World Health Organization

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No. 2

Laboratory-based evaluation of 19 commercially available rapid diagnostic tests for tuberculosis

Special Programme for Research & Training in Tropical Diseases (TDR) sponsored by
UNICEF/UNDP/World Bank/WHO

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Abbreviations

AACC	American Association for Clinical Chemistry
BCG	Bacille Calmette-Guerin
BELAC	Belgian Organisation for Accreditation
CRF	case report form
CXR	chest radiography
DECs	disease-endemic countries
ELISA	enzyme-linked immunosorbent assay
HIV	human immunodeficiency virus
ICT	immunochromatographic
ISO	International Organization for Standardization
IUATLD	International Union Against Tuberculosis and Lung Disease
KG	kit group
MTB	*Mycobacterium tuberculosis*
NTM	nontuberculous mycobacteria
PCR	polymerase chain reaction
POC	point of care
RDT	rapid diagnostic test
ROC	receiver-operator curve
Rx	treatment
RDT	rapid diagnostic test
RT	room temperature
SG	subgroup
SOP	standard operating procedure
TB	tuberculosis
TDR	Special Programme for Research and Training in Tropical Diseases
USAID	United States Agency for International Development
WHO	World Health Organization

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Executive summary

Accurate and prompt tuberculosis (TB) diagnosis is critical to disease control. Simple user-friendly and affordable detection tools could save lives and reduce overall costs borne by patients and health systems. Rapid, accessible serologic tests for tuberculosis are on the market, largely in developing countries, but little reliable information about their content and performance is available. Therefore, TB case detection remains dependent upon sputum smear microscopy, radiography and clinical symptomatology. In recent years, remarkable efforts have been made globally to improve access to, and the quality of, tuberculosis diagnostic services and to identify promising new diagnostic tools. Global case notification rates have increased and more than 15 diagnostic candidates are in the pipeline. However, still less than 20% of TB patients receive a microbiologically confirmed diagnosis. To this end, in cooperation with rapid TB test manufacturers, WHO/TDR sponsored an evaluation of commercially available rapid TB tests to assess their performance, reproducibility and operational characteristics and to identify promising candidates.

Using 355 well-characterized archived serum samples, 19 rapid TB tests were evaluated at the Prince Leopold Institute of Tropical Medicine Mycobacteriology Unit. The sensitivity of these rapid tests ranged from 0.97% to 59.7%; specificity ranged from 53% to 98.7%, compared against a combined reference standard of mycobacterial culture and clinical follow-up. In general, tests with high specificity (>95%) had very low sensitivity (0.97-21%). Test performance was poorer in patients with sputum smear-negative TB (sensivity & specificity: p=0.0006) and in HIV-positive patients (sensivity: p=<0.0001, specificity: p=0.44). The average difference in test sensitivity between the HIV-negative (n=198) and the HIV-positive population (n=157) was +22%; the maximum difference was +43%. Several tests showed high reliability; the average inter-reader variability kappa was 0.77 and the overall lot-to-lot and run-to-run variability ranges were 0-25% and 0-26%, respectively. Twelve of the tests (63%) were rated as very easy to use and therefore appropriate for use in primary health-care settings in developing countries. None of the assays performed well enough to replace microscopy. However, smear microscopy combined with most rapid tests improved overall diagnostic sensitivity from 75% (smear alone) up to 89% (smear plus rapid test). This gain is equivalent to the detection of 57% (29/51) of the smear negative, culture positive TB cases but concomitantly yielded an unacceptable overall false positive rate of 42% (63/149).

Background

The Special Programme for Research and Training in Tropical Diseases (TDR) is an independent global programme of scientific collaboration. Established in 1975 and cosponsored by the United Nations Children's Fund (UNICEF), the United Nations Development Programme (UNDP), the World Bank and the World Health Organization (WHO), its vision is to foster an effective global research effort on infectious diseases of poverty, in which disease-endemic countries (DECs) play a pivotal role.

TDR uses a three-pronged strategy to achieve its vision and aims to:

- provide a collaborative framework and information service for research partners;
- empower scientists from disease-endemic countries as research leaders;
- support research on neglected priority needs.

For effective delivery of this strategy TDR has restructured its operations to a limited number of clearly delineated business lines. One of these focuses on the delivery of accessible quality-assured diagnostics. Diagnostic activities range from convening expert consultations to define diagnostic needs and product specifications to facilitating test development and evaluation to assess introduction in DECs. TB-focused activities include funding of TB diagnostics research; facilitating test development by providing test developers with clinical reference materials (TB Specimen Bank & TB Strain Bank), conducting evaluations of new and improved diagnostics; and building laboratory capacity for diagnostic trials in DECs.

Experts from WHO's Global TB monitoring and surveillance project estimate the annual total number of TB cases to be 8.8 million (1). If recent trends continue, the projected global number of new cases will increase to 10 million in 2015. This is despite the implementation of a global strategy for diagnosing and treating TB in over 182 countries. TB control is undoubtedly constrained by the inadequacy of available diagnostic tools. The cornerstone of pulmonary TB diagnosis worldwide is sputum smear microscopy. Although simple and relatively inexpensive, the WHO-recommended method requires high-quality microscopes, experienced microscopists, exacting quality management and multiple sputum examinations. Specificity is over 95% in high-prevalence settings but sensitivity ranges between 40% and 80% (3 smears combined). Sensitivity is particularly restricted in the setting of noncavitary parenchymal (i.e. children, HIV-infected persons) or extrapulmonary disease. The majority of TB patients (90%) live in low- and middle-income countries where diagnosis relies upon identification of acid-fast bacilli in unprocessed sputum smears using a conventional light microscope. Mycobacterial culture methods partially overcome the problem of low sensitivity but this advantage is offset by the delay (results take weeks); dedicated equipment and technical expertise required; and additional cost. Molecular amplification techniques (e.g. PCR) have been commercialized for TB but the equipment, personnel and financial investments required are too high for the majority of laboratories in the developing world.

Simple, accurate, inexpensive and, ideally, point-of-care (POC) diagnostic tools for TB are needed urgently. POC serological based tests have been developed successfully for many diseases (e.g. HIV and malaria) and are very attractive. Test formats (e.g. immunochromatographic [ICT] test) are suitable for resource-limited areas as these tests can be performed without specialized equipment and with minimal training. The development of immune-based tests for the detection of TB antibodies, antigens and immune complexes has been attempted for decades. Their performance is appraised critically in several descriptive reviews and textbook chapters (2-11). The most common of these tests rely on detection of an antibody immune response to *Mycobacterium tuberculosis* (MTB), as opposed to the T-cell based cellular immune response (e.g. interferon gamma release assays), or direct detection of antigens in specimens other than serum, e.g. lipoarabinomannan (LAM) detection in urine (12,13) and pleural fluid (14).

Progress in antibody detection has been limited by the heterogeneity of host immunological responses to TB

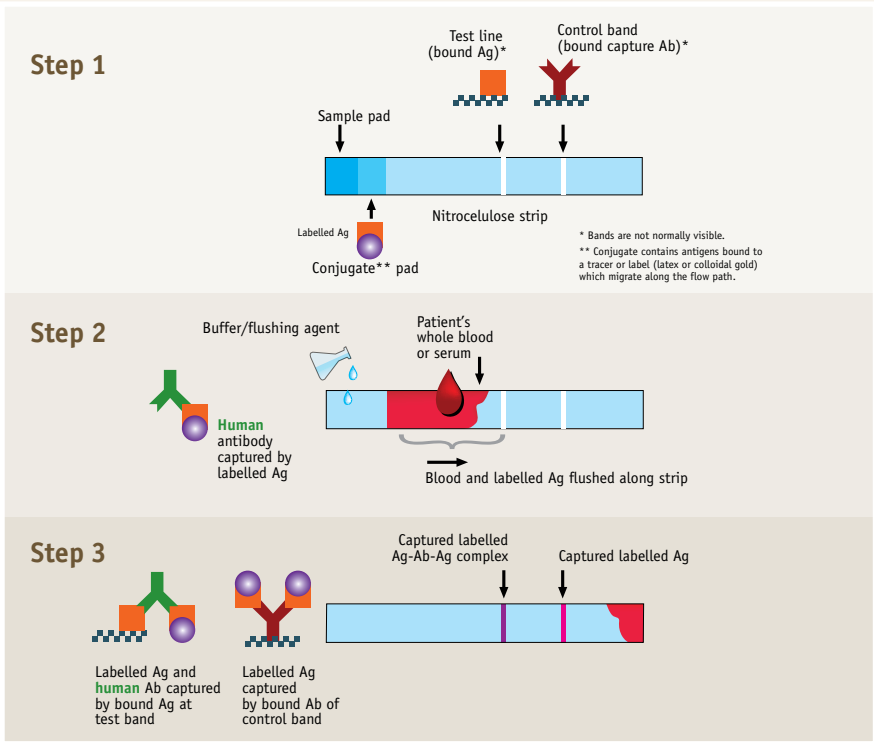
antigen. Furthermore, the profile of antigenic proteins of MTB recognized by antibodies differs at different stages of infection and disease progression (15-18). Thus, an accurate diagnostic test for TB will almost certainly need to be based on a combination of antigens.

It is estimated that over 40 rapid serologic TB tests that use various antigenic compositions to detect patients' antibodies are currently commercially available in many low- and middle-income countries. These may be suitable to diagnose TB in primary health-care settings but there are limited data on their performance characteristics in both HIV-infected and non-infected patient populations. Available data are limited to those found on package inserts – typically favourable but based on a small number of patients. These tests differ in a number of their features including antigen composition; antigen source (e.g. native or recombinant); chemical composition (e.g. protein, carbohydrate or lipid); extent and manner of purification of the antigen(s); and class of immunoglobulin detected (e.g. IgG, IgM or IgA). An ICT test format (Fig. 1) is common for rapid diagnostic TB tests. Antigens are precoated in lines across a membrane (e.g. nitrocellulose) to which samples are applied. Antigen-antibody reactions are visualized on the lines using anti-human antibody bound to substances such as colloidal gold. The test takes minutes to perform.

TDR has received repeated requests for information on the performance of rapid serologic TB tests and their potential for use in primary health-care settings in developing countries. The evaluation of the performance and reliability of rapid serologic TB diagnostics was identified as an important priority. Objective evaluation of the performance of these tests will provide national TB programmes with the critical preliminary information required to develop guidelines for appropriate use. Hence, the TDR rapid test evaluations will be conducted as a laboratory-based evaluation of test performance and reliability using well-characterized archived serum specimens from diverse geographical locations.[1] The results of the evaluation will inform the needs and content of field trials.

1 Brazil, Canada, the Gambia, Kenya, South Africa, Spain, Uganda, United Republic of Tanzania.

Figure 1. Mode of action of common tuberculosis rapid diagnostic test (RDT) format



1) Labelled antigen(s) (Ag), specific for target human antibody (Ab), is present on the lower end of the nitrocellulose strip (conjugate pad). Antigen(s) also specific for the target antibody is bound to the strip in a thin (test) line and antibody specific for labelled antigen(s) is bound at the control line.

2) Whole blood or serum and buffer, which have been placed on the strip or in the well are mixed with labelled antigen(s) and drawn up the strip across lines of bound antigen(s) and capture Ab.

3) If antibody is present, some labelled antigen(s) will be trapped on the test line. Other labelled antigen(s) is trapped on the control line by capture antibody.

Objectives

1 To compare the performance and reproducibility[2] of rapid MTB-specific antibody detection tests using archived serum samples from the WHO/TDR TB Specimen Bank.

2 To assess the operational characteristics of rapid MTB tests, including ease of use, technical complexity and inter-reader variability.

2 **Lot-to-lot reproducibility:** will the test give the same results with tests of different manufacturing lots using the same specimens?
Operator reproducibility: will the test give the same results on the same specimen if it is performed by two different operators?
Run-to-run variability: will tests performed on the same specimen on different days give the same results?

1. Evaluation methodology

1.1 General principles

The design and conduct of this evaluation was carried out in accordance with the best practice guidelines published by the TDR Diagnostics Evaluation Expert Panel (19). More specifically the evaluation follows the following guiding principles.

- A diagnostic test should be evaluated for a clearly defined indication i.e. to further knowledge about test performance.
- A diagnostic test should be evaluated using the methods and equipment fit for that purpose. Staff performing the evaluation should be qualified and competent to undertake the task and demonstrate that they can perform the test properly.
- The study population is the eventual target population for the diagnostic test.
- Tests are compared with reference standard (microbiological identification and clinical follow-up).
- Outcome measures are defined.
- Quality assurance procedures are incorporated including study quality control, external quality monitoring and study quality improvement.

1.2 Test selection

Over a two-year period (2003-2005), TDR compiled an inventory of commercially available serological tests for tuberculosis. Tests were identified via several mechanisms including web searches; international conferences (MEDICA, AACC); correspondence with directors of TB-laboratories in different countries; and company approaches to TDR. This process identified over 40 serological tests for TB, both ELISA and lateral flow ICT formats. All tests detect antimycobacterial antibodies in serum.

The first meeting of the ad hoc committee of TB immunologists and clinical-trial experts was convened to set criteria for the tests to be included in the evaluation. The following operational characteristics were established.

- **Rapid** – test result is available in less than 15 minutes.
- **Simple** – test can be performed in one or two steps[3], requiring minimal training and no equipment.
- **Easy to interpret** – card or strip format with visual readout.

Only test manufacturing companies were invited to participate, not distributors. This strategy was intended to reduce duplicate testing of identical products under different labels. Distributor products were eliminated from the list if the manufacturer could be determined with a high level of certainty.

Letters of invitation and the study protocol were sent to 27 companies whose products met the outlined inclusion criteria. In certain cases (2), identical products were revealed by identical package inserts and subsequent company disclosure of information. Companies interested in participating were asked to donate tests for evaluation and to sign an agreement for the results to be published in a WHO/TDR report and made available to health departments of WHO Member States. Nineteen companies agreed to participate:

1. **ABP Diagnostics Ltd** USA
2. **Advanced Diagnostics Inc.** UK
3. **American Bionostica Inc.** USA
4. **Ameritek** USA
5. **Bio-Medical Products Corporation** USA
6. **Chembio Diagnostic Systems Inc.** USA
7. **CTK Biotech Inc.** USA
8. **Hema Diagnostic Systems, LLC.** USA
9. **Laboratorios Silanes** Mexico
10. **Millennium Biotechnology Inc.** USA
11. **Minerva BiOTECH Corporation** Canada
12. **Mossman Associates Inc.** USA
13. **Pacific Biotech Co. Ltd.** Thailand

3 TB-Spot Version 2.0 (Stimulus Specialty Diagnostics, a division of Span Diagnostics Ltd) and MycoDot (Mossman Associates) are exceptions to this rule.

14. **Premier Medical Corporation** USA
15. **Princeton BioMeditech Corporation** USA
16. **Span Diagnostics Ltd.** India
17. **Standard Diagnostics Inc.** Republic of Korea
18. **Unimed International Inc.** USA
19. **VEDA.LAB** France

Six declined in writing:

- Clinotech Diagnostics and Pharmaceuticals Inc., Canada
- Dialab GmbH, Austria
- JAJ International Inc., USA
- NUBENCO Medical International, USA
- Oncoprobe Biotech Inc., Taiwan
- VicTorch Meditek Inc., USA

The characteristics of the tests are summarized in a table in Annex 1 (page 42).

Participating companies received preliminary results of test sensitivity, specificity and reproducibility following analysis of 298 patient serum specimens. At the conclusion of the evaluation, based on 355 patient serum specimens, participating companies received a courtesy draft of the report prior to publication. Under the terms of the confidentiality agreement with WHO, the companies could review the data and data analyses and provide comments to TDR. They could not modify any of the conclusions.

1.3 Site and personnel selection

The laboratory-based evaluation was conducted at the Prince Leopold Institute of Tropical Medicine Mycobacteriology Unit, a WHO Collaborating Centre for the Diagnosis and Surveillance of Mycobacterium Ulcerans Infection and the Coordinating Centre for the WHO/IUATLD Supranational Reference Laboratory Network for Tuberculosis[4] in Antwerp, Belgium. This laboratory also houses the WHO/TDR TB Strain Bank and was chosen because of its highly trained staff – experienced in routine, reference and research diagnostic methods for MTB. The laboratory holds International Organization for Standardization (ISO) certifications (EN ISO/IEC 15189;2003 and EN ISO/IEC 17025;2005) and is part of the Belgian Organisation for Accreditation (BELAC) external quality-assurance programme.

The principal investigator, Dr Francoise Portaels, holds a PhD in microbiology. Her responsibilities included:

- participation in the development of the consensus evaluation protocol;
- ensuring that the evaluation was conducted according to the final protocol;
- transferring data to TDR;
- participation in the data analysis and compilation of results.

The technical supervisor, Dr Anandi Martin, holds a PhD in microbiology. Her responsibilities included:

- maintaining the log of serum and test kit shipment receipts and ensuring proper storage;
- overseeing preparation of serum aliquots, study-code assignment (001 to 355) and tube labelling;
- ensuring that both technicians were blinded to the reference test results for the evaluation panel;
- supervising the performance of rapid test evaluations;
- ensuring that the rapid tests' results were read independently by technicians 1 and 2;
- signing off the lab books of each technician at the end of each day;
- collating the results from the two technicians and entering them into the Excel spreadsheet provided by TDR;
- entering the reference test result (i.e. final diagnosis) in the case report form (CRF).

Technician 1 was Cécile Uwizeye. Her responsibilities included:

- performing rapid tests in accordance with manufacturers' directions;

4 A network of laboratories established in 1994 to support the Global Project on Anti-tuberculosis Drug Resistance Surveillance. It provides external quality assurance in DST methods to over 150 countries.

- recording results in own laboratory record book;
- placing completed tests on a tray for technician 2 to read;
- assessing the operational characteristics of each rapid test according to the scheme provided.

Technician 2 was Natacha Koczorowski. Her responsibilities included:

- reading results of rapid tests
- recording results in own laboratory record book.

1.4 Collection of specimens and quality assurance

Archived serum samples from the WHO/TDR TB Specimen Bank were used to evaluate the tests. Formally launched by WHO/TDR in June 2000, the WHO/TDR TB Specimen Bank contains well-characterized samples from symptomatic respiratory patients with and without TB and HIV, from Brazil, Canada, Colombia, the Gambia, Kenya, Peru, South Africa, Spain, Uganda, the United Republic of Tanzania and Viet Nam. Blood, urine, sputum and saliva are collected on site from patients presenting at collaborating health clinics and showing symptoms of pulmonary tuberculosis. TB is diagnosed or excluded on the basis of smear microscopy, culture, radiography and clinical follow-up two to three months after the original visit. A final diagnosis is assigned according to a standardized classification scheme (Table 1). Aliquots of sputum, serum, saliva and urine are frozen at -70 °C at collection sites. The samples are shipped in liquid nitrogen to a central repository where they are transferred, without thawing, to storage at -70 °C. Each sample is linked by a unique numerical code to detailed clinical and microbiological information. Details of microbiological methods are available on the TDR web site (**http://www.who.int/tdr/diseases/tb/specimen.htm,** accessed 22 September 2008). The history of BCG vaccination was not available for all patients.

All collection sites were assessed for proficiency at conducting routine and reference TB diagnostic testing; received training in protocol procedures; and underwent clinical monitoring.

1.5 Preparation and validation of evaluation panels

For the purposes of this evaluation, only samples in the WHO/TDR TB Specimen Bank matching diagnostic codes 1 (smear positive, culture positive) and 2 (smear negative, culture positive) were included as reference standard, TB positive. Code 4 (smear negative, culture negative, no initial TB treatment and improved clinical condition, based on clinical, radiographic and microscopic evaluation, after 2-3 months follow up) samples were included as reference standard, TB negative. All samples were collected between 1999 and 2005.

Well in advance of the start of the evaluation all samples were shipped frozen on dry ice from the central WHO/TDR TB Specimen Bank repository in aliquots of 0.5 ml. On receipt the samples were unpacked and transferred to -20 °C without thawing. The evaluation site received two (0.5 ml) aliquots per patient for the performance evaluation and an additional three (0.5 ml) aliquots of serum from 56 patients for reproducibility testing.

The evaluation took place over several months due to the large volume of tests and samples. As it is not appropriate to leave sera thawed for several months, groups of serum aliquots were thawed systematically, realiquoted and labelled in volumes required for each kit group (plus an additional 20% volume). These were refrozen until required for testing, up to a maximum of four months at -20 °C.

With one exception[5], two tests were evaluated in parallel, forming kit groups (KGs) and labelled (A-I). Tests requiring

5 KG-D comprised three tests.

Table 1. WHO/TDR TB Specimen Bank: diagnostic classification scheme

No.	Diagnosis	Smear	Culture	Caveat/ description	Initial CXR	Clinical CXR improved at f/u without TB Rx	3rd (repeat) f/u sputum smear	Clin / CXR response to TB treatment	Alternative cause of Sx confirmed
1	TB, smear positive	Pos.	Pos.	Must have at least 2 pos. smears					
2	TB, culture positive, AFB-negative	Neg.	Pos.	≥1 neg. smear and ≥ 1 pos. culture	Pos. or	→	→	Pos.	
3	TB, culture negative	Neg.	Neg.	2 neg smear, neg. or 1+ cx, pos CXR and response to TB Rx	Pos. or	→	→	Pos.	
4	Non-TB, untreated	Neg.	Neg.	Not treated initially for TB		Yes	Neg. or ND		
5	Non-TB, treated	Neg.	Neg.	Treated initially for TB			Neg. **and** →	Neg. **and** →	Yes
6	Indeter-minate, treated	Neg.	Neg. or pos. (1+)	Treated initially for TB				Neg. or ND	No
7	Indeter-minate, untreated	Neg.	Pos.	Not treated initially for TB		Yes or ND	ND or neg.		
8	Indeter-minate	Other combinations have insufficient follow-up or inadequate data							

Patients must have the smear and culture results as listed, plus other relevant criteria as noted. Necessary or alternative criteria are indicated with and/or in bold. There are other types of indeterminate cases; these are examples. Response time for follow-up CXR and exam is ideally two months (20).

larger volumes of sera were matched randomly with tests requiring fewer sera. In one instance two tests with multiple steps and similar formats were evaluated in parallel (KG-F). Each KG required 60-105 µl serum per patient (Table 2). Approximately 20-30% of additional sera was added to each aliquot to ensure that there was sufficient.

Table 2. Composition of kit groups (KGs)

KG	Company 1	Volume required (µl)	Company 2	Volume required (µl)	Company 3	Volume required (µl)	Total volume required (µl)	Volume aliquoted (µl)
A	Premier Medical	100	Millennium Biotechnology	5	-	-	105	125
B	Standard Diagnostics	100	American Bionostica	5	-	-	105	125
C	Pacific Biotech	100	Bio-Medical Products Corp.	3	-	-	103	123
D	Advanced Diagnostics	5	ABP Diagnostics	5	Lab. Silanes	10	20	40
E	CTK Biotech	50	Chembio Diagnostic	30	-	-	80	100
F	Span Diagnostics	50	Mossman Associates	40	-	-	90	110
G	Unimed International	50	Hema Diagnostic	10	-	-	60	80
H	Ameritek USA	60	Princeton BioMeditech	25	-	-	85	105
i	Minerva BiOTECH	20	VEDA.LAB	25	-	-	45	65
				Total	-	10	**693**	**873**

1.6 Blinding to reference standard results and results between tests

After pooling two 0.5 ml serum aliquots from the same patient, the laboratory supervisor ensured that each aliquot was coded with a unique three digit study ID between 001 and 355. For reliability testing, three 0.5 ml aliquot were pooled and labelled between 501 and 556. Specimens were numbered randomly in order to ensure that the diagnosis category cannot be deduced from the numbering. The laboratory supervisor ensured that both technicians were blinded and did not have access to the reference test results.

In order to avoid comparison of results between tests, the same sera were not used on the two tests in each KG during one evaluation session (day). To this end, all aliquots in each KG were subdivided into ten groups of between 25 and 40 serum samples. The aliquots were labelled further according to the subgroup (SG) 1-10. Different subgroups were used for the two tests during each day of evaluation to ensure that the results of different tests were not compared.

Three aliquots from the same patient were pooled to one aliquot (1.5 ml) for reproducibility testing. The fifty-six 1.5 ml aliquots were labelled from 501 to 556 and subdivided into seven groups (01-07) of eight aliquots.

2. Ethical considerations

Each WHO/TDR TB Specimen Bank collection site obtained approval from the WHO Research Ethics Review Committee and a local institutional review board or ethics committee for specimen collection and archiving of clinical materials for the purpose of facilitating commercial development and evaluation of diagnostics for tuberculosis. Specimens are unlinked to personal identifiers so that sera cannot be traced to individual patients. The protocol for the laboratory-based evaluation of commercially available rapid TB tests using archived samples from the WHO/TDR TB Specimen Bank was approved by the WHO Research Ethics Review Committee.

3. Performing rapid tests

Two lots of rapid test kits were shipped directly from the manufacturers to the evaluation centre. All kits were received by 19 January 2005, with two exceptions – a "lot 2" delivery from Mossman Associates, for reproducibility testing, was received 4 July 2005; the CTK Biotech test kits were received 3 May 2005. The lot number, quantities and expiry dates were recorded (Annex 2). Products were stored according to manufacturers' instructions prior to, and during, the evaluation.

Performance of rapid tests was in accordance with the following general guidelines for use of test kits and biosafety.

3.1 General guidelines for test kit use

1. Record lot number and expiry date on CRF: kits should not be used beyond their expiry dates.[6]
2. Ensure correct storage conditions: do not use the kit if a desiccant included in the package has changed colour.
3. Test kits stored in a refrigerator should be brought to room temperature (approximately 30 minutes) before use. Test kits that are too cold may produce false-negative results.
4. Damaged kits should be discarded.
5. A test kit should be used immediately after opening.
6. Reagents from one kit should not be used with those of another.
7. Use a new pipette or dropper for each specimen in order to avoid cross contamination.
8. Test should be performed exactly as described in the product insert/instructions.[7]

3.2 General biosafety guidelines

1. Treat all specimens as potentially infectious.
2. Wear protective gloves and laboratory gown while handling specimens.
3. Do not eat, drink or smoke in the laboratory.
4. Do not wear open-toed footwear in the laboratory.
5. Clean up spills with appropriate disinfectants, e.g. 1% bleach.
6. Decontaminate all materials with an appropriate disinfectant.
7. Dispose of all dry waste consumables, including test kits, in a biohazard container.

6 We requested, and were granted, a certificate of expiry extension from Span Diagnostics for a two-month period (until December 2005).

7 Some manufacturers recommended use of fresh serum or serum frozen <1 year, that had not undergone repeat freeze-thaw cycles. It was not possible to comply with these recommendations as archived samples collected between 1999 and 2005 were used and two freeze-thaw cycles were required. The potential impact on test performance and reproducibility is believed to be minimal and is discussed elsewhere in this report.

3.3 Preparing tests and serum samples for testing

At the beginning of each day all tests and serum samples were brought to room temperature before use. When a precipitate was visible, the serum was clarified by centrifuging at 12 000 g for five minutes prior to testing.

3.4 Test sequence

As described, the 19 tests were divided into 9 KGs (8 of 2 tests; 1 of 3 tests) and 10 SGs (each between 25 and 40 sera). Each day two tests were evaluated using batches of between 25 and 40 samples from different SGs. In order to avoid comparison of results between tests, each KG was evaluated with the full panel of sera before moving to the next. It took a maximum of ten days to complete each KG with a full panel of sera. The evaluation of test kits on all 355 serum specimens was completed in December 2005.

3.5 Standard operating procedures (SOPs) for tests under evaluation (1-19)

Annex 3 (page 49) contains a descriptive and illustrated summary of the test procedures for each of the tests covered in this report. For full details and any questions regarding the SOPs, please refer to the product insert for each test kit.

3.6 SOP for determining inter-observer variability

1. Each test should be performed and read by technician 1 according to the instructions described. Results should be recorded in a laboratory record book.
2. The test should then be mounted onto a numbered folder and handed to technician 2.
3. Technician 2 will interpret the test result immediately and independently.
4. Technician 2 will record the results in a separate laboratory record book.

3.7 Handling of indeterminate results

Indeterminate results were recorded as such. The test was repeated if sufficient test kits and sera were available after the evaluation was completed.

3.8 SOP for performing reproducibility testing

Two technicians independently read and recorded the results of each testing.
Two technicians independently repeated each test of **two** different lot numbers on **eight** samples over **three** subsequent days.

Aliquots (1.5 ml) of serum for reliability testing were prepared from eight patient samples.

Two or (in one case) three kits were evaluated in parallel. The tests were performed according to manufacturers' recommendations. The two lot numbers of each test were performed in parallel on identical samples by both technicians.

3.9 Assessing operational characteristics

Each rapid test was assessed for the following operational characteristics by technician 1 and technician 2 after 25 repetitions.

1. Clarity of kit instructions (maximum possible score – 3).
2. Technical complexity or ease of use (maximum possible score – 3).
3. Ease of interpretation of results (maximum possible score – 3).

An additional point was given to the rapid tests that do not require any additional equipment or supplies. Ten was the maximum possible score. This score indicates that a test has operational characteristics suggesting suitability for use in primary health-care facilities in resource-limited settings. Scores from the two technicians were averaged and data were recorded on the operational characteristics form (Annex 4).

4. Pilot phase

Two technicians performed each of the tests under evaluation with two positive and one negative sera from the evaluation panel, under the supervision of the technical supervisor. Each KG was piloted in parallel. Technicians were blinded to the reference material status. The tests results were read by both technicians. When results were invalid, the tests were repeated with new devices. The supervisor and technicians proceeded with the evaluations only when they were confident about each component of the testing procedure.

5. Statistical methods

5.1 Sample size

Sample size calculations were made according to a prediction that sensitivity and specificity of tests would be 50% and 50%, respectively, against reference materials; and allowing a 10% margin of error. Each rapid test was to be evaluated using a panel of 400 serum samples divided into 4 diagnostic categories, each comprising 100 samples:

1. **TB positive, HIV positive**
2. **TB positive, HIV negative**
3. **TB negative, HIV positive**
4. **TB negative, HIV negative**

The target sample size allowed a determination of sensitivity and specificity of the test with a 95% ± 10% confidence interval.

Unfortunately, it was not possible to obtain the target sample size in each diagnostic category as the WHO/TDR TB Specimen Bank had insufficient samples of symptomatics who were confirmed TB negative, HIV positive. Despite enrolment and clinical follow up of new pulmonary symptomatics from several regions of the world, target numbers could not be achieved prior to test kit expiry dates. The final sample sizes across diagnostic categories were:

1. **TB positive, HIV positive:**	**107**
2. **TB positive, HIV negative:**	**99**
3. **TB negative, HIV positive:**	**50**
4. **TB negative, HIV negative:**	**99**
Total	**355**

These sample sizes allowed a point estimate determination of sensitivity and specificity with 95% ±10-14% confidence intervals.

Box 1. Sensitivity and specificity calculations

		Reference test results +	Reference test results -
Rapid test results	+	a	b
	-	c	d
		a+c	b+d

Rapid test sensitivity = a/(a+c)
Rapid test specificity = d/(b+d)

a = true-positive result
b = false-positive result
c = false-negative result
d = true-negative result

5.2 Sensitivity and specificity

The overall sensitivity and specificity of the rapid tests compared to the reference test were calculated (Box 1). Test performance within HIV positive and negative subgroups was also performed. The test for homogeneity of kappa statistics is often used to determine the combined correlation of test sensitivity and specificity against the reference standard in order to estimate overall test performance. However, significant differences in the kappa (ranging from 0.01 to 0.21) made it inappropriate to apply this as an estimate of overall test performance for all tests. Instead, overall performance is illustrated using receiver operating characteristic (ROC) curves. No discrepant analysis was performed.

5.2.1 Comparative sensitivity and specificity

The test for homogeneity of kappa statistics compares the 19 tests separately for TB cases and non TB cases. This is followed by two tests at a time to provide pair-wise comparisons. A Bonferroni correction is applied to the significance level – tests are deemed significantly different only when the calculated p-value exceeds 0.00028 (correcting for 171 pairs).

5.3 Test reproducibility

The reproducibility of each test was evaluated. Two technicians read the results of each test performed (inter-reader reproducibility). Two technicians (operator-to-operator reproducibility) also performed tests from two different lot numbers (lot-to-lot reproducibility) over three subsequent days (run-to-run reproducibility) using eight unique samples. This resulted in a total of 96 replications (2 technicians x 2 lots x 3 days x 8 samples).

5.3.1 Inter-reader reproducibility

The kappa statistic reflecting the agreement between reader 1 and reader 2 is estimated along with its 95% confidence intervals. Generally, kappa statistics greater than 0.70 are deemed to have excellent agreement, those less than 0.40 are poor. McNemar's test for correlated proportions is used to test for systematic differences between reference and test results. There are separate analyses of TB-positive; TB-negative; HIV-positive; and HIV-negative samples.

5.3.2 Operator-to-operator, run-to-run and lot-to-lot reproducibility

The variability of each rapid test was calculated as follows:

- **Operator-to-operator** – the number of test results which differ between 2 readers of rapid test results x 100/total number of tests performed using the same 8 serum specimens.
- **Run-to-run** – number of test results which differ between days x 100/total number of tests performed on the same 8 serum specimens on 3 successive days.
- **Lot-to-lot** – number of differing test results between 2 lots x 100/total number of tests performed on the 2 lots using the same 8 serum specimens.

6. Data management

6.1 Data entry

The results of the evaluation and the reproducibility testing were recorded in the laboratory notebooks of each of the two technicians. The technical supervisor signed off the results (source documents) daily. These were entered into the hard copy CRFs (Annexes 5 and 6) and then into a corresponding Excel spreadsheet. Any repeat tests and the reason for repeating were entered on the spreadsheet. Only the technical supervisor and the principal investigator had access to the electronic record files. The scoring scheme for the operational characteristics of each rapid test were completed by the two technicians and entered into the corresponding Excel file (Annex 4) by the technical supervisor.

The final diagnostic code assignment for each patient sample was verified against the WHO/TDR TB Specimen Bank database and hard copy CRFs. Discrepancies were resolved through direct contact with the WHO/TDR TB Specimen Bank collection site and subsequent review of raw data.

All source documents and two electronic records of study data were kept in secure areas until the conclusion of the evaluation, data analysis and report publication.

7. Quality assurance

Prior to the initiation of the trial WHO/TDR staff assessed the study laboratory to ensure proper storage of patient samples and test kits and proficiency in performing the tests under evaluation. During the study, a TDR-designated consultant independently assessed that protocol and laboratory procedures were in accordance with the study protocol and that Good Clinical Practice, Good Laboratory Practice and Good Clinical Laboratory Practice were observed.

8. Results

A total of 355 sera from 8 geographically diverse collection sites were used to evaluate the 19 rapid tuberculosis tests. Of these, 206 (58%) were reference standard TB positive and 149 (42%) were reference standard TB negative. The average patient age was 35 years and the distribution of males to females was 58% (206) to 42% (149); 44% (157) of samples were from HIV positive patients. Of the TB positive samples, 44% (155/206) were smear positive, culture positive and 14% (51/206) were smear negative, culture positive. Table 3 shows the distribution of specimen/patient characteristics – age; sex; sputum smear and HIV status; and geography.

Table 4 shows the overall sensitivity and specificity of each test compared to the reference standard. Figures 2a-2b and 3a-3b show the range of sensitivity and specificity of rapid tests, the comparison of performance indicators across tests and how performance compares with a selection of rapid serologic assay reports published between 1990 and 2006.

Table 3. Archive specimen (patient) characteristics

Country number (%)	Sample collection period	Age (mean)	Age (median)	Sex		Final diagnosis			HIV status No./%		TB & HIV status					
				Male	Female	ss+/cx+	ss-/cx+	Non TB	Pos.	Neg.	SS+/ HIV+	SS+/HIV-	SS-/CX+/ HIV+	SS-/CX+/ HIV-	Non TB/ HIV+	Non TB/ HIV-
Brazil n= 21 (6%)	2000-2001	39	38	9 (43%)	12(57%)	2(10)	1(5)	18(86)	0(0)	21(100)	0(0)	2(10)	0(0)	1(5)	0(0)	18(86)
Canada n= 42 (12%)	2001-2002	61	67	24(57)	18(43)	0(0)	0(0)	42(100)	1(2)	41(98)	0(0)	0(0)	0(0)	0(0)	1(2)	41(98)
Gambia n= 103 (30%)	1999-2000	30	29	58(56)	45(44)	30(29)	19(18)	54(52)	22(21)	81(79)	3(3)	27(26)	2(2)	17(17)	17(17)	37(36)
Kenya n= 42 (12%)	2005	34	33	26(62)	16(38)	19(45)	16(38)	7(17)	40(95)	2(5)	19(45)	0(0)	14(33)	2(5)	7(17)	0(0)
South Africa n= 15 (4%)	1999, 2005	36	34	8(53)	7(47)	12(80)	1(7)	2(13)	15(100)	0(0)	12(80)	0(0)	1(7)	0(0)	2(13)	0(0)
Spain n=23 (6%)	2003	50	48	19(83)	4(17)	4(17)	7(30)	12(52)	9(39)	14(61)	0(0)	4(17)	0(0)	7(30)	9(39)	3(13)
United Republic of Tanzania n= 33 (9%)	2002	37	35	17(52)	16(48)	12(36)	7(21)	14(42)	33(100)	0(0)	12(36)	0(0)	7(21)	0(0)	14(42)	0(0)
Uganda n= 76 (21%)	1999	29	29	45(59)	31(41)	76(100)	0(0)	0(0)	37(49)	39(51)	37(49)	39(51)	0(0)	0(0)	0(0)	0(0)
Overall n=355	**1999-2005**	**35**	**38**	**206 (58%)**	**149 (42%)**	**155 (44%)**	**51 (14%)**	**149 (42%)**	**157 (44%)**	**198 (56%)**	**83**	**72**	**24**	**27**	**50**	**99**
											Overall TB positive: 206 (58%)				**Non TB: 149 (42%)**	

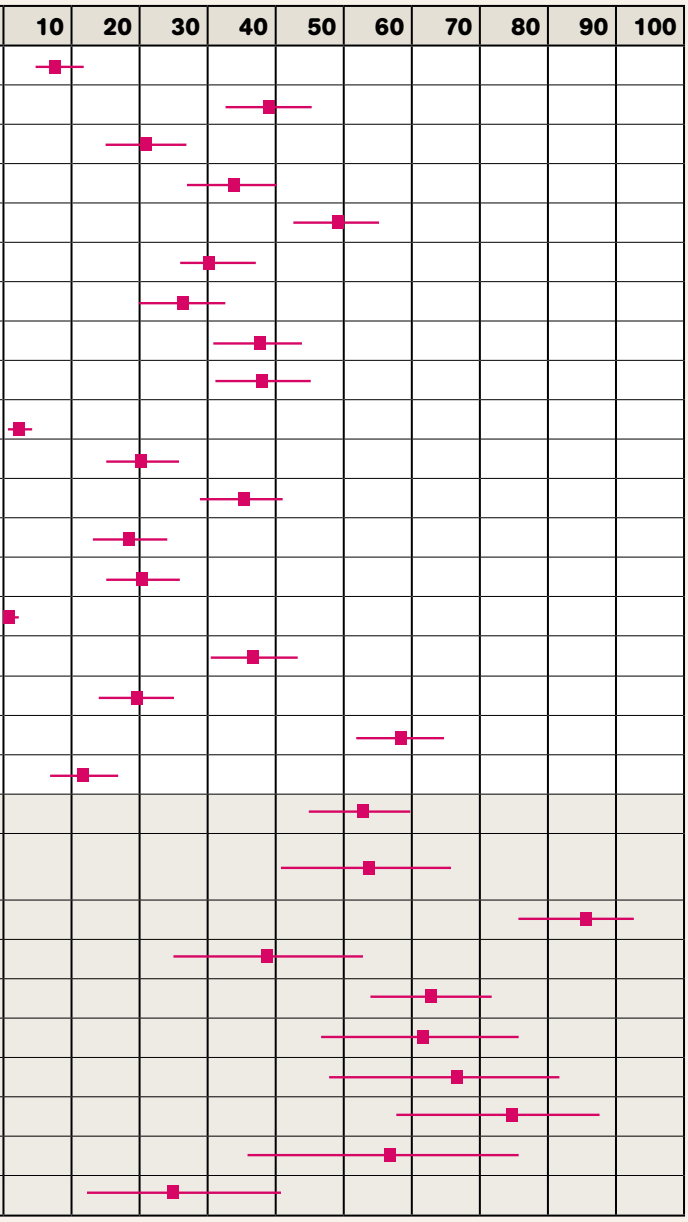
ss+: sputum smear positive; ss-: sputum smear negative; cx+: culture positive; cx-: culture negative; HIV+: HIV positive; HIV-: HIV negative

Table 4. Performance of rapid diagnostic tests for pulmonary tuberculosis

Manufacturer	Test	Sensitivity 95% CI	Specificity 95% CI
ABP Diagnostics	TB Rapid Screen Test	7.77 (4.11- 11.43)	95.3 (91.90-98.70)
Advanced Diagnostics	Tuberculosis Rapid Test	39.71 (33.00-46.42)	53.02 (45.01-61.03)
American Bionostica	ABI Rapid TB Test	20.39 (14.89-25.89)	79.87 (73.43-86.31)
Ameritek USA	dBest One Step Tuberculosis Test	33.82 (27.33- 40.31)	68.24 (60.74-75.74)
Bio-Medical Products	Rapid TB Test	49.03 (42.20-55.86)	57.05 (49.10-65.00)
Chembio Diagnostic Systems	TB STAT-PAK II	31.55 (25.20-37.90)	82.55 (76.46-88.64)
CTK Biotech	Onsite Rapid Test	26.70 (20.66-32.74)	69.13 (61.71-76.55)
Hema Diagnostic Systems	Rapid 1-2-3 HEMA Tuberculosis Test	35.92 (29.37-42.47)	72.48 (65.31-79.65)
Laboratorios Silanes	TB-Instantest	37.86 (31.24-44.48)	69.8 (62.43-77.17)
Millennium Biotechnology	Immuno-Sure TB Plus	2.43 (0.33-4.53)	98.66 (96.81-100)
Minerva BiOTECH	V Scan	21.36 (15.76-26.96)	89.26 (84.29-94.23)
Mossman Associates	MycoDot's 9 Easy Steps	36.41 (29.84-42.47)	86.58 (81.11-92.05)
Pacific Biotech	BIOLINE Tuberculosis Test	19.42 (14.02- 24.82)	94.63 (91.01-98.25)
Premier Medical	First Response Rapid TB Card	21.46 (15.84- 27.08)	95.24 (91.80-98.68)
Princeton BioMeditech	BioSign M.tuberculosis Test	0.97 (0-2.31)	98.66 (96.81-100)
Span Diagnostics	TB Spot ver. 2.0	38.35 (31.71-44.99)	77.85 (71.18-84.52)
Standard Diagnostics	SD TB Rapid Test	20.59 (15.04-26.14)	95.95 (92.77-99.13)
Unimed International	FirstSign MTB Test	59.71(53.01-66.41)	57.72 (49.79-65.65)
VEDA.LAB	TB-Rapid Test	12.62 (8.09-7.15)	97.99 (95.74-100)

Figure 2a. Sensitivity of commercial rapid test for diagnosis of pulmonary tuberculosis in this study compared with a selection of rapid serologic assay studies published 1990-2006 (22)

Manufacturer	Sensitivity 95% CI
ABP Diagnostics	7.77 (4.11-11.43)
Advanced Diagnostics	39.71 (33.00-46.42)
American Bionostica	20.39 (14.89-25.89)
Ameritek USA	33.82 (27.33-40.31)
Bio-Medical Products	49.03 (42.20-55.86)
Chembio Diagnostic Systems	31.55 (25.20-37.90)
CTK Biotech	26.70 (20.66-32.74)
Hema Diagnostic Systems	35.92 (29.37-42.47)
Laboratorios Silanes	37.86 (31.24-44.48)
Millennium Biotechnology	2.43 (0.33-4.53)
Minerva BiOTECH	21.36 (15.76-26.96)
Mossman Associates	36.41 (29.84-42.47)
Pacific Biotech	19.42 (14.02-24.82)
Premier Medical	21.46 (15.84-27.08)
Princeton BioMeditech	0.97 (0-2.31)
Span Diagnostics	38.35 (31.71-44.99)
Standard Diagnostics	20.59 (15.04-26.14)
Unimed International	59.71 (53.01-66.41)
VEDA.LAB	12.62 (8.09-17.15)
Kaolin agglutination test†-a	54 (46-61)
Kaolin agglutination test†- b	55 (42-67)
ICT* - a	87 (77-94)
ICT* - b	40 (26-54)
ICT* - c	64 (55-73)
MycoDot ¥ -a	63 (48-77)
MycoDot ¥ -b	68 (49-83)
MycoDot ¥ -c	76 (59-89)
MycoDot ¥ -d	58 (37-77)
MycoDot ¥ -e	26 (13-42)



† Hitech Laboratories, Bombay, India

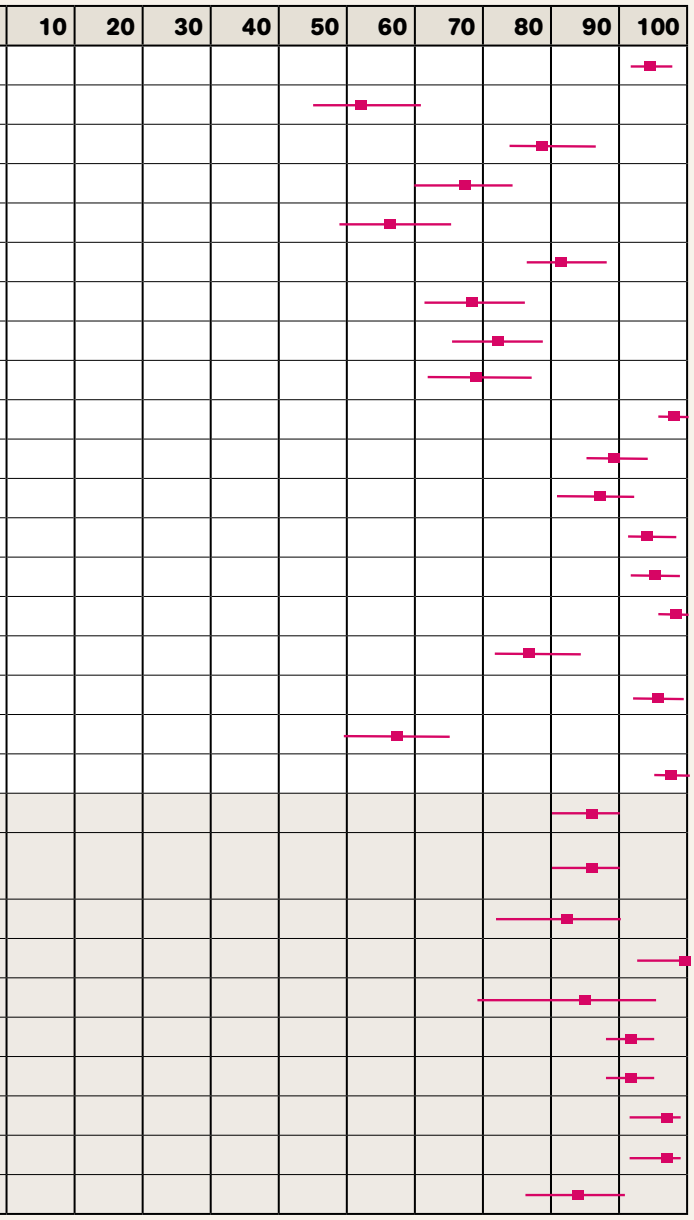
* ICT Diagnostics, Balgowlah, New South Wales, Australia

¥ Mossman Associates, Blackstone, Massachusetts, USA

= published studies (22)

Figure 2b. Specificity of commercial rapid test for diagnosis of pulmonary tuberculosis in this study compared with a selection of studies published 1990-2006 (22).

Manufacturer	Specificity 95% CI
ABP Diagnostics	95.3 (91.90-98.70)
Advanced Diagnostics	53.02 (45.01-61.03)
American Bionostica	79.87 (73.43-86.31)
Ameritek USA	68.24 (60.74-75.74)
Bio-Medical Products	57.05 (49.10-65.00)
Chembio Diagnostic Systems	82.55 (76.46-88.64)
CTK Biotech	69.13 (61.71-76.55)
Hema Diagnostic Systems	72.48 (65.31-79.65)
Laboratorios Silanes	69.8 (62.43-77.17)
Millennium Biotechnology	98.66 (96.81-100)
Minerva BiOTECH	89.26 (84.29-94.23)
Mossman Associates	86.58 (81.11-92.05)
Pacific Biotech	94.63 (91.01-98.25)
Premier Medical	95.24 (91.80-98.68)
Princeton BioMeditech	98.66 (96.81-100)
Span Diagnostics	77.85 (71.18-84.52)
Standard Diagnostics	95.95 (92.77-99.13)
Unimed International	57.72 (49.79-65.65)
VEDA.LAB	97.99 (95.74-100)
Kaolin agglutination test†-a	86 (80-90)
Kaolin agglutination test†- b	86 (80-90)
ICT* - a	82 (72-90)
ICT* - b	100 (93-100)
ICT* - c	85 (69-95)
MycoDot ¥ -a	92 (88-95)
MycoDot ¥ -b	92 (87-95)
MycoDot ¥ -c	97 (92-99)
MycoDot ¥ -d	97 (92-99)
MycoDot ¥ -e	84 (76-91)



† Hitech Laboratories, Bombay, India

* ICT Diagnostics, Balgowlah, New South Wales, Australia

¥ Mossman Associates, Blackstone, Massachusetts, USA

= published studies (22)

Figure 3a. **Comparative sensitivity performance of commercial rapid tests for diagnosis of pulmonary tuberculosis**

	Sensitivity	UniMed International	Bio-Medical Products	Advanced Diagnostics	Span Diagnostics	Laboratorios Silanes	Mossman Associates	Hema Diagnostic Systems	Ameritek, USA
UniMed International	0.6								
Bio-Medical Products	0.49	0.0127							
Advanced Diagnostics	0.4	<0.0001	0.029						
Span Diagnostics	0.38	<0.0001	0.0076	0.718					
Laboratorios Silanes	0.38	<0.0001	0.0038	0.6171	0.9028				
Mossman Associates	0.36	<0.0001	0.001	0.425	0.4652	0.7055			
Hema Diagnostic Systems	0.36	<0.0001	0.003	0.4631	0.5831	0.6276	0.9055		
Ameritek, USA	0.34	<0.0001	0.002	0.2273	0.2786	0.4367	0.6464	0.7532	
Chembio Diagnostic Systems	0.32	<0.0001	<0.0001	0.0558	0.0614	0.1385	0.1736	0.2987	0.4602
CTK Biotech	0.27	<0.0001	<0.0001	0.0018	0.0036	0.0106	0.0168	0.0282	0.0548
Standard Diagnostics	0.21	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0011
Premier Medical	0.21	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0021
Minerva BiOTECH	0.21	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	0.0003	<0.0001	0.0041
American Bionostica	0.2	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0005
Pacific Biotech	0.19	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0003
VEDA.LAB	0.13	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
ABP Diagnostics	0.08	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Millennium Biotechnology	0.02	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Princeton BioMediTech	0.01	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Chembio Diagnostic Systems	CTK Biotech	Standard Diagnostics	Premier Medical	Minerva BiOTECH	American Bionostica	Pacific Biotech	VEDA.LAB	ABP Diagnostics	Millennium Biotechnology	Princeton BioMediTech
0.1489										
0.0004	0.0741									
0.0014	0.159	1								
0.0063	0.1658	0.8728	1							
0.0028	0.0741	0.7855	0.7893	0.7995						
<0.0001	0.0394	0.5271	0.3458	0.505	0.7855					
<0.0001	<0.0001	0.0131	0.0094	0.0126	0.0017	0.0348				
<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0001	0.0412			
<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0116		
<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.001	0.2568	

<0.0005

>=0.05

Figure 3b. **Comparative specificity performance of commercial rapid tests for diagnosis of pulmonary tuberculosis**

	Specificity	Princeton BioMediTech	Millennium Biotechnology	VEDA.LAB	Standard Diagnostics	ABP Diagnostics	Pacific Biotech	Premier Medical	Minerva BiOTECH
Princeton BioMeditech	0.99								
Millennium Biotechnology	0.99	1							
VEDA.LAB	0.98	0.5637	0.6547						
Standard Diagnostics	0.96	0.1025	0.1573	0.2568					
ABP Diagnostics	0.95	0.0588	0.0956	0.0455	0.7389				
Pacific Biotech	0.95	0.0339	0.0578	0.0956	0.3173	0.763			
Premier Medical	0.95	0.0588	0.0956	0.1573	0.5637	0.7389	1		
Minerva BiOTECH	0.89	0.0005	0.001	0.0016	0.0253	0.0495	0.0881	0.0495	
Mossman Associates	0.87	<0.0001	0.0001	<0.0001	0.001	0.0046	0.0073	0.0029	0.4795
Chembio Diagnostic Systems	0.83	<0.0001	<0.0001	<0.0001	<0.0001	0.0003	0.0002	<0.0001	0.0956
American Bionostica	0.80	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0308
Span Diagnostics	0.78	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0095
Hema Diagnostic Systems	0.72	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0006
Laboratorios Silanes	0.70	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
CTK Biotech	0.69	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Ameritek USA	0.68	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
UniMed International	0.58	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Bio-Medical Products	0.57	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Advanced Diagnostics	0.53	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Mossman Associates	Chembio Diagnostic Systems	American Bionostica	Span Diagnostics	Hema Diagnostic Systems	Laboratorios Silanes	CTK Biotech	Ameritek USA	UniMed International	Bio-Medical Products	Advanced Diagnostics
0.2568										
0.0588	0.4652									
0.0046	0.2498	0.6015								
0.0033	0.0321	0.0782	0.2763							
<0.0001	0.0056	0.0053	0.0768	0.5862						
<0.0001	0.0032	0.0183	0.0796	0.8886	0.8886					
<0.0001	<0.0001	0.0131	0.0321	0.4669	0.6858	0.884				
<0.0001	<0.0001	<0.0001	<0.0001	0.0052	0.0314	0.0243	0.0356			
<0.0001	<0.0001	<0.0001	<0.0001	0.0016	0.0038	0.029	0.0629	0.9093		
<0.0001	<0.0001	<0.0001	<0.0001	0.0004	0.0007	0.0041	0.0105	0.431	0.4142	

<0.0005

>=0.05

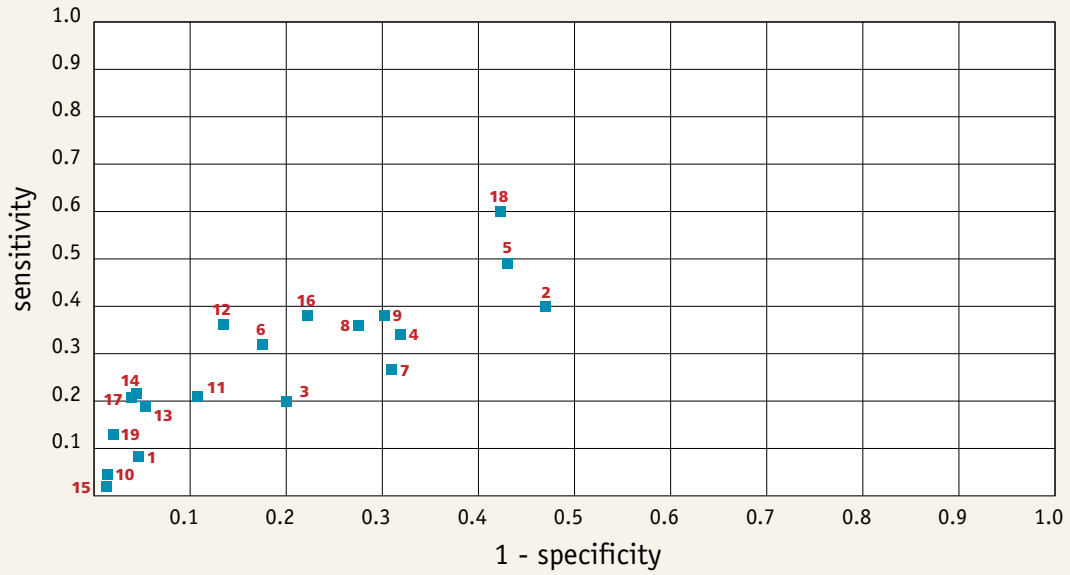
8.1 Test performance

FirstSign MTB Card Test (Unimed International) and Rapid TB Test (Bio-Medical Products) had the highest sensitivity: 59.7% (53.0%-66.4% 95% CI) and 49.0 (42.2-55.8 95% CI), respectively (p=0.0127); with corresponding specificity of 57.7% (49.8%-65.6% 95% CI) and 57.0(49.1-65.0 95% CI), respectively (p=0.9093). Immu-Sure TB plus (Millennium Biotechnology), BioSign M.tuberculosis (Princeton BioMeditech), TB-Rapid Test (VEDA.LAB), TB Rapid Test (Standard Diagnostics), TB Rapid Screen Test (ABP Diagnostics), BIOLINE Tuberculosis Test (Pacific Biotech), First Response Rapid TB Card (Premier Medical) and V Scan (Minerva BiOTECH) had the highest specificity (p≥0.0005): 98.7% (96.8%-100% 95% CI), 98.7% (96.8%-100% 95% CI), 98 (95.7-100), 95.9 (92.8-99.1), 95.3 (91.9-98.7), 94.6 (91.0-98.2), 95.2(91.8-98.7), 89.26(84.29-94.23), respectively. Corresponding point estimate sensitivity ranged between 1 and 21% for this group of tests.

8.1.1 Overall performance

Overall performance is illustrated using receiver operating characteristic (ROC) curves (Figs. 4-7b). Tests with the best overall performance are located in the upper left hand corner of the graph.

Figure 4. ROC curve of commercial rapid tests for the diagnosis of pulmonary tuberculosis (all patients, n=355)



1. ABP Diagnostics **2.** Advanced Diagnostics **3.** American Bionostica **4.** Ameritek USA **5.** Bio-Medical Products **6.** Chembio Diagnostic Systems **7.** CTK Biotech **8.** Hema Diagnostic Systems **9.** Laboratorios Silanes

Figure 5a. ROC curve of commercial tests for the diagnosis of pulmonary tuberculosis – sputum smear-positive patients (n=304)

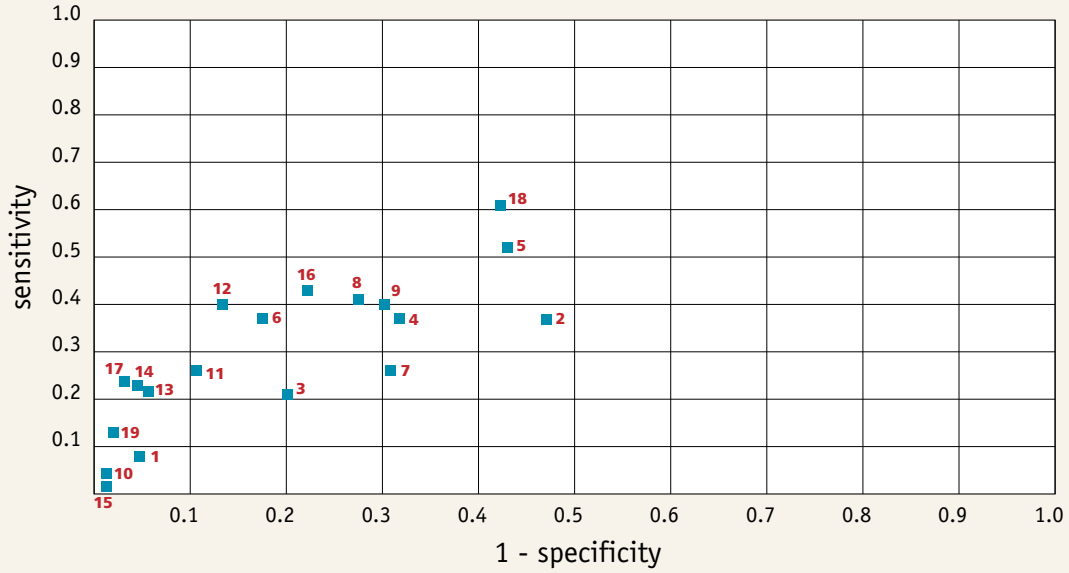
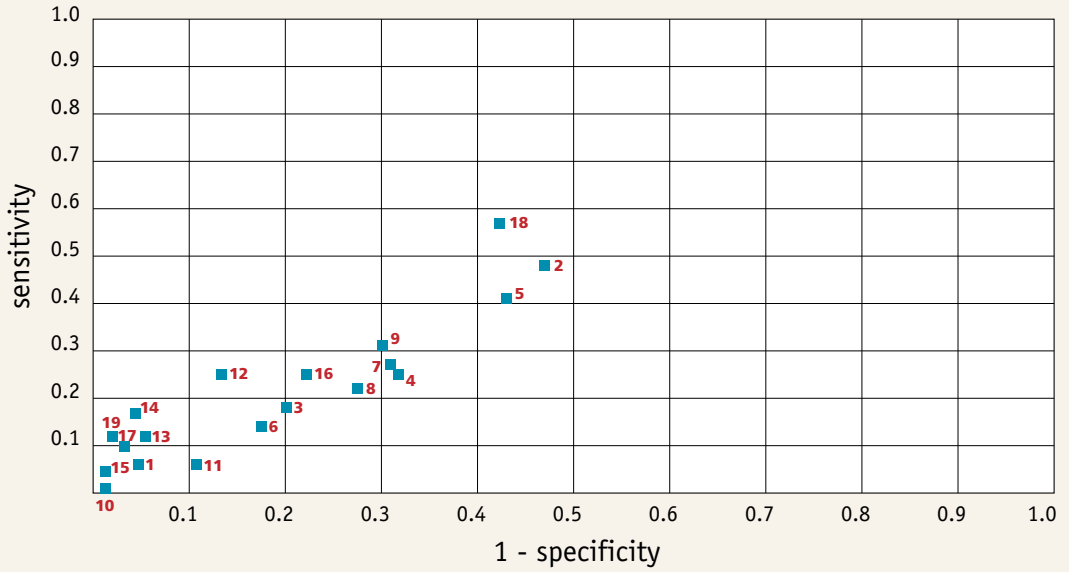


Figure 5b. ROC curve of commercial tests for the diagnosis of pulmonary tuberculosis – sputum smear-negative patients (n=300)



10. Millennium Biotechnology **11.** Minerva BiOTECH **12.** Mossman Associates **13.** Pacific Biotech **14.** Premier Medical **15.** Princeton Biomeditech **16.** Span Diagnostics **17.** Standard Diagnostics **18.** Unimed International **19.** VEDA.LAB

Figure 6a. ROC curve of commercial tests for the diagnosis of pulmonary tuberculosis – HIV-negative patients (n=198)

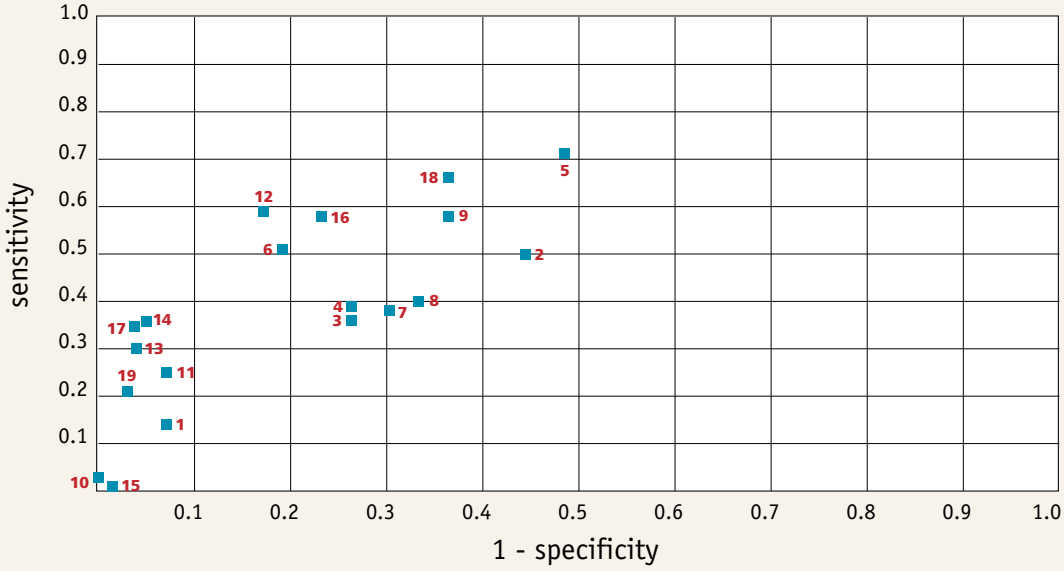
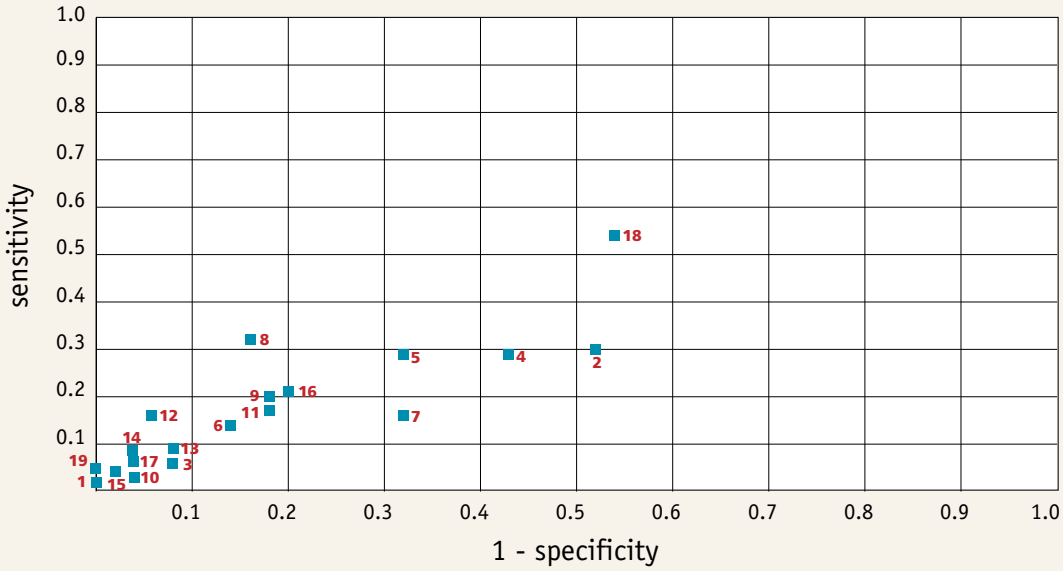


Figure 6b. ROC curve of commercial tests for the diagnosis of pulmonary tuberculosis – HIV-positive patients (n=157)



1. ABP Diagnostics **2.** Advanced Diagnostics **3.** American Bionostica **4.** Ameritek USA **5.** Bio-Medical Products **6.** Chembio Diagnostic Systems **7.** CTK Biotech **8.** Hema Diagnostic Systems **9.** Laboratorios Silanes

Figure 7a. ROC curve of commercial tests for the diagnosis of pulmonary tuberculosis – sputum smear-positive and HIV-negative patients (n=171)

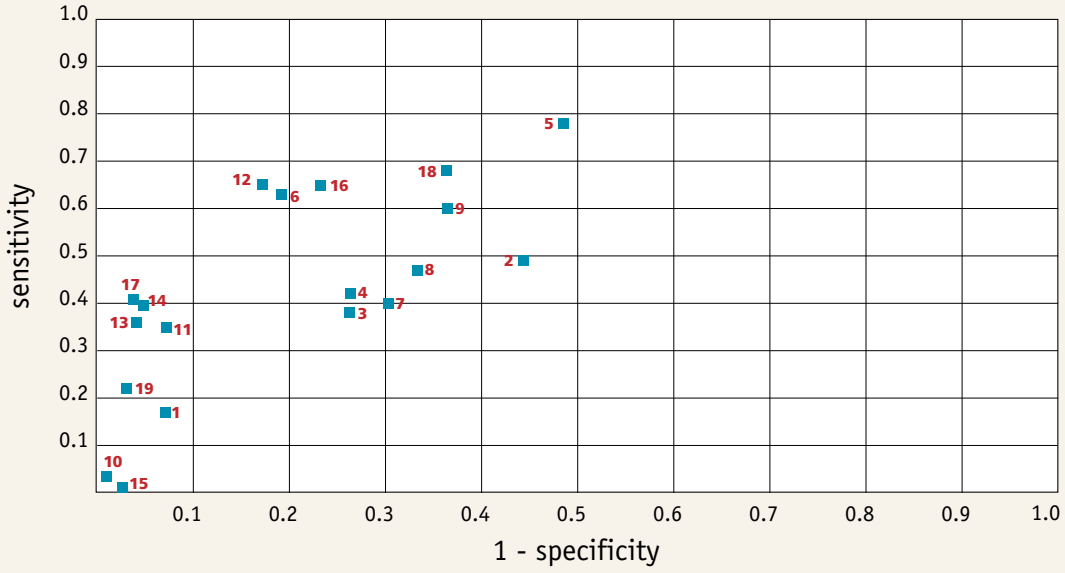
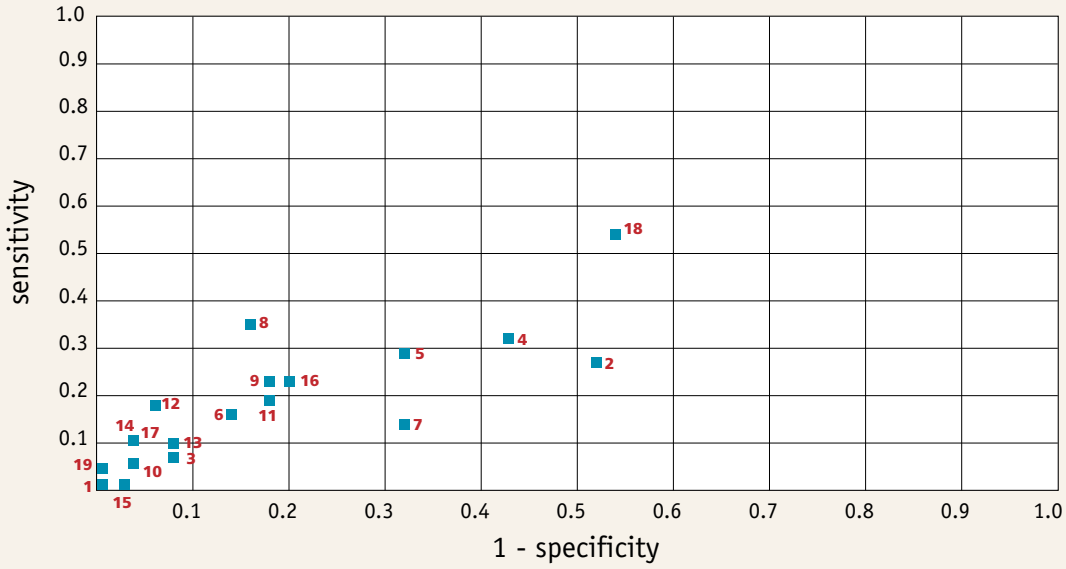


Figure 7b. ROC curve of commercial tests for the diagnosis of pulmonary tuberculosis – sputum smear-positive and HIV-positive patients (n=133)



10. Millennium Biotechnology **11.** Minerva BiOTECH **12.** Mossman Associates **13.** Pacific Biotech **14.** Premier Medical **15.** Princeton Biomeditech **16.** Span Diagnostics **17.** Standard Diagnostics **18.** Unimed International **19.** VEDA.LAB

8.1.2 HIV's impact on test performance

Qualitative, visual inspection of ROC diagrams illustrates that test performance is significantly compromised in specimens from HIV-positive patients. A discriminant analysis and a logistic regression both indicate that sensitivity and specificity independently separates (discriminant) or predicts (logistic) HIV groupings. The p-values are as follows.

For all TB samples:

	discrim: partial r2	p-value	logistic: OR	p-value
sensitivity	0.5030	<0.0001	0.798 (0.682, 0.933)	0.0047
specificity	0.3057	0.0004	0.809 (0.679, 0.965)	0.0182

When test performance is compared in HIV-negative (n=198) and HIV-positive (n=157) populations, the difference in test sensitivity ranges between -1% and +43% (Table 5) and differences in test specificity range between -18% and +18%. In HIV-negative samples only, test sensitivity was the highest for Rapid TB Test (Bio-Medical Products) at 71%, followed by FirstSign MTB Card Test (Unimed International) at 66% (Table 5).

Table 5. Difference in test sensitivity and specificity in HIV negative and HIV positive sample populations

Manufacturer	HIV-negative samples only (n=198)		HIV-positive samples (n=157)		HIV negative – HIV positive	
	sensitivity	specificity	sensitivity	specificity	difference sensitivity	difference specificity
Millenium Biotechnology	2%	100%	3%	96%	-1%	-4%
Premier Medical	35%	95%	8%	96%	27%	1%
Standard Diagnostics	35%	96%	7%	96%	28%	0%
American Bionostica	36%	74%	6%	92%	31%	18%
Pacific Biotech	30%	96%	9%	92%	21%	-4%
Bio-Medical Products Corp.	71%	52%	29%	68%	42%	16%
Advanced Diagnostics	50%	56%	30%	48%	20%	-8%
ABP Diagnostics	14%	93%	2%	100%	12%	7%
Laboratorios Silanes	58%	64%	20%	82%	38%	18%
CTK Biotech	38%	70%	16%	68%	22%	-2%
Chembio Diagnostic Systems	51%	81%	14%	86%	36%	5%
Span Diagnostics	58%	77%	21%	80%	37%	3%
Mossman Associates	59%	83%	16%	94%	43%	11%
Unimed International	66%	64%	54%	46%	11%	-18%
Hema Diagnostic Systems	40%	67%	32%	84%	9%	17%
Ameritek USA	39%	74%	29%	57%	10%	-17%
Princeton BioMeditech	1%	99%	1%	98%	0%	-1%
Minerva BiOTECH	25%	93%	18%	82%	7%	-11%
VEDA.LAB	21%	97%	5%	100%	17%	3%

8.1.3 Impact of smear status on test performance

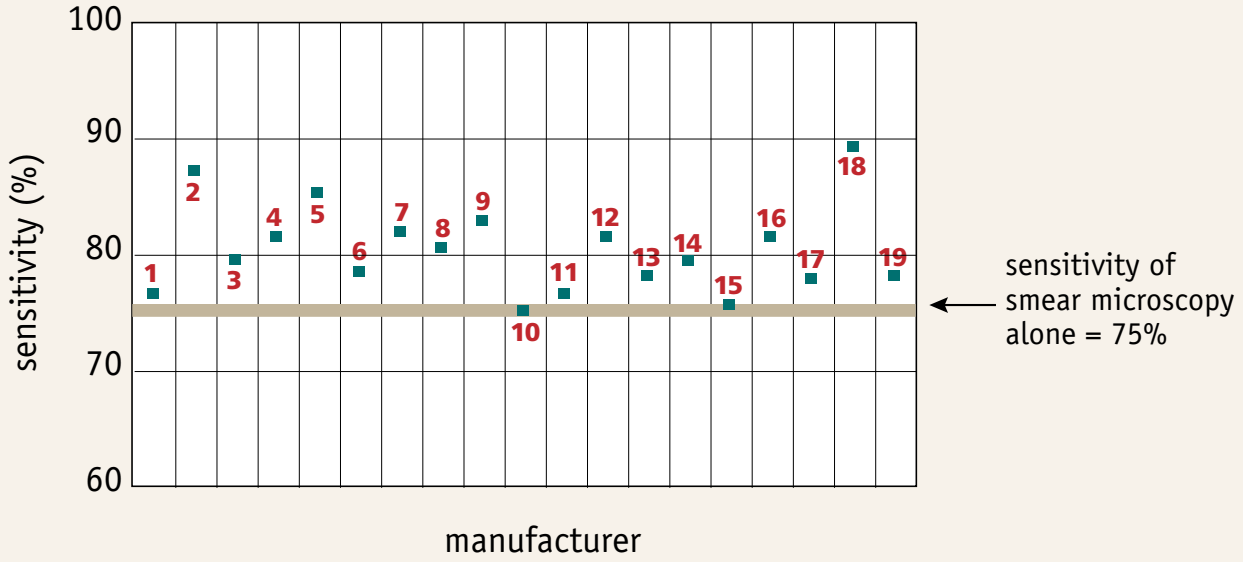
Negative smear status, like HIV, has a negative impact on test performance. For smear-positive samples:

	discrim: partial r2	p-value	logistic: OR	p-value
sensitivity	0.4770	<0.0001	0.823 (0.722, 0.938)	0.0034
specificity	0.2436	0.0019	0.843 (0.732, 0.972)	0.0183

8.1.4 Impact of combined smear microscopy and rapid test

Overall smear microscopy detected 75% (155/206) of all TB cases. Rapid tests on average detected an additional 9 TB cases (median 10). Of the 51 cases missed by smear microscopy, three tests (Rapid TB, Bio-Medical Products; Tuberculosis Rapid Test, Advanced Diagnostics; FirstSign MTB, Unimed International) detected 21 (41%),24 (47%), 29 (57%) additional TB cases, respectively. This increased overall combined smear and rapid test sensitivity to 85%, 87% and 89%, respectively. Figure 8 illustrates the overall sensitivity gains of a combined smear microscopy, rapid test approach by the manufacturer. However, each of these tests (Rapid TB, Bio-Medical Products; Tuberculosis Rapid Test, Advanced Diagnostics; FirstSign MTB, Unimed International) yielded an unacceptably high number of false positives: 64 (43%), 70(47%) and 63(42%), respectively.

Figure 8. Sensitivity of smear microscopy (75%) and combined smear microscopy and rapid test by manufacturer (n=206)



1. ABP Diagnostics **2.** Advanced Diagnostics **3.** American Bionostica **4.** Ameritek USA **5.** Bio-Medical Products **6.** Chembio Diagnostic Systems **7.** CTK Biotech **8.** Hema Diagnostic Systems **9.** Laboratorios Silanes **10.** Millennium Biotechnology **11.** Minerva BiOTECH **12.** Mossman Associates **13.** Pacific Biotech **14.** Premier Medical **15.** Princeton BioMeditech **16.** Span Diagnostics **17.** Standard Diagnostics **18.** Unimed International **19.** VEDA.LAB

8.2 Indeterminate and missing results

Overall difficulties of technician and test origin accounted for 0.2% (13/6840) indeterminate results. Results are described in Table 6 and were eliminated from the final analysis.

Table 6. Indeterminate results

Manufacturer	Test	Sample ID	Sample origin	Smear status	HIV status	Reference result	Problem
Premier Medical	First Response Rapid TB Card Test	500687	Canada	negative	negative	negative	no migration
		49160	United Republic of Tanzania	negative	positive	negative	no migration
		01950	Kenya	negative	positive	positive	no migration
Standard Diagnostics	One step Tuberculosis antibody test: SD TB Rapid Test	100313	Gambia	positive	negative	positive	no reaction
		100330	Gambia	negative	negative	positive	no reaction
		49160	United Republic of Tanzania	negative	positive	negative	no migration
Ameritek USA	dBest One Step TB Test	600944	Spain	negative	positive	negative	no migration
		601010	Spain	positive	negative	positive	no migration
		49072	United Republic of Tanzania	positive	positive	positive	no migration
Advanced Diagnostics	TB Rapid Test (strip)	01975	Kenya	negative	negative	positive	reader 1 result missing
		302301	South Africa	positive	positive	positive	reader 1 + 2 result missing
Chembio Diagnostic Systems	TB STAT-PAK II	100460	Gambia	negative	negative	negative	reader 2 result missing
Laboratorios Silanes	TB-Instantest	100487	Gambia	negative	negative	negative	reader 2 result entered as 9

Notes:
(11/6840 tests = 0.2%) this ratio only include problems with reader1
(13/6840 tests = 0.2%) this ratio include problems with reader 1 and or reader 2

8.3 Reproducibility

8.3.1 Inter-reader reproducibility

Each test result during the evaluation was interpreted by two technicians. Inter-reader reliability was measured for 19 tests and 355 test results and analysed separately for TB and non-TB samples and HIV-positive and HIV-negative samples. A kappa value of 0.70 is considered excellent. Results are summarized in Tables 7a-7c.

Table 7a. Inter-observer reliability in all samples tested

	all n=355 inter-reader reliability		
	kappa	(95% CI)	McNemars
ABP Diagnostics	0.72	0.57-0.87	1
Advanced Diagnostics	0.86	0.81-0.92	0.414
American Bionostica	0.90	0.85-0.96	0.763
Ameritek USA	0.84	0.78-0.90	0.05
Bio-Medical Products	0.73	0.65-0.80	<0.0001
Chembio Diagnostic Systems	0.85	0.79-0.92	0.108
CTK Biotech	0.72	0.65-0.80	0.086
Hema Diagnostic Systems	0.65	0.58-0.73	<0.0001
Laboratorios Silanes	0.73	0.66-0.81	0.016
Millennium Biotechnology	0.49	0.14-0.84	0.414
Minerva BiOTECH	0.73	0.65-0.82	0.0003
Mossman Associates	0.82	0.75-0.89	0.004
Pacific Biotech	0.91	0.84-0.97	0.157
Premier Medical	0.87	0.79-0.94	0.248
Princeton BioMeditech	0.54	0.18-0.90	0.18
Span Diagnostics	0.75	0.67-0.82	0.001
Standard Diagnostics	0.89	0.82-0.96	0.317
Unimed International	0.81	0.75-0.87	0.732
VEDA.LAB	0.76	0.64-0.88	0.109

Table 7b Inter-observer reliability in TB and non-TB samples

	TB samples n=206 inter-reader reliability			Non-TB samples n=149 inter-reader reliability		
	kappa	(95% CI)	McNemars	kappa	(95% CI)	McNemars
ABP Diagnostics	0.74	0.57-0.91	0.48	0.65	0.34-0.97	0.317
Advanced Diagnostics	0.88	0.81-0.94	0.248	0.84	0.75-0.93	1
American Bionostica	0.93	0.86-0.99	0.18	0.87	0.77-0.97	0.414
Ameritek USA	0.81	0.73-0.89	0.018	0.88	0.79-0.96	1
Bio-Medical Products	0.68	0.58-0.78	0.0003	0.79	0.68-0.88	0.003
Chembio Diagnostic Systems	0.86	0.79-0.94	0.083	0.83	0.71-0.95	0.705
CTK Biotech	0.77	0.67-0.87	0.251	0.67	0.54-0.79	0.201
Hema Diagnostic Systems	0.67	0.57-0.77	<0.0001	0.63	0.51-0.75	<0.0001
Laboratorios Silanes	0.70	0.58-0.81	0.297	0.76	0.67-0.86	0.016
Millennium Biotechnology	0.43	0.02-0.84	0.655	0.66	0.04-1.00	0.317
Minerva BIOTECH	0.8	0.71-0.90	0.004	0.58	0.39-0.77	0.02
Mossman Associates	0.83	0.75-0.91	0.012	0.75	0.58-0.91	0.157
Pacific Biotech	0.91	0.84-0.98	0.414	0.88	0.72-1.00	0.157
Premier Medical	0.88	0.81-0.96	1	0.76	0.54-0.99	0.046
Princeton BioMeditech	0.57	0.13-1.0	0.083	0.49	0.0-1.0	1
Span Diagnostics	0.73	0.64-0.83	0.001	0.74	0.61-0.87	0.285
Standard Diagnostics	0.88	0.80-0.96	0.48	0.91	0.72-1.00	0.317
Unimed International	0.76	0.67-0.85	0.414	0.86	0.78-0.94	0.527
VEDA.LAB	0.75	0.62-0.89	0.248	0.74	0.40-1.0	0.157

Table 7c Inter-observer reliability in HIV-positive and HIV-negative samples

	HIV positive n=157 inter-reader reliability			HIV negative n=198 inter-reader reliability		
	kappa	(95% CI)	McNemars	kappa	(95% CI)	McNemars
ABP Diagnostics	0.66	0.22-1.0	0.157	0.72	0.56-0.88	0.527
Advanced Diagnostics	0.88	0.80-0.96	0.095	0.85	0.77-0.92	0.796
American Bionostica	0.89	0.75-1.0	1	0.89	0.83-0.96	0.739
Ameritek USA	0.83	0.73-0.92	0.564	0.84	0.76-0.92	0.032
Bio-Medical Products	0.54	0.42-0.67	0.0002	0.84	0.76-0.92	0.004
Chembio Diagnostic Systems	0.84	0.72-0.96	1	0.85	0.77-0.93	0.052
CTK Biotech	0.68	0.54-0.82	0.808	0.74	0.64-0.84	0.041
Hema Diagnostic Systems	0.65	0.53-0.78	0.001	0.64	0.54-0.74	<0.0001
Laboratorios Silanes	0.57	0.42-0.72	0.014	0.8	0.71-0.88	0.371
Millennium Biotechnology	0.56	0.12-1.0	0.08	0.39	0-0.94	0.564
Minerva BIOTECH	0.72	0.59-0.85	0.004	0.75	0.63-0.87	0.02
Mossman Associates	0.67	0.48-0.86	0.058	0.85	0.77-0.92	0.032
Pacific Biotech	0.72	0.54-0.90	0.157	1	1.00-1.00	n/a
Premier Medical	0.71	0.48-0.93	1	0.91	0.84-0.98	0.102
Princeton BioMeditech	0.66	0.04-1.0	0.317	0.49	0.07-0.92	0.046
Span Diagnostics	0.66	0.52-0.80	0.039	0.77	0.68-0.86	0.01
Standard Diagnostics	0.81	0.61-1.0	0.083	0,9	0.82-0.98	1
Unimed International	0.72	0.61-0.83	1	0.88	0.81-0.94	0.564
VEDA.LAB	0.59	0.22-0.95	1	0.79	0.66-0.91	0.058

8.3.2 Lot-to-lot, operator-to-operator and run-to-run reproducibility

For the 19 rapid tests, reproducibility was also measured separately by determining lot-to-lot, operator-to-operator and run-to-run variation. The results are summarized in Table 8 and Figures 9a-9c. Seven manufacturers' tests demonstrated 0% operator-to-operator, lot-to-lot and run-to-run variability. In contrast, the Tuberculosis Rapid Test (Advanced Diagnostics) had the highest discordant operator-to-operator variability at 79% (38/48); TB-Spot Ver. 2.0 (Span Diagnostics) had the highest lot-to-lot and run-to-run variability at 25% (12/48) and 26.5% (17/64), respectively. Overall, lot-to-lot, operator-to-operator and run-to-run variability ranges were 0-25%, 0-79% and 0-26%, respectively.

Table 8. Test reproducibility (operator-to-operator; lot-to-lot and run-to-run) – discordant results (%)

Manufacturer	Operator-to-operator n=48		Lot-to- lot n=48		Run-to-run Consecutive n=64	
ABP Diagnostics	2	4.17%	4	8.33%	1	1.56%
Advanced Diagnostics	38	79.17%	6	12.50%	14	21.88%
American Bionostica	0	0.00%	0	0.00%	0	0.00%
Ameritek USA	0	0.00%	0	0.00%	0	0.00%
Bio-Medical Products	12	25.00%	8	16.67%	14	21.88%
Chembio Diagnostic Systems	0	0.00%	0	0.00%	0	0.00%
CTK Biotech	11	22.92%	7	14.58%	6	9.38%
Hema Diagnostic Systems	16	33.33%	8	16.67%	13	20.31%
Laboratorios Silanes	21	43.75%	9	18.75%	14	21.88%
Millennium Biotechnology	0	0.00%	0	0.00%	0	0.00%
Minerva BIOTECH	0	0.00%	0	0.00%	0	0.00%
Mossman Associates	5	10.42%	3	6.25%	3	4.69%
Pacific Biotech	0	0.00%	0	0.00%	0	0.00%
Premier Medical	3	6.25%	1	2.08%	0	0.00%
Princeton BioMeditech	1	2.08%	1	2.08%	2	3.12%
Span Diagnostics	18	37.50%	12	25.00%	17	26.56%
Standard Diagnostics	3	6.25%	3	6.25%	3	4.69%
Unimed International	10	20.83%	2	4.17%	3	4.69%
VEDA.LAB	0	0.00%	0	0.00%	0	0.00%

Note: [ ] those with excellent reproducibility

Manufacturers were ranked for overall reproducibility based on the sum of ranks of each of the three measures of reproducibility (Table 9). Seven tests scored equally and ranked first.

Table 9. Summary test reproducibility results

Manufacturer	Test	Percentage concordance across 3 measures of reproducibility			Rank
ABP Diagnostics	TB Rapid Screen Test	4.17%	8.33%	2.08%	4
Advanced Diagnostics	Tuberculosis Rapid Test	79.17%	12.50%	18.75%	9
American Bionostica	ABI Rapid TB Test	0.00%	0.00%	0.00%	1
Ameritek USA	dBest One Step TB Test	0.00%	0.00%	0.00%	1
Bio-Medical Products	Rapid TB Test	25.00%	16.67%	22.90%	10
Chembio Diagnostic Systems	TB STAT-PAK II	0.00%	0.00%	0.00%	1
CTK Biotech	TB Onsite Rapid Test	22.92%	14.58%	10.40%	8
Hema Diagnostic Systems	Rapid 1-2-3 HEMA TB Test	33.33%	16.67%	22.90%	11
Laboratorios Silanes	TB-Instantest	43.75%	18.75%	20.80%	12
Millennium Biotechnology	Immu-Sure TB Plus	0.00%	0.00%	0.00%	1
Minerva BIOTECH	V Scan	0.00%	0.00%	0.00%	1
Mossman Associates	MycoDot 9 Easy Steps	10.42%	6.25%	4.17%	6
Pacific Biotech	BIOLINE Tuberculosis Test	0.00%	0.00%	0.00%	1
Premier Medical	First Response Rapid TB Card	6.25%	2.08%	0.00%	2
Princeton BioMeditech	BioSign M.tuberculosis Test	2.08%	2.08%	2.08%	3
Span Diagnostics	TB-Spot Ver. 2.0	37.50%	25.00%	20.80%	12
Standard Diagnostics	SD TB Rapid Test	6.25%	6.25%	4.17%	5
Unimed International	FirstSign MTB Card Test	20.83%	4.17%	6.25%	7
VEDA.LAB	TB-Rapid Test	0.00%	0.00%	0.00%	1

Figure 9a. **Test reproducibility results (discordance %): operator-to-operator variability (n=48)**

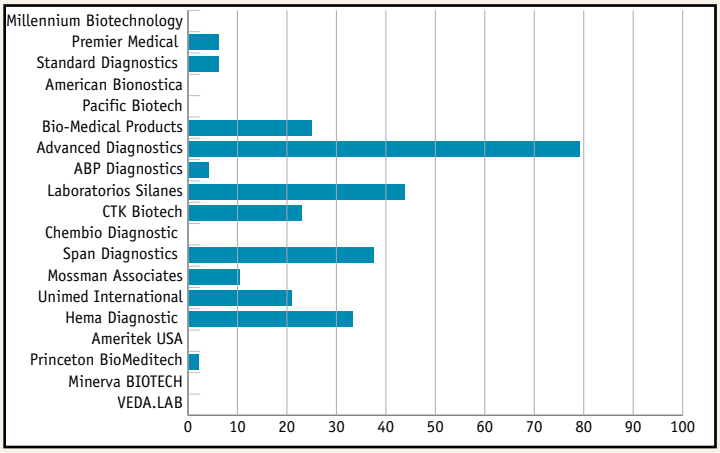


Figure 9b. **Test reproducibility results (discordance %): lot-to-lot variability (n=48)**

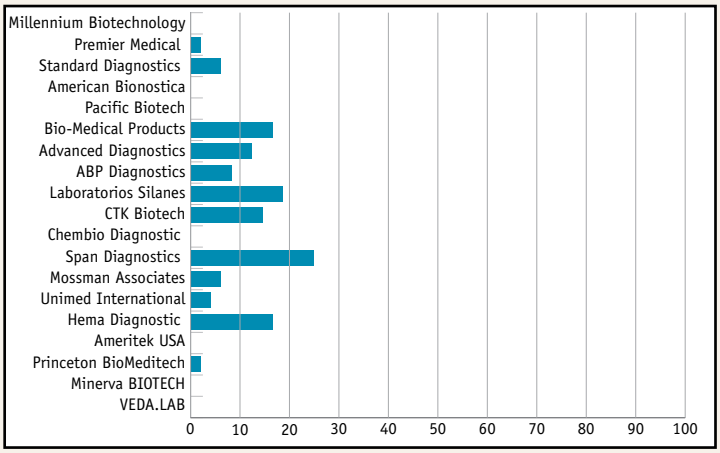
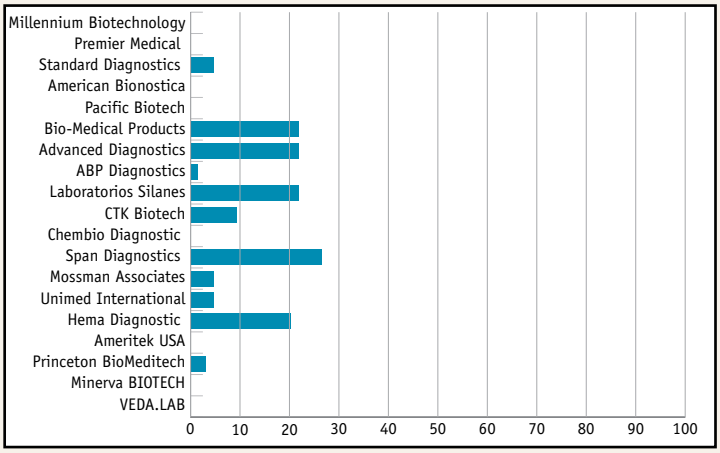


Figure 9c. **Test reproducibility results (discordance %): run-to-run variability (n=64)**



8.4 Operational characteristics

Five manufacturers gained the best score on the questionnaire (6/10) – First Response Rapid TB Card (Premier Medical), SD TB Rapid Test (Standard Diagnostics), ABI Rapid TB Test (American Bionostica), BIOLINE Tuberculosis Test (Pacific Biotech) and BioSign M.tuberculosis (Princeton BioMeditech). TB-Spot Ver. 2.0 (Span Diagnostics) received the lowest score (2.5/10). First Response Rapid TB Card (Premier Medical) and BIOLINE Tuberculosis Test (Pacific Biotech) scored highest for clarity of kit instructions (2.5/3). Given the similar test formats, it is not surprising that several tests scored equally for technical complexity (Table 10). SD TB Rapid Test (Standard Diagnostics), ABI Rapid TB Test (American Bionostica), dBest One Step TB test (Ameritek USA) and BioSign M.tuberculosis (Princeton BioMeditech) scored highest (2/3) for ease of interpretation of results.

In general, none of the tests received excellent (perfect scores) in any area (clarity of instructions, technical complexity, ease of interpretation of results) and all required equipment that was not provided. Nonetheless, technical complexity was rated "very easy" in 63% (12/19) of the tests evaluated, therefore appropriate for use in primary health-care settings in developing countries. Technical complexity was attributed to inadequate space for labelling and incomplete migration of specimens.

Table 10. Summary of operational test performance characteristics

	ABP Diagnostics	Advanced Diagnostics	American Bionostica	Ameritek USA	Bio-Medical Products	Chembio Diagnostic Systems	CTK Biotech	Hema Diagnostic Systems	
Mean Score:									
Clarity of kit instructions	1.5	2	2	2	2	2	2	2	2
Technical complexity	2	0	2	0	2	2	2	1.5	0
Ease of interpretation of results	1.5	1.5	2	2	1	1	1.5	0	1
Equipment required; not provided	0	0	0	0	0	0	0	0	0
Total mean score	5	3.5	6	4	5	5	5.5	3.5	3
Comments		No space for label/ID	Buffer volume inadequate	Poor migration	Signal colour variation	Signal colour variation; excessive buffer per kit	The micropipette provided was not useful	Signal intensity low or diffuse; sample loop difficult to use.	No space for label/ ID; excess diluent per kit

Millennium Biotech-nology	Minerva BiOTECH	Mossman Associates	Pacific Biotech	Premier Medical	Princeton BioMeditech	Span Diagnostics	Standard Diagnostics	Unimed International	VEDA.LAB
2	2	1.5	2.5	2.5	2	1.5	2	2	2
2	1	1	2	2	2	0.5	2	2	2
0	1	0.5	1.5	1.5	2	0.5	2	1.5	1
0	0	0	0	0	0	0	0	0	0
4	4	3	6	6	6	2.5	6	5.5	5
Signal intensity low or diffuse	Signal intensity low or diffuse	Control quantity inadequate; signal intensity low or diffuse; a hole present in one microwell plate	Discrimi-nation between positive and nega-tive results sometimes difficult	Clear positive results	Excessive buffer per kit	Controls difficult to open; signal colour variation and intensity low or diffuse			Signal intensity low or diffuse

9. Evaluation strengths and limitations

This study is a landmark in the field of rapid TB test evaluations. This is the first head-to-head comparison of multiple (19) commercially available rapid tests for TB using several hundred (355) well-characterized reference specimens from a range of endemic and non-endemic countries, collected using a standard protocol. General guidelines for diagnostic evaluations were followed in the design, conduct, monitoring and reporting of the trial.

Use of archived specimens had several advantages including convenience, speed and low cost. The use of frozen sera that passed through two freeze-thaw cycles and were between one month and six years of age (stored at -70 °C) could, theoretically, compromise test sensitivity, but it is unlikely. One biobank reports stability of IgG stored over 12 years at -80 °C, and over at least 30 freeze-thaw cycles (F.Betsou, unpublished data). One report evaluated the impact of multiple freeze-thaw cycles and various temperatures (-20 °C , 4 °C, 25 °C, 37 °C) on the reactivity of HIV antibodies using current ELISA, recombinant and Western blot methodologies. Twenty consecutive freeze-thaw cycles and storage of specimens at -20 °C and 4 °C for 57 days resulted in no loss of HIV antibody reactivity nor any false positive samples (21). A recent systematic review of serological based tests for TB (combined total of nine tests), reported that 87% of studies since 1990 used frozen sera (22).

Sera from TB negative patients represented the appropriate control population for rapid TB tests, more specifically – pulmonary symptomatics rather than healthy controls. Test specificity is higher if healthy controls are used. Furthermore, TB was excluded with high confidence on the basis of detailed microbiological work up and clinical follow-up of these patients after two to three months.

The WHO/TDR TB Specimen Bank protocol requires prospective enrolment of consecutive symptomatic patients. To this end, the natural distribution of disease severity amongst TB patients should be represented. Furthermore, the evaluation included 44% and 14% of sputum smear-positive and sputum smear-negative patients, respectively, reflecting advanced and less advanced disease states.

Samples from patients diagnosed with nontuberculous mycobacteria (NTM) infections (potentially causing cross reactivity and loss of specificity) were excluded. However, those with concomitant or subclinical NTM infections could not be excluded.

The proportion of HIV-positive samples included in the evaluation (44%) is not representative of the respiratory symptomatic pool in all geographical settings. For this reason, overall test performance is lower for all tests (particularly sensitivity) than might be expected in populations with much lower HIV prevalence in respiratory symptomatics. Furthermore, the high incidence of HIV in Africa means that sub-Saharan Africa (SSA) is the originator of the majority of WHO/TDR TB Specimen Bank samples from TB-positive and TB-negative patients who are HIV positive. Specific antibody responses to mycobacterial antigens vary in different human populations, so too may the sensitivity of assays.

In addition, it proved unexpectedly difficult to acquire samples from pulmonary symptomatics who were also HIV positive and TB negative. For TB to be excluded with high confidence smear-negative symptomatics had to demonstrate clinical and/or radiographic improvement two to three months after the original consultation, in the absence of TB treatment. SSA has high incidence of, and mortality from, TB and HIV coinfection; smear-negative pulmonary TB cases match or exceed smear-positive cases; and there are few sophisticated facilities for TB or alternative diagnoses. Therefore, the majority of TB symptomatics are treated syndromically, i.e. without microbiological confirmation of their disease. The WHO/TDR TB Specimen protocol enrolls consecutive, symptomatic patients and specifies the laboratory and follow-up procedures but not the decision to treat. The precise distribution of TB-positive, TB-negative, HIV-positive and HIV-negative patients cannot be predicted or demanded.

Prolonged and careful follow-up of the target population is required to determine true specificity. Patients with active TB do not have uniform disease progression and there is always the possibility that a two to three month follow-up visit (to exclude TB) is inadequate. However, many previous studies include healthy control subjects rather than pulmonary symptomatics. This yields higher test specificity.

Sometimes the antigen composition of the tests and/or their preparation is considered proprietary information. Unfortunately, we could not determine the antigen composition of all tests and therefore cannot comment on the performance of specific antigens or antibody class combinations.

Conclusions

Currently marketed rapid serologic TB tests vary widely in performance but generally perform poorly compared to a combined reference standard using well-characterized archived serum specimens.

Overall sensitivity ranged from 1% to 60% (mean=27%) and was higher in sputum smear-positive than smear-negative patients (sensitivity & specificity: p=<0.0006) and amongst HIV-negative samples (2%-71%; n=198; sensitivity: p=<0.0001, specificity: p=0.44).

The average difference in test sensitivity between the HIV-negative (n=198) and the HIV-positive population (n=157) was +22%; the maximum difference was +43%.

The majority of products had poor specificity (<80%) when tested in TB suspects from endemic settings. Tests with specificity over 90% detected less than 30% of all TB patients.

The final sample size was insufficient to definitively determine the accuracy of commercial tests in HIV-positive patients. However, based on our results, it appears that HIV co-infection diminishes the performance of existing assays.

None of the assays perform well enough to replace microscopy. Smear microscopy combined with most rapid tests improved overall diagnostic sensitivity from 75% (155/206) (smear alone) up to 89% (184/206) (smear plus rapid test). The latter detected 57% (29/51) of the smear negative, culture positive TB cases but had an associated, unacceptably high false positive rate of 42%.

Some products show high lot-to-lot, run-to-run, operator-to-operator and inter-reader reproducibility.

The majority of tests had very low technical complexity. If performance was acceptable, they would be appropriate for use in primary health-care settings in developing countries.

Our evaluation did not permit an analysis of how specific antigen or antigen combinations performed because of the proprietary nature of this information. The way forward clearly needs to include a review of the literature targeting the utility of specific antigens, in addition to activities to support the discovery of new antigens with immunodiagnostic potential.

These tests are sold and used in disease-endemic countries, without evidence of effectiveness. Clearly this reinforces the need for greater regulatory oversight, and the introduction, of quality standards for diagnostic tests, particularly for diseases that have a significant public health impact. Individual countries need to strengthen the design, conduct and reporting of diagnostic test evaluations. In turn, these can guide national and local procurement and clinical practice.

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Annexes

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Annex 1.
Characteristics of rapid tuberculosis diagnostics evaluated*

Product name	TB Rapid Test	Immu-Sure TB Plus	TB-Spot Ver. 2.0	ABI Rapid TB Test
Company/manufacturer	Standard Diagnostics, Inc.	Millennium Biotechnology, Inc.	Span Diagnostics Ltd.	American Bionostica, Inc.
Assay type	One step qualitative immunochromatographic assay	Lateral flow rapid test	Immunodot assay on plastic comb	Immunochromatographic test
Solid phase (strip, cassette)	Cassette	Cassette	Polystyrene comb	Strip or cassette
Specimen type (whole blood, plasma, serum)	Serum,plasma	Serum or whole blood	Whole blood, plasma or serum	Blood, plasma, serum
Number of tests per kit	30 tests	20 tests	24 or 48 tests	25 tests
Shelf life (months, temp °C)	18 months, 2~30 °C	24 months	12 months, 2-8 °C	18 months
Supplies/equipment required but not provided	None	Pipette(s) - 5 µl and/or 10 µl	Micropipettes, timer	None
Number of samples per run (minimum-maximum)	Min. number per run: 1 Max. number per run: 1	Min. number per run: 1 Max. number per run: 1	Min. number per run: 1 Max. number per run: X	Min. number per run: 1 Max. number per run : 1
Number of steps to perform test	1	2	8 (plus buffer preparation)	2
Volume of samples	100 µl	5 µl for serum, 10 µl for whole blood	50 µl	5 µl
Incubation temp (°C)	Ambient - room temperature	Ambient - room temperature	Ambient - room temperature	Ambient - room temperature
Total time to perform assay (h.min)	15 minutes	25 minutes or less	20 minutes	10-20 minutes
Reading endpoint stability (h.min ±min)	15-30 minutes	30 minutes	Indefinitely stable	20 minutes
Price per test (US$ from manufacturer)	US$ 0.70/test (FOB)	Volume dependent - can be as low as US$ 0.50/ test in large quantity	Pricing is volume related and ranges from US$ 0.60-1.00 per test	Price depends on purchase quantities and customer type (ie. end-user, distributor, OEM**)

* one manufacturer (Minerva BIOTECH Corporation) did not provide product characteristic information.
** OEM = **??**

TB Onsite Rapid Test	BIOLINE Tuberculosis Test	First Response Rapid TB Card	MycoDot's 9 Easy Steps	dBest One Step Tuberculosis Test
CTK Biotech, Inc.	Pacific Biotech Co. Ltd.	Premier Medical Corporation	Mossman Associates Inc.	Ameritek USA
Lateral flow immunoassay	Immunochromatographic assay	Lateral flow immunochromatographic assay	Serological TB test	Rapid test
Cassette	Cassette	Cassette	Comb - 8 individual tests on the comb can be cut into individual teeth with scissors.	Strip and cassette
Plasma, serum	Serum, plasma	Whole blood,serum,plasma	Whole blood, serum or heparin-derived plasma can be used. Plasma derived by the addition of divalent cation chelators such as sodium citrate or EDTA must not be used.	Whole blood, plasma and serum
25 tests	40 tests	30 tests	96 tests	60 cassettes or 100 strips
18 months, 4-8 °C	24 months, 4–30 ºC	12 months	12 months, 2-8º C	36 months, 0-37º C
Sample collection tube, timer	Autopipette	Lancet	micropipettes (40-200 µl); graduated cylinder (10-120 ml)	None
Min. number per run: 1 Max. number per run: 1	Min. number per run: 1 Max. number per run: 1	Min. number per run: 1 Max. number per run: 1	Min. number per run: 1; Max : 288 tests with little experience and 384 tests with experience	Min. number per run: 1 Max. number per run: 1
2	1	1	8 (plus buffer preparation)	1
50-90 µl	100 µl	60 µl of whole blood or 100 µl of serum/plasma	40 µl of patient serum or 60 µl of whole blood	60 µl = two drops blood
Ambient - room temperature	15-30 °C	Ambient - room temperature	Ambient - room temperature	20-37 °C
11 minutes	5-20 minutes	10 minutes	20 minutes	3-5 minutes
10 minutes + 10 minutes	Information not provided	30 minutes	Permanent	5 minutes
US$ 0.50	US$ 0.60	US$ 0.60	Ranges from US$ 1.00 for developing world countries to US$ 2.00 for developed world countries. Volume discounts available	US$ 0.50/strip, US$ 0.90/cassette, US$1.20/ whole blood cassette

(continued)

Annex 1 (continued)

Product name	RAPID 1-2-3 HEMA TB Test	Tuberculosis Rapid Test	BioSign M.tuberculosis Test	FirstSign - MTB Card Test
Company/manufacturer	Hema Diagnostic Systems, LLC	Advanced Diagnostics, Inc.	Princeton BioMeditech Corporation	Unimed International, Inc.
Assay type	Immunochromatographic, lateral flow (non-sandwich) assay	Lateral flow immunochromatographic test	Rapid immunochromatographic assay	Double antigen sandwich immunochromatographic assay
Solid phase (strip, cassette)	Strip	Strip and cassette format	Cassette	Cassette
Specimen type (whole blood, plasma, serum)	Whole blood, serum	Whole blood or serum	Whole blood, plasma, serum	Whole blood, plasma or serum
Number of tests per kit	25, 50 or 100 tests	25 cassettes or 50 strips	35 tests	5, 10, 25, 50 or 100 tests
Shelf life (months, temp °C)	18 months, 2-25 ºC	18 months, <30 °C	12 months	24,4-30 ºC
Supplies/equipment required but not provided	None	None	Timer, pipette for sample transfer	None
Number of samples per run (minimum-maximum)	Min. number per run: 1 Max. number per run: 1	Min. number per run: 1 Max.number per run: 1	Min. number per run: 1 Max. number per run: 1	Min. number per run: 1 Max. number per run: 1
Number of steps to perform test	2	2	2	2
Volume of samples	5-10 µl	10 µl	35 µl whole blood, 25 µl serum or plasma	50 µl
Incubation temp (°C)	Ambient - room temperature	Room temperature (<30 °C)	Ambient - room temperature	25-30 °C
Total time to perform assay (h.min)	20 minutes	15-20 minutes	8 minutes	15 minutes
Reading endpoint stability (h.min ±min)	20 minutes	20 minutes	20 minutes	1hour +/- 15mins
Price per test (US$ from manufacturer)	US$ 1.75-1.78	variable	US$ 1.50	US$ 1.10

TB STAT-PAK II	TB Rapid Screen Test	TB-Rapid Test	TB-Instantest	Rapid TB Test
Chembio Diagnostic Systems, Inc.	ABP Diagnostics, Ltd.	VEDA.LAB	Laboratorios Silanes SA de CV	Bio-Medical Products, Corp
Lateral flow immunochro-matographic test	Lateral flow immunochro-matographic (qualitative) assay	Immunochromatographic rapid test	Lateral flow immunochro-matographic assay	Immunochromatographic test
Cassette	Cassette	Cassette	Strip	Cassette
Serum, plasma or whole blood	Plasma or serum	Whole blood, serum, plasma	Whole blood, serum, plasma	Plasma or serum
20 tests	40 tests	20 tests	10, 25 or 50 tests	20 tests
18 months, 5-30 ºC	24 months 4-30 ºC	18 months, 4-30 ºC	15 months	18 months,4-30 °C.
Sample pipettes for 30 µl; lancets for whole blood collection	5 µl pipette and a laboratory timer	Timer	Timer	Timer
Min. number per run: 1 Max.number per run: 1	Min. number per run: 1 Max. number per run: 1	Min. number per run: 1 Max. number per run: 1	Min. number per run: 1 Max. number per run: 1	Min. number per run: 1 Max. number per run: 1
2	2	2	2	2
30 µl	5 µl	25 µl serum or plasma 50 µl whole blood	10 µl	3 µl
Ambient - room temperature	Ambient - room temperature	Ambient - room temperature	2-30 °C	Room temperature
20 minutes	20-22 minutes	15 minutes	15-20 minutes	15 minutes
60 minutes	20 minutes	20 minutes	20 minutes	20 minutes
US$ 2.00	Supplied by ABP	average € 0.74	Upon request	US$ 1.75

Annex 2.

Record of test kit storage conditions, lot numbers, expiry dates and quantities received

Manufacturer	Name of test	Storage Conditions	Lot 1		
			Lot No.	Quantity	
ABP Diagnostics	TB Rapid Screen Test	Room temperature	04110902	600	
Advanced Diagnostics	Tuberculosis Rapid Test (STRIP)	Room temperature	412059	600	
American Bionostica	ABI Rapid TB Test	Room temperature	5003	600	
Ameritek, USA	dBest One step TB Test Disk	Room temperature	080412-A	600	
BioMedical Products	Rapid TB Test (Cassette)	Room temperature	01200502	600	
Chembio Diagnostic Systems	TB STAT-PAK II	Room temperature	TB112904/1	600	
CTK Biotech	TB Onsite Rapid Test	Room temperature	F0407B5	600	
Hema Diagnostics Systems	Rapid 1-2-3 HEMA TB Test	Room temperature	4345	600	
Laboratorios Silanes	TB-Instantest	Room temperature	05A025	600	
Millennium Biotechnology	Immu-Sure TB Plus	Room temperature	A0904MTB	600	
Minerva BIOTECH	V Scan	Room temperature	TB6-2004	600	
Mossman Associates	MycoDot's 9 Easy Steps	2-8 °C	5781	672	
Mossman Associates	MycoDot's 9 Easy Steps	2-8 °C	new lot n° 2 (arrived 4 July 2005)		
Pacific Biotech	BIOLINE Tuberculosis Test	Room temperature	04183	600	
Premier Medical	First Response Rapid TB Card	Room temperature	42J0104	600	
Princeton BioMeditech	BioSign M. tuberculosis Test	Room temperature	TB344L10	600	
Span Diagnostics*	TB-Spot Ver. 2.0	2-8 °C	TBS-05	600	
Standard Diagnostics	SD TB Rapid Test	Room temperature	046009	720	
Unimed International	FirstSign MTB Card Test	Room temperature	A31002	600	
VEDA.LAB	TB-Rapid Test	Room temperature	22124	600	

* We requested, and were granted, a certificate of expiry extension from Span Diagnostics for a two-month period (until December 2005).

		Lot 2			Total
	Expiry date	Lot no.	Quantity	Expiry date	
	11/2006	04081206	120	11/2006	720
	05/2006	501001	100	06/2006	700
	06/2006	5001	100	06/2006	700
	07/2006	080412-B	100	07/2006	700
	06/2006	01200501	100	06/2006	700
	02/2006	TB112904	100	02/2006	700
	10/2006	F0419B1	100	10/2006	700
	01/2006	4247	100	01/2006	700
	04/2006	05B025	100	05/2006	700
	08/2006	A1104MTB	100	10/2006	700
	05/2006	TB10-2004	100	05/2006	700
	11/2005	5143	192	05/2005	864
		0605	100	10/2005	100
	07/2006	04245	120	09/2006	720
	02/2006	42K0204	120	02/2006	720
	11/2005	TB344L20	100	11/2005	700
	10/2005	TBS-06	120	01/2006	720
	07/2007	046008	120	04/2006	840
	11/2006	A31003	100	11/2006	700
	08/2006	24015-01	100	09/2006	700

Annex 3.

Standard operating procedures (SOPs) for rapid TB tests

1. ABP Diagnostics Ltd:
TB Rapid Screen Test 49

2. Advanced Diagnostics, Inc.:
Tuberculosis Rapid Test 50

3. American Bionostica, Inc:
ABI Rapid TB Test 51

4. Ameritek USA:
dBest One Step Tuberculosis Test 52

5. Bio-Medical Products Corporation:
Rapid TB Test 53

6. Chembio Diagnostic Systems, Inc:
TB STAT-PAK II 54

7. CTK Biotech, Inc:
TB Onsite Rapid Test 55

8. Hema Diagnostic Systems, LLC:
Rapid 1-2-3 HEMA Tuberculosis Test . 56

9. Laboratorios Silanes SA de CV:
TB-Instantest 65

10. Millennium Biotechnology:
Immu-Sure TB Plus 57

11. Minerva BiOTECH Corporation:
V Scan 58

12. Mossman Associates, Inc.:
MycoDot's 9 Easy Steps 59

13. Pacific Biotech Co. Ltd.:
BIOLINE Tuberculosis Test 60

14. Premier Medical Corporation:
First Response Rapid TB Card 61

15. Princeton BioMeditech Corporation:
BioSign M.tuberculosis Test 62

16. Span Diagnostics Ltd.:
TB-Spot Ver. 2.0 63

17. Standard Diagnostics, Inc.:
SD TB Rapid Test 64

18. Unimed International, Inc:
FirstSign MTB Card Test 66

19. VEDA.LAB:
TB-Rapid Test 67

1. ABP Diagnostics Ltd: TB Rapid Screen Test

STANDARD OPERATING PROCEDURE

Conditions:

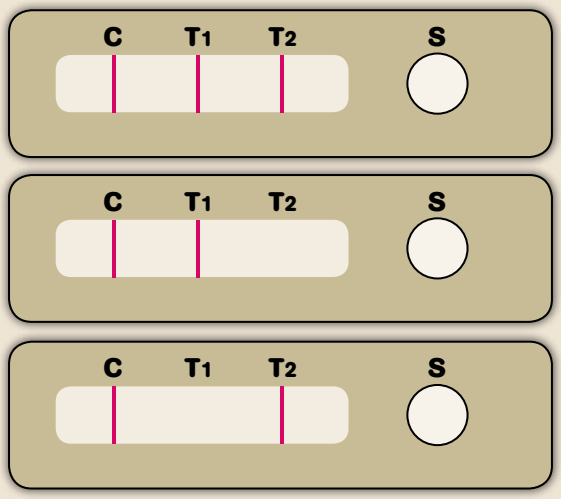
- Store test kit at 4-28 °C; shelf life 24 months
- Sera stored at 2-8 °C for up to two weeks
- Frozen serum can be stored at -20 °C for up to one year

Steps:

1) Apply 5 µl of sample to middle of membrane
2) Wait 1 minute and let sample solution absorb on membrane
3) Add 2 drops (40-80 µl) of chasing buffer into sample well
4) Read result at 5 minutes and 20 minutes
5) Interpret results as follows:

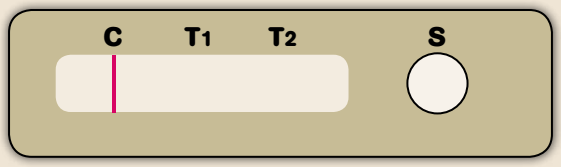
EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- Centrifuge
- Sample container
- Timer
- Pipette
- Gloves



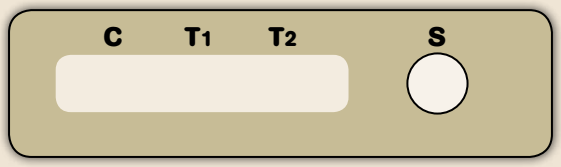
Positive result:

One or two lines in test area plus control line appear in test area of cassette. This indicates that specimen contains detectable amount of TB antibody.



Negative result:

Only one pink band appears on test region of cassette. No detectable TB antibody in specimen.



Invalid result:

No coloured band appears on test region. This indicates a possible error in performing test. Test should be repeated using a new device.

6) Discard cassette after 20 minutes.

2. Advanced Diagnostics, Inc.: Tuberculosis Rapid Test

STANDARD OPERATING PROCEDURE

Conditions:

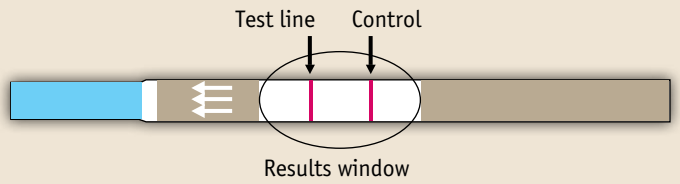
- Store test kit at room temperature (15-30 °C) or refrigerated (2-8 °C)
- Test strip, reagents and specimen warmed to room temperature before use
- Use fresh specimens, evaluated immediately after collection

Steps:

1) Apply 5 µl of sample (serum, plasma, whole blood) to upper area of sample pad
2) Add 100 µl of TB developer solution to lower area of sample pad
3) Read result within 15 minutes
4) Interpret results as follows:

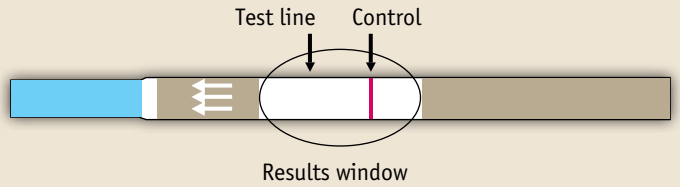
EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- Timer
- Sample container
- Gloves



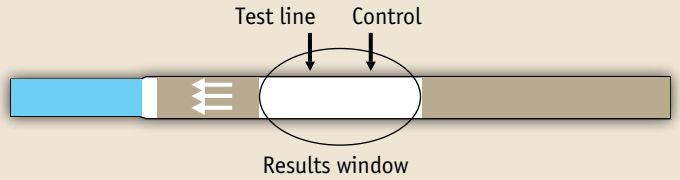
Positive result:

Two coloured lines appear in results window – one in control area, one in test area. Result can be read as soon as a distinctive pink-purple line appears in test area. In most strong positive cases, test line will appear before control line. With very strong positive specimens control line may be lighter than test line. With some weak positive cases, test line may appear after control line; control line may become darker than test line.



Negative result:

Only one coloured line in results window – in control area, with no distinctive coloured line in test area. Indicates that no active M. tuberculosis infection was detected.



Invalid result:

A distinct coloured line should always appear in control area. Test is invalid if no line forms in control area.

3. American Bionostica, Inc: ABI Rapid TB Test

STANDARD OPERATING PROCEDURE

Conditions:

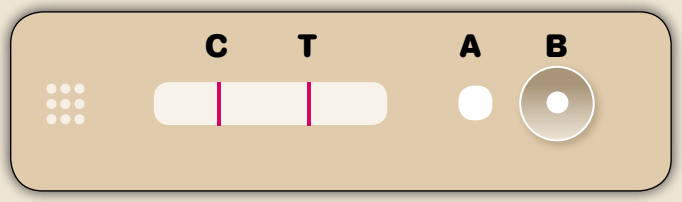
- Use fresh specimens
- Test cassette, reagents and specimen warmed to room temperature before use

Steps:

1) Using disposable micropipette, add approximately 5 µl of serum, plasma or whole blood to sample port on test cassette (A, figure below)
2) Add 110 µl (4-5 drops) of TB test buffer to buffer port of cassette. If solution does not flow up membrane, add 1 or 2 more drops of buffer solution (B, figure below)
3) Read results after 15-20 minutes
4) Interpret results as follows:

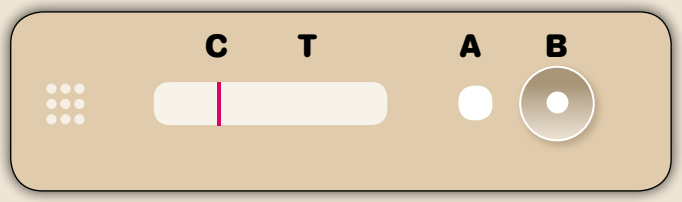
EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- Timer
- Gloves
- Lancets (if using whole blood from finger prick)



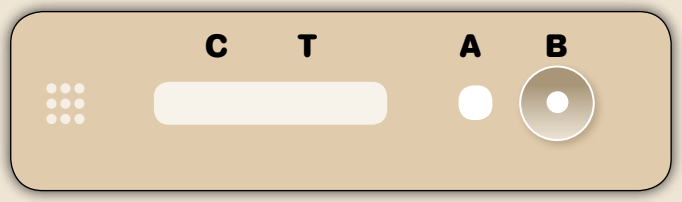
Positive result:

Two coloured lines appear in result window – one in control area, one in test area. Test result can be read as soon as a distinctive pink-purple line appears in test area.



Negative result:

Only one coloured line appears in results window – in control area. No distinctive coloured line in test area.



Invalid result:

A distinct coloured line should always appear in control area. Test is invalid if no control line appears.

4. Ameritek USA: dBest One Step Tuberculosis Test

STANDARD OPERATING PROCEDURE

Conditions:

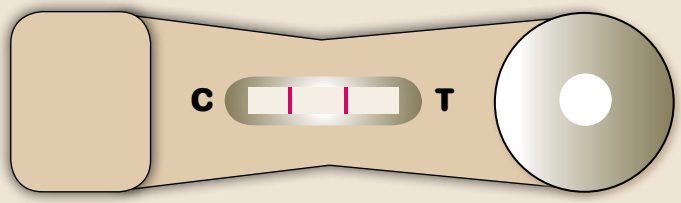
- Tests stored at room temperature 4-30 °C
- If not used immediately specimens should be stored at 2-8 °C
- Freezing is recommended for storage ≥ 3 days

Steps:

1) Using sample dropper, add 1 hanging drop into sample well. Once absorbed, add a second drop, and repeat once again (total 3 drops)
2) Purple colour will move across results window in centre of test disk
3) Interpret tests as follows after 10-15 minute:

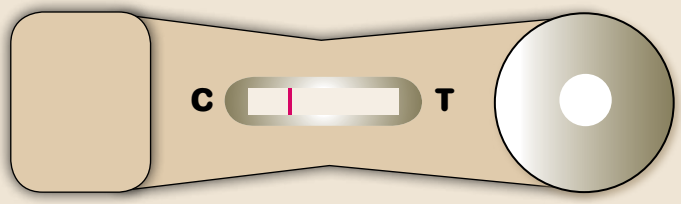
EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- Centrifuge (for serum, plasma)
- Timer
- Gloves
- Lancets (if using whole blood from finger prick)



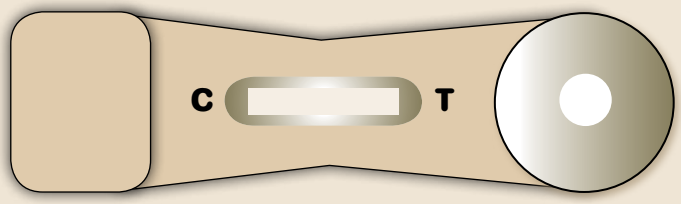
Positive result:

Two colour bands (T and C) within result window, regardless of which appears first.



Negative result:

Only one band within result window.



Invalid result:

After performing the test, if no purple colour band is visible within result window.

5. Bio-Medical Products Corporation: Rapid TB Test

STANDARD OPERATING PROCEDURE

Conditions:

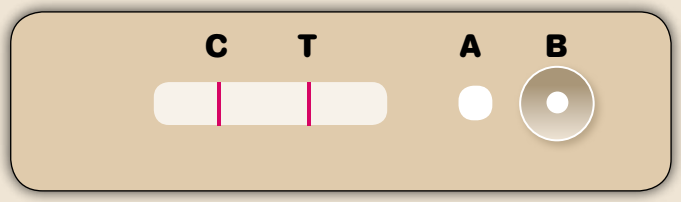
- Store at room temperature (4-30 °C); shelf life 24 months
- If not used immediately specimens should be stored at 2-8 °C for up to 2 weeks. Serum may be frozen at -20 °C for up to one year

Steps:

1) Apply 3 µl of sample to light blue line printed on centre area of membrane
2) Wait one minute and let sample absorb
3) Add two drops (40-80 µl) of chasing buffer into sample well (S)
4) Read result after 15 minutes
5) Interpret results as follows:

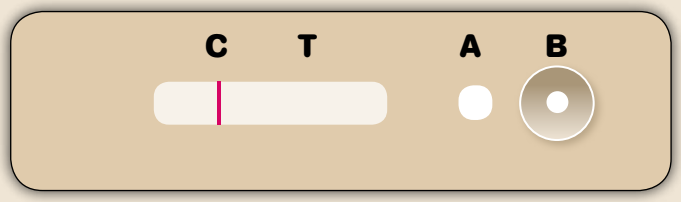
EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- Centrifuge
- Timer
- Gloves
- Lancets (if using whole blood from finger stick)



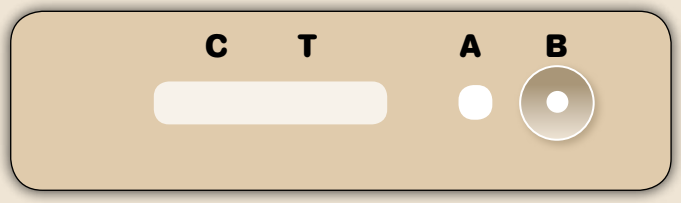
Positive result:

Two pink bands appear on test region of cassette.



Negative result:

Only one pink band appears on test region of cassette.



Invalid result:

No coloured band appears on test region.

6. Chembio Diagnostic Systems, Inc: TB STAT-PAK II

STANDARD OPERATING PROCEDURE

Conditions:

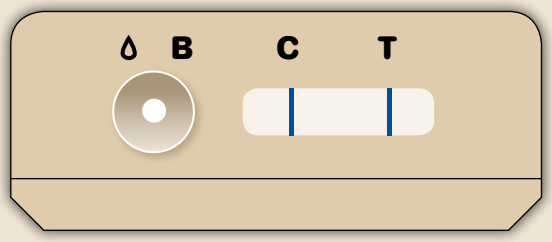
- Store tests at 8-30 °C
- Specimens ideally tested immediately after collection. Otherwise, refrigerate (2-8 °C) for up to 3 days, then freeze (≤ -20 °C). Avoid repeat freeze-thaw cycles
- Test, reagents and specimen warmed to room temperature before use
- Use of control materials along with test samples is recommended

Steps:

1) Add 30 µl of specimen to sample area using disposable pipette
2) Slowly add 3 drops (approx 100 µl) of diluent
3) Interpret results as follows, 20 minutes after addition of diluent:

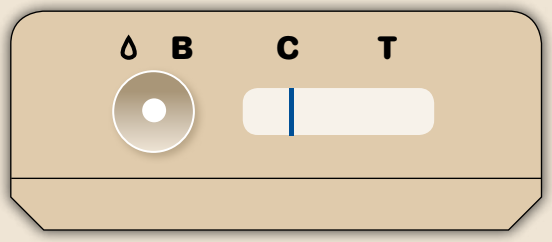
EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- Timer
- Sterile single use lancets (for whole blood samples only)
- Sterile alcohol swabs (for whole blood samples only)
- Pipettes for 30 µl
- Gloves



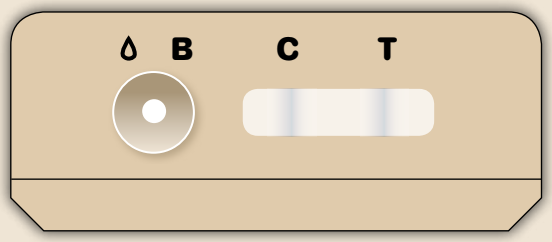
Positive result:

Two blue lines – one in test area, one in control area. Even a very faint line in test area of device within 20 minutes is indicative of a positive result.



Negative result:

One blue coloured line in control area, no coloured line in test area.



Invalid result:

Blue line should always appear in control area, whether or not test line develops. If no distinct line in control area, test is inconclusive.

7. CTK Biotech, Inc: TB Onsite Rapid Test

STANDARD OPERATING PROCEDURE

Conditions:

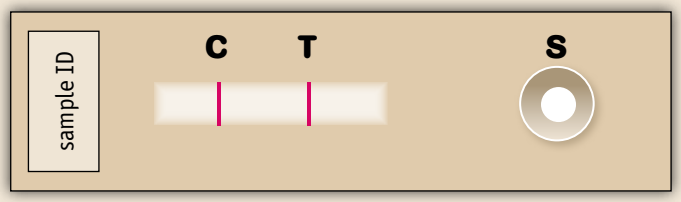
- Tests stored at room temperature (4-30 °C)
- Test, reagents and specimen warmed to room temperature before use

Steps:

1) Using pipette dropper provided, collect 50-90 µl of specimen and dispense into sample well
2) Add one drop (30 µl) of saline or phosphate buffered saline into sample well
3) Interpret results as follows, 5-10 minutes after adding specimen:

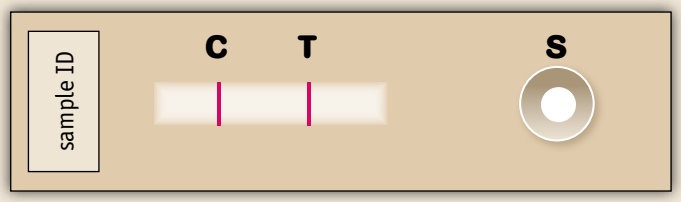
EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- Timer
- Container for specimen collection
- Centrifuge
- Saline, PBS
- Gloves



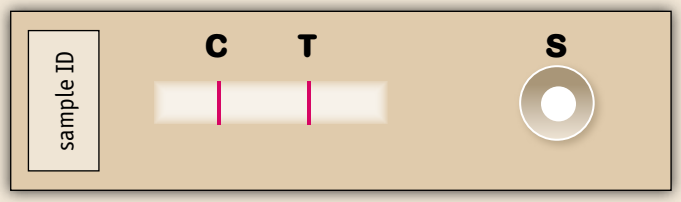
Positive result:

Both C (control) and T (test) lines are present.



Negative result:

Only C (control) line is present.



Invalid result:

If no C (control) line develops.

8. Hema Diagnostic Systems, LLC: Rapid 1-2-3 HEMA Tuberculosis Test

STANDARD OPERATING PROCEDURE

Conditions:

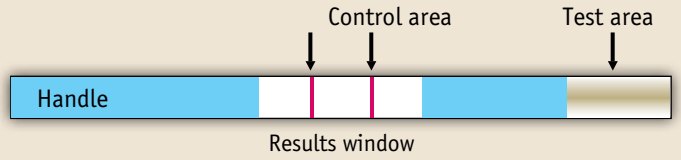
- Preferred temperature 18-20 °C, relative humidity <40%
- Reopening dessicant canisters up to 3 times per day, <15 seconds per opening
- Shelf life – 1 month, if above conditions met
- If temperature >28 °C and relative humidity >40%, same practice applies but shelf life limited to 14 days
- Test, reagents and specimen warmed to room temperature before use

Steps:

1) Using transfer device add 10 µl of serum to sample pad
2) Add buffer solution
3) Interpret results as follows after 15 minutes (but not longer than 20 minutes after adding developer solution):

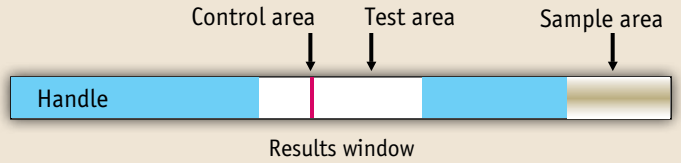
EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- Timer
- Gloves
- Container for specimen collection



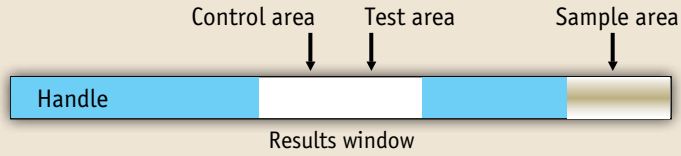
Positive result:

Two pink-purple lines appear in results window – one in control area, one in test area. Any line, regardless of its intensity should be considered positive.



Negative result:

Only one pink-purple line in results window – in control area.



Invalid result:

No pink-purple lines appear in control area.

9. Laboratorios Silanes SA de CV: TB-Instantest

STANDARD OPERATING PROCEDURE

Conditions:

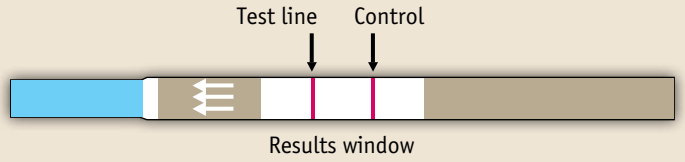
- Specimens ideally should be tested immediately after collection. Otherwise, refrigerate (4 °C) for up to 5 days. Alternatively, use frozen serum samples not subjected to more than one freeze-thaw cycle

Steps:

1) Use pipette to add 10 µl of serum to sample window
2. Immediately add 4-5 drops of TB-Instantest diluent to sample window
3. Interpret results after 15 minutes (maximum 20 minutes) as follows:

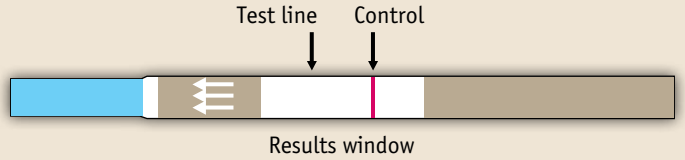
EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- Timer
- Gloves
- Container for specimen collection
- Centrifuge (serum, plasma samples)



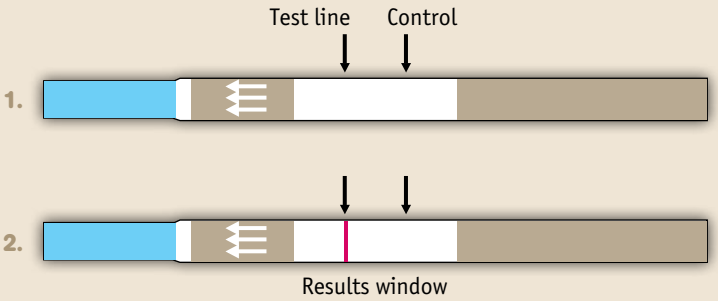
Positive result:

Two colour bands (of any intensity) within result window, no matter which band appears first.



Negative result:

One purple colour band in the control zone of the results window.



Invalid result:

1. The appearance of two lines in the results window can not be observed.

2. The control line can not be observed.

10. Millennium Biotechnology: Immu-Sure TB Plus

STANDARD OPERATING PROCEDURE

Conditions:

- Unopened – stable at temperatures 8-30 °C, shelf life 24 months
- Specimens ideally tested immediately after collection
- Otherwise, refrigerate (2-8 °C) for up to 3 days, then freeze (≤ -20 °C). Whole blood samples should not be frozen
- Use of control materials along with test samples is recommended

Steps:

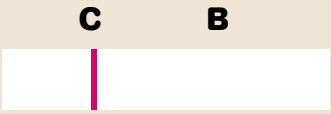
1) Use pipette to add 5 µl of serum to well of test card
2) Add 5 drops of diluent (using dropper bottle provided) to well of test card
3) If dye has not cleared the membrane after 15 minutes, add one more drop of diluent to test well
4) Interpret results as follows up to 25 minutes after diluent is added:

EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- Timer
- Gloves
- Container for specimen collection
- Pipette (5-10 µl)

Positive result:

Two pink/purple bands appear – one in test (B) area, one in control (C) area.



Negative result:

Only one pink/purple bank appears in C (control) area of test card.

Invalid result:

Only one band appears in test (B) area or no band appears in control (C) area.

11. Minerva BiOTECH Corporation: V Scan

STANDARD OPERATING PROCEDURE

Conditions:

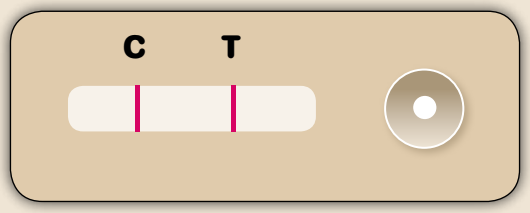
- Tests stored at 8-30 °C; shelf life 24 months.
- Store serum and plasma specimens at -20 °C

Steps:

1) Add 1 drop of sample followed by 8 to 10 drops of buffer into sample well
2) Interpret results as follows after 15 minutes and before 30 minutes:

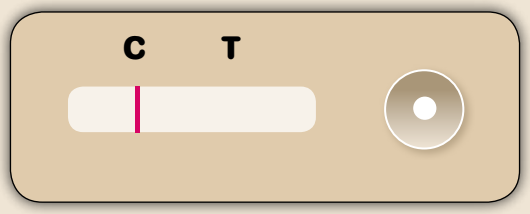
EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- Timer
- Gloves
- Container for specimen collection
- Centrifuge (serum, plasma samples)



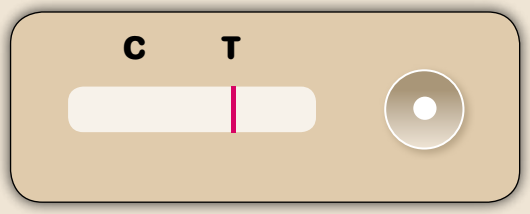
Positive result:

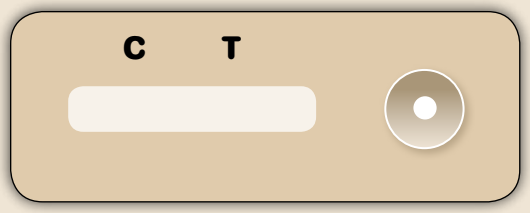
Two purplish-red lines – one in control zone, one in test zone.



Negative result:

Only one purplish-red line in control zone.





Invalid result:

If control line does not appears in control zone; if both test and control lines do not appear.

12. Mossman Associates, Inc.: MycoDot's 9 Easy Steps

STANDARD OPERATING PROCEDURE

Conditions:

- Specimens can be kept at 2-8 °C for short-term storage. However, they must be frozen (≤ -20°C) for long-term storage
- Store kit components at 2-8 °C
- Pouch containing antigen-coated combs should be brought to room temperature before opening to prevent condensation
- Unused antigen combs should be stored in aluminium pouches with silica gel bag and tightly closed in zipper seal bag to protect from moisture during storage
- Once diluted, rinse buffer is stable for one week if stored 2-8 °C
- All samples and kit components should be at room temperature prior to testing
- Positive and negative assay controls supplied are to be routinely tested each day test is performed, or as lab protocol dictates

EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- Pipette and disposable tips capable of delivering 10-80 µl
- 100 ml graduated cylinder
- Distilled water
- Timer capable of timing 6 and 10 minutes
- Gloves
- Container for specimen collection
- Paper towels or other absorbent pad
- Centrifuge (serum or plasma samples)

Steps:

1) Add 160 µl of sample diluent to first row of wells on microtiter plate
2) Add 160 µl of signal generating reagent to second row of wells on microtiter plate
3) Add 40 µl of serum to each sample diluent well. Pipette back and forth to mix thoroughly
4) Remove a test comb from foil pouch and incubate at room temperature for 6 minutes with first row of diluted samples, gently rock comb back and forth 8–10 times
5) Remove comb and allow to drain on paper towel
6) Rinse teeth of comb in diluted rinse buffer
7) Incubate combs for 10 minutes at room temperature in signal-generating reagent
8) Repeat Steps 5 and 6
9) With reference comb, interpret results after comb has air dried, ideally using white background and fluorescent light:

Positive result:

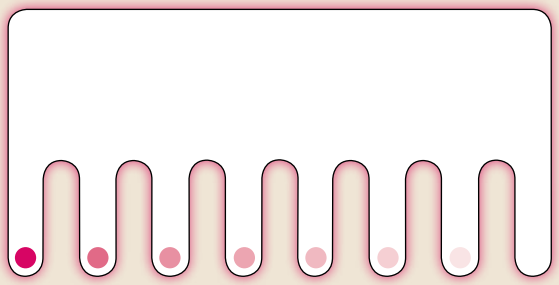
A coloured spot as, or more intense, than weakest positive spot on reference comb.

Negative result:

A spot less intense than weakest positive spot on reference comb, or no spot at all.

Invalid result:

For borderline reactions, it is recommended that a fresh sample be drawn after 2-4 weeks and retested.



13. Pacific Biotech Co. Ltd.: BIOLINE Tuberculosis Test

STANDARD OPERATING PROCEDURE

Conditions:

- Unopened: stable at temperatures 4-30 °C, shelf life 24 months
- Specimens ideally tested immediately after collection. Otherwise, refrigerate (2-8 °C) for up to 3 days, then freeze (≤ -20 °C)
- Test, reagents and specimen warmed to room temperature before use

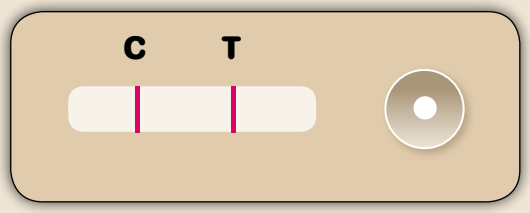
Steps:

1) Add 100 µl of serum to sample well

2) Interpret results after 5-20 minutes, as follows:

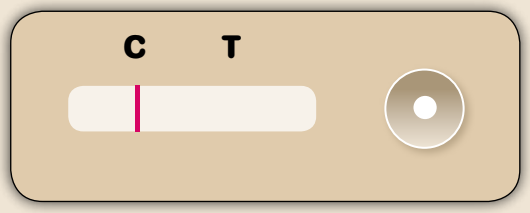
EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- Timer
- Gloves
- Container for specimen collection
- Centrifuge (serum, plasma samples)
- Pipette



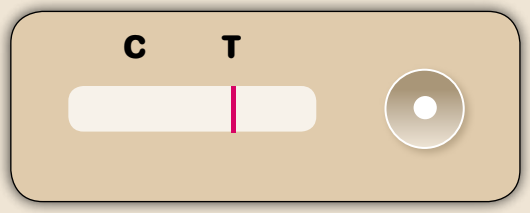
Positive result:

Two colour bands (T and C) within result window, no matter which band appears first.



Negative result:

Only one purple colour band (control band) within result window.



Invalid result:

If no purple colour band within control region after performing the test.

14. Premier Medical Corporation: First Response Rapid TB Card

STANDARD OPERATING PROCEDURE

Conditions:

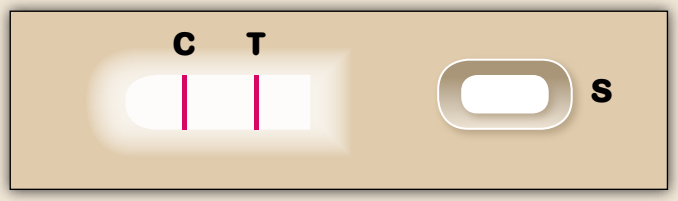
- Store test device at room temperature
- Specimens ideally should be tested immediately after collection. Otherwise, refrigerate (2-8 °C) for up to 3 days, then freeze (≤ -20 °C)
- Test, reagents and specimen warmed to room temperature before use

Steps:

1) Add 100 µl of serum into the sample well (S) with micropipette
2) Interpret test results at 15 minutes and not more than 30 minutes after sample application as follows:

EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- Timer
- Gloves
- Container for specimen collection
- Centrifuge (serum, plasma samples)
- Pipette



Positive result:
Two colour bands (T and C) within result window, no matter which band appears first.

Negative result:
Only one purple colour band within result window.

Invalid result:
If no C (control) line develops.

15. Princeton BioMeditech Corporation: BioSign M. tuberculosis Test

STANDARD OPERATING PROCEDURE

Conditions:

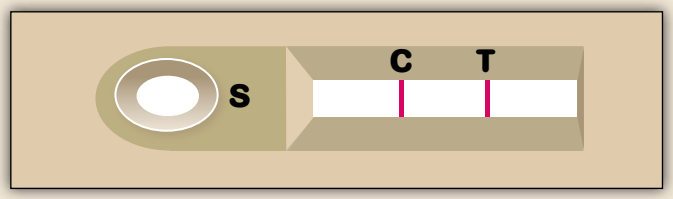
- Test device should be stored at room temperature (2-30 °C)
- Test, reagents and specimen warmed to room temperature before use

Steps:

1) Add 25 µl of serum to upper portion of sample well (S)
2) Add 2 drops of developer solution to lower part of sample well
3) Interpret test results after 8 minutes, as follows:

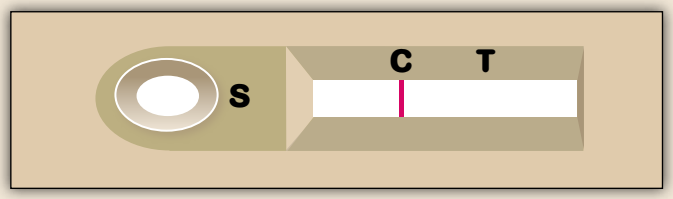
EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- Timer
- Gloves
- Container for specimen collection
- Centrifuge (serum, plasma samples)
- Lancet (for whole blood samples)
- Pipette or micropipette



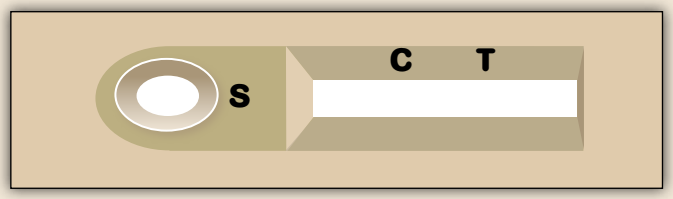
Positive result:

Two coloured lines in reading window – one in control (C) area, one in the lower, test area (T).



Negative result:

One coloured line in control area (C) and no distinctive coloured line in test area.



Invalid result:

No lines form in control area (C).

16. Span Diagnostics Ltd.: TB-Spot Ver. 2.0

STANDARD OPERATING PROCEDURE

Conditions:

- Specimens can be kept at 2-8 °C for short-term storage. For long-term storage they must be frozen (≤ -20 °C)
- Store kit components at 2-8 °C
- Pouch containing antigen-coated combs should be brought to room temperature before opening to prevent condensation
- Unused antigen combs should be stored in aluminum pouches with silica gel bag and tightly closed in zipper seal bag to protect from moisture during storage
- All samples and kit components are at room temperature prior to testing
- Positive and negative assay controls supplied are to be routinely tested each day test is performed, or as lab protocol dictates

Steps:

1) Preparation of wash buffer: dilute washing buffer 1:5 with distilled water
2) Fill wash reservoir/tray with washing buffer. Once diluted, rinse buffer is stable for one week if stored 2-8 °C
3) Add 3 drops (150 µl) of sample diluent and 4 drops (200 µl) of colloidal gold signal reagent to designated wells
4) Add sample controls to sample diluent wells
5) Add 50 µl of serum to each sample diluent well
6) Place comb in respective wells for 6 minutes at room temperature
7) Wash comb to remove unbound antibody
8) Incubate comb with colloidal gold signal reagent for 10 minutes at room temperature
9) Wash comb again in buffer to remove unbound colloidal gold signal reagent
10) With reference comb, interpret results after comb has air dried, ideally using white background and fluorescent light:

EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- Micropipette and disposable tips capable of delivering 50-100 µl
- 100 ml graduated cylinder
- Distilled water
- Timer
- Gloves
- Container for specimen collection
- Paper towels or other absorbent pad
- Discard jar with appropriate disinfectant (5% sodium hypochlorite)
- Centrifuge (serum or plasma samples).

Positive result:

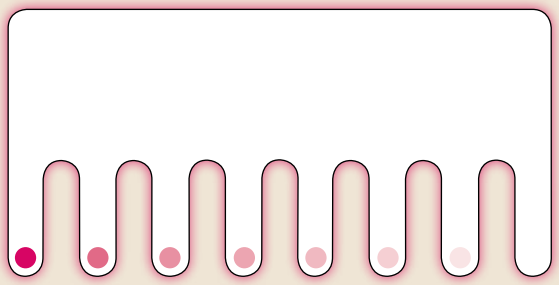
A coloured spot as intense, or more intense, than weakest positive spot on reference comb.

Negative result:

A spot less intense than weakest positive spot on reference comb, or no spot at all.

Invalid/indeterminate result:

For borderline reactions, it is recommended that a fresh sample be drawn after 2-4 weeks and retested.



17. Standard Diagnostics, Inc.: SD TB Rapid Test

STANDARD OPERATING PROCEDURE

Conditions:

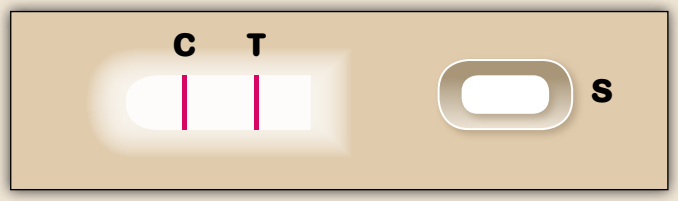
- Test device should be stored at room temperature (2-30 °C)
- Specimens ideally should be tested immediately after collection. Otherwise, refrigerate (2-8 °C) for up to 3 days, then freeze (≤ -20 °C)
- Test, reagents and specimen warmed to room temperature before use

Steps:

1) Add 100 µl of serum to the sample well (S) with micropipette
2) Interpret results as follows, 15 minutes after sample application:

EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- Timer
- Gloves
- Container for specimen collection
- Centrifuge (serum, plasma samples)
- Pipette or micropipette



Positive result:

Presence of two colour bands (T and C) within result window, no matter which band appears first. Depending on the TB antibodies' concentration, intensity of the control line and test line may vary.

Negative result:

Presence of only one purple colour band within result window.

Invalid result:

If no purple colour band is visible within result window.

18. Unimed International, Inc: FirstSign MTB Test

STANDARD OPERATING PROCEDURE

Conditions:

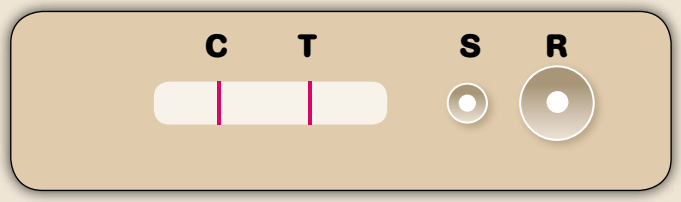
- Test device should be stored at room temperature (2-30 °C)
- Specimens ideally should be tested immediately after collection but may be refrigerated (2-8 °C) for up to 24 hours
- Test, reagents and specimen warmed to room temperature before use

EQUIPMENT REQUIRED BUT NOT SUPPLIED:

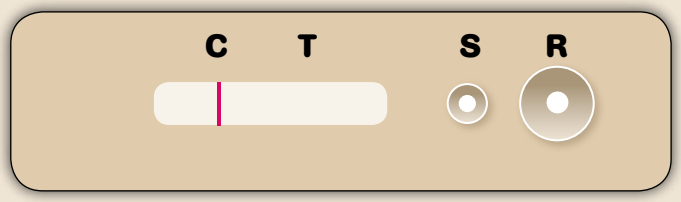
- Timer
- Gloves
- Container for specimen collection
- Centrifuge (serum, plasma samples)

Steps:

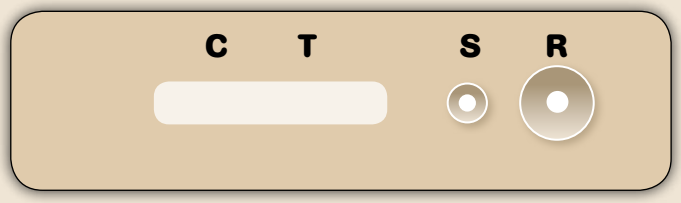
1) Using sample dropper, add one drop of serum to sample port A.
2) Dispense 5 drops of sample running buffer into port B.
3) Interpret results after 15 minutes as follows:



Positive result:
Two pink-purple bands appear in results window (C and T).



Negative result:
One pink-purple band appears in results window (C).



Invalid result:
No bands appear in results window.

19. VEDA.LAB: TB Rapid Test

STANDARD OPERATING PROCEDURE

Conditions:

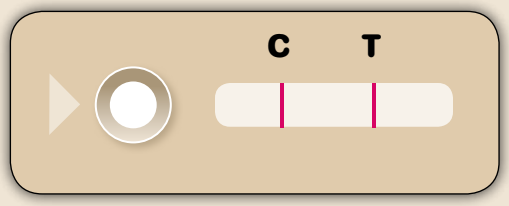
- Test device should be stored at room temperature (2-30 °C) and is stable for 18 months under these conditions
- Specimens ideally tested immediately after collection. Otherwise, refrigerate (4 °C) serum and plasma for up to 48 hours or freeze samples
- Avoid repeat freeze-thaw cycles
- Test, reagents and specimen warmed to room temperature before use

Steps:

1) Using serum dropper, add one drop (25 µl) to sample well
2. Dispense 2-4 full drops (150 µl) of diluent into sample well
3. Interpret results after 10-15 minutes only, as follows:

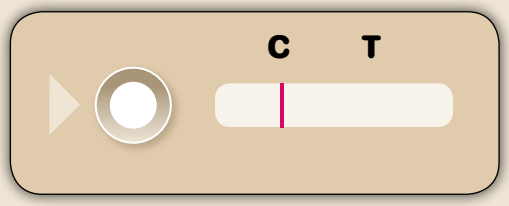
EQUIPMENT REQUIRED BUT NOT SUPPLIED:

- Timer
- Gloves
- Container for specimen collection
- Centrifuge (serum, plasma samples)



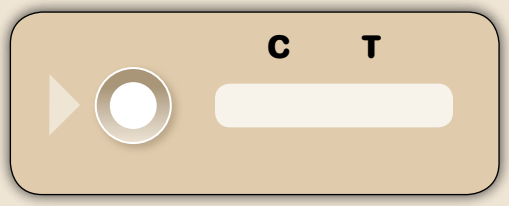
Positive result:

In addition to control band, a clearly distinguishable band shows in test window.



Negative result:

One coloured band shows in control window.



Invalid result:

No bands appear in results window.

Annex 4. Operational characteristics form

To be completed for each test evaluated after 25 repetitions

Name of test: .....

Manufacturer: .....

Date of evaluation: .....

1. Clarity of kit instructions

- **difficult to follow** 0 ❑
- **fairly clear** 1 ❑
- **very clear** 2 ❑
- **excellent** 3 ❑

2. Technical complexity

- **complex** 0 ❑

  If yes, why? (check all that apply)

  - Small volumes ❑
  - Multiple steps ❑
  - Short time intervals between steps ❑
  - Test difficult to manipulate ❑
  - No space for labelling ❑
  - Incomplete migration of samples ❑
  - Other:..... ❑

- **fairly easy** 1 ❑
- **very easy** 2 ❑
- **excellent** 3 ❑

3. Ease of interpretation of results

- **difficult** 0 ❑

  If yes, why? (check all that apply)

  - Signal intensity low or diffuse ❑
  - Signal colour variation ❑

- **fairly easy** 1 ❑
- **very easy** 2 ❑
- **unambiguous** 3 ❑

4. Equipment required but not provided e.g. micropipette

- **yes** 0 ❑
- **no** 1 ❑

If no, what is required?

.....

.....

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Comments: .....

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Annex 5. Laboratory data collection form: performance

Name of test: .....

Manufacturer: .....

Date of evaluation: .....

LOT number: .....

Expiry date: .....

Study ID (001-400)	Kit group (A-I)	Subgroup (01-10)	Date of testing	Test Results		Reference test result (see Table 1)
				Reader 1	Reader 2	

Annex 6. Laboratory data collection form

Name of test: .....

Manufacturer: .....

Date of evaluation: .....

LOT number 1: .....

LOT number 2: .....

Study ID (001-400)	Group (01-07)	LOT number	Test Results					
			Day 1		Day 2		Day 3	
			Reader 1	Reader 2	Reader 1	Reader 2	Reader 1	Reader 2
		1						
		2						
		1						
		2						
		1						
		2						
		1						
		2						
		1						
		2						
		1						
		2						
		1						
		2						
		1						
		2						

Comments: .....

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