A revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med*. 2011;155(8):529-536. doi:10.7326/0003-4819-155-8-201110180-00009

24. Wells G, Shea B, O’Connell D, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality if nonrandomized studies in meta-analyses. (Available from: URL: http://www.ohri.ca/programs/clinical\_epidemiology/oxford.asp). doi:10.2307/632432

25. Moberg J, Oxman AD, Rosenbaum S, et al. The GRADE Evidence to Decision (EtD) framework for health system and public health decisions. *Heal Res Policy Syst*. 2018;16(1). doi:10.1186/s12961-018-0320-2

26. Moher D, Liberati A, Tetzlaff J, Altman D. Preferred Reporting Items for Systematic Reviews and MetaAnalyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1. *PLoS Med*. 2009. doi:10.7326/0003-4819-151-4-200908180-00135

**Appendix 2: Search Strategy**

Databases:

* Embase Classic+Embase 1947 to present
* Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily 1946 to Present

|  |  |
| --- | --- |
|  | Search term |
| 1 | exp TUBERCULOSIS/ or tuberculosis.mp. or exp MYCOBACTERIUM TUBERCULOSIS/ or tb.mp. |
| 2 | exp Recombinant Proteins/ or (recombinant or novel or dppd or esat 6 or esat6 or cfp 10 or cfp10 or early secretory antigenic target\* or culture filtrate protein\* or rd\* or region of difference).mp. |
| 3 | skin test\*.mp. or Skin Tests/ |
| 4 | (c tb or diaskintest).mp. |
| 5 | 1 and 2 and 3 |
| 6 | 4 or 5 |

* e-library “Diaskintest” used as search term in Russian and English letters

Appendix 2 – sample quality assessment form based on QUADAS-223

