Recent advances in the diagnosis of *Mycobacterium tuberculosis*

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Abstract

Molecular technologies offer the greatest potential for laboratories in resource-rich countries because they have the highest sensitivity and specificity.

Continued use of new technologies will be crucial in elucidating the true epidemiology and pathogenesis of a disease, including the less well studied diseases.

Continued development of affordable, sensitive, and specific diagnostic tools will be required for use in resource-poor settings, where the incidence of disease is highest.

Keywords ***Mycobacterium*, tuberculosis diagnosis, BACTEC, QuantiFERON, PCR.**

Introduction

Given the limited advances in tuberculosis (TB) diagnosis for more than a century, the development of new diagnostic tools has become a central part of the TB research agenda in recent years.

Many organizations have acknowledged the urgent need for improved TB diagnostics, and have advocated for additional research.[1-5] Recommendations stemming from these groups have been incorporated into TDR's (Special Programme for Research and Training in Tropical Diseases) strategic plan for TB diagnostics research, and a targeted diagnostics research agenda aims at stopping TB, with a Partnership second Global Plan to stop TB implemented during 2006 – 2015.[6]

Several promising TB diagnostic tests are currently under development and some are even under use in many countries, varying according to the level of the health system where they could be implemented.

Unfortunately, tests that would have the greatest impact on TB control (point-of-care tests) are only in early development stages and are put to little use in some countries. New diagnostic tests that would increase the sensitivity or simplicity of diagnosing active disease are in later stages of development but these tools will probably only be implemented at district or central referral laboratories; nonetheless, they are expected to have a measurable impact on TB control.

Rapid implementation of proven new technologies will also be critical to meet the urgent public health need and TB control targets.

Over the past years the quality of information on TB micro- and macro- epidemiology has increased substantially. There is, however, consensus on the need for more and better TB prevalence surveys. Tuberculin skin testing is still the method in use, despite its shortcomings. Novel diagnostic methods for use in TB epidemiological studies are highly desirable.[7,8] This review examines the recent advances in the diagnosis of *Mycobacterium tuberculosis* in humans.

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Available techniques for the detection of *Mycobacterium* species

Septi-chek AFB

Septi-chek AFB (Roche Diagnostic Systems, Nutley, N.J.) is a test which allows simultaneous detection of *Mycobacterium tuberculosis* and non-tuberculosis mycobacteria and it consists of a capped bottle containing 30 mL of Middlebrook 7H9 broth under enhanced CO_2 and a paddle with agar media enclosed in a plastic tube. The paddle is covered on one side with non-selective Middlebrook 7H11 agar and on the other side it is divided into two sections, one which allows the differentiation of M *tuberculosis* from other mycobacteria (7H11 agar with NAP – para-nitro-a-acetylamino-b-hydroxy-propiophenone), and the other which ensures detection of contaminants (chocolate agar). The bottle also contains enrichment broth with glucose, glycerin, oleic acid, pyridoxal, catalase, albumin, polyoxy-ethylene 40 stearate, azlocillin, nalidixic acid, trimethoprim, polymyxin B and amphotericin B.[9]

This method requires about 3 weeks of incubation, but the non-radiometric approach has the potential to expedite processing, obviate CO_2 incubation requirements thus facilitating early detection of positive cultures.[9]

Septi-chek AFB can be used for the detection and isolation of mycobacteria from sputum, bronchoalveolar lavage or aspirate (three early morning samples on successive days, not to exceed 10 mL each), urine (three clean samples or catheterized morning urine specimens on successive days), stool, body fluids (pleural effusion, ascites, synovial fluid or cerebrospinal fluid), biopsy tissues, wounds and skin.[10]

The unique advantage of this technique is the simultaneous detection of M *tuberculosis*, non-tuberculosis mycobacteria (NTM), other respiratory pathogens and even contaminants. A historical multicentric study conducted in four medical centers has shown that the system gives better results compared to conventional mycobacterial isolation media and BACTEC 460 TB System, supporting the growth of bacteria from small inocula and shortening the time required for recovery of mycobacteria from clinical specimens.[11,12]

Microcolony detection on solid media

Microcolony detection is a method which aims to identify the characteristic strings and tangles of M *tuberculosis* through the use of simple light microscopy[13] on plates with a thin layer of Middlebrook 7H11 agar medium (Difco). This method allows detection of microcolonies of M *tuberculosis* in less than seven days but despite the relatively low costs associated with this approach, it appears to be less efficient.[9]

Microscopic observation broth-drug susceptibility assay (MODS)

The microscopic observation broth-drug susceptibility assay (MODS) is a relatively inexpensive method that yields results rapidly, in roughly 9.0-9.5 days.[14] Although this technique has a sensitivity of 92%, comparable to that of mycobacteria growth indicator tubes (MGIT, 93%) and polymerase chain reaction (PCR, 90%),[14] it requires a relatively high technical skill, P2 bio-safety cabinets, Middlebrook 7H9 broth, oleic acid dextrose catalase (OADC) and a series of antimicrobial supplements.[9] It could be considered, however, as an alternative for susceptibility testing in developing countries, since the results are generally obtained within two weeks if the initial patient sample is used directly for susceptibility testing.[14]

BACTEC 460TB

BACTEC 460TB (Becton Dickinson, Sparks, MD, USA) has been long considered the best method for rapid testing of susceptibility of M *tuberculosis* to major anti-tuberculous drugs such as rifampicin, isoniazid, ethambutol, pyrazinamide and streptomycin in clinical laboratories.[15] An Indian study showed that the BACTEC 460TB radiometric method obtained 87% of the positive results within seven days and 96% within 14 days.[11,16] Therefore, by facilitating early diagnosis, the BACTEC 460TB method is considered cost effective in countries endemic for tuberculosis.[11]

This radiometric technique uses ^{14}C labeled palmitic acid in 7H12 medium to detect the metabolism rather than the visible growth of mycobacteria in half the time required by

conventional culture methods.[17] The metabolism of the ^{14}C labeled substrate leads to the production of $^{14}CO_2$ which is measured and reported in terms of growth index.[9]

ESP blood culture system

The ESP system (Difco Laboratories, Detroit, Mich.) was designed to detect consumption and/or production of gas by microbes rather than only the production of CO_2, as is the case with BACTEC. Through the ESP system, the blood culture bottles are monitored every 12-24 minutes to detect changes in the oxygen consumption and gas production, changes which are associated with microbial growth.[18] It is soon to be replaced with ESP Culture System II.

ESP Culture System II for growth and detection of mycobacteria

ESP Culture System II (Difco Laboratories, Detroit, Mich.) is a fully automated continuous monitoring culture system used for the growth and detection of different microorganisms (including *M tuberculosis*). This test interprets mycobacterial growth by evaluating gas consumption, which leads to changes in the pressure above the culture medium.[19]

A study on 2283 specimens found no significant difference between the recovery rates of BACTEC 460TB and Middlebrook 7H11/7H11 selective agar systems. This study reported a mean time for recovery of all mycobacteria (including *M tuberculosis* and *Mycobacterium avium* complex) ranging from 10.9 to 15.5 days.[19] These positive results suggest that the ESP culture system II is a reliable non-radiometric alternative to BACTEC 460TB. However, as with other liquid culture systems, it should only be used in combination with a solid medium, and not as a stand-alone system.[19]

MB/BacT

MB/BacT (Organon Teknika, Turnhout, Belgium) is a non-radiometric continuous monitoring system designed for the isolation of mycobacteria from clinical specimens. It utilizes a colorimetric sensor and reflected light to continuously monitor the CO_2 concentration in the culture medium.[20]

When comparing the performance of MB/BacT with that of BACTEC 12B media for BACTEC 460, a Swiss study showed that the mean time for the detection of *M tuberculosis* from sputum, cerebrospinal fluid (CSF) and urine samples was 17.5 (±6.4) days for MB/BacT, 14.3 (±8.2) for BACTEC and 24.2 (±7.5) days for egg-based media cultures.[11] The study concluded that MB/BacT is an acceptable alternative for BACTEC 460 despite some minor disadvantages such as increased contamination and slightly longer time for detection of growth.

One other study performed in the Philippines showed that the detection time for MB/BacT system was shorter with two weeks compared to the conventional Löwenstein-Jensen (LJ) egg culture.[21]

Identification of mycobacterial species

There are several methods which allow identification of mycobacterial species, ranging from biochemical typing to gas chromatography and high pressure liquid chromatography (HPLC).

Chromatography

The technique behind chromatography allows separation of complex mixtures based on the differential affinities of substances for two different media, a gas or liquid mobile medium and a stationary adsorbing medium through which they pass. It is used in reference laboratories for epidemiologic studies, since it can identify different species of *M tuberculosis* after isolation by any type of culture technique, based on the difference between species regarding the length of the colic acid residues in the cell wall, and it can provide results in as little as two hours.[22]

Chromatography is a highly reproducible technique, but the initial cost of the equipment is high. The evolution of technology can clearly been seen in the evolution of chromatographic techniques, which range from historical methods such as column chromatography to recent ones such as high pressure liquid chromatography (HPLC).[22]

TB PNA FISH

Fluorescence in situ hybridization (FISH) using peptide nucleic acid (PNA) probes allows differentiation between tuberculous and nontuberculous mycobacteria in smears of mycobacterial cultures. PNA molecules are pseudopeptides with DNA-binding capacity[23] in which the sugar phosphate backbone of DNA has been replaced by a polyamide backbone.[11]

The *M tuberculosis* complex-specific PNA probes showed sensitivities of 84-97% while the nontuberculous mycobacteria-specific PNA probes showed diagnostic sensitivities of 64-91%. Both types of probes showed diagnostic specificities and predictive values of 100%.[23]

Nucleic acid amplification (NAA)

Nucleic acid amplification allows both detection and identification of *M tuberculosis* through enzymatic amplification of bacterial deoxyribonucleic acid (DNA). The most widely used technique is PCR, but transcription mediated amplification (TMA) and strand displacement amplification (SDA) are also commercially used. The sensitivity of this test is higher than that of smear microscopy but it is slightly lower than that of culture techniques.[24]

Nucleic acid tests are currently used mostly for confirmation of smear-positive results or for primary case diagnosis, when combined with other methods. The main advantage of these tests is that they offer quick results,[25] paired with a high level diagnostic accuracy. Because of their price and complexity, the use of these methods is still limited to developed countries, but their introduction to developing countries is improving gradually.

A positive direct amplified test in conjunction with smear positive for acid-fast bacilli are highly predictive for tuberculosis but mycobacterial cultures are still needed for species identification, confirmation or susceptibility testing.[11]

The advantages of nucleic acid amplification tests include the fact that results are available quite rapidly, in a matter of hours, with high specificity (98-100)%,[24,26] and sensitivity (higher than 95% acid-fast bacilli positive sputum, and between 60-70% in smear-negative, culture-positive specimens).[27,28] Newer amplification tests may display better sensitivity in smear-negative specimens while retaining high specificity.[24,29-31]

Nucleic acid amplification tests could also be used for detecting *M tuberculosis* in specimens other than sputum (e.g., blood, lymph, cerebrospinal fluid, urine, bronchial aspirate and lavage, bone marrow, gastric aspirate), although, to date, results have varied widely.[24,32,33] The disadvantages include the high costs and a possibly lower specificity in clinical conditions.[24]

Xpert MTB/RIF

Xpert MTB/RIF is an automated molecular test for *M tuberculosis* and its resistance to rifampin, based on the Cepheid GeneXpert system. It uses hemi-nested real-time PCR assay to amplify a specific sequence of the *rpoB* gene, which is then probed with molecular beacons for mutations within the rifampin-resistance determining region, providing a result within two hours.[34]

PCR

PCR allows sequences of DNA to be amplified in vitro even when only a few copies of mycobacteria are present, so that the amount of amplified NDA can be rapidly visualized and identified.[11] The most common target used for PCR is insertion sequence IS6110.[11,35] This sequence is specific for *M tuberculosis* and offers multiple targets for amplification, being present up to 20 times in the genome.[11]

A recent study performed in Bangladesh reported the sensitivity and specificity of PCR using the IS6110 to be 94.74% and 100% respectively, concluding that the PCR technique is a rapid and alternative method to Löwenstein-Jensen culture for the diagnosis of pulmonary tuberculosis.[35]

Transcription mediated amplification (TMA). AMPLIFIED MTD (*Mycobacterium Tuberculosis* Direct) Test

TMA can identify the presence of genetic information unique to *M tuberculosis* directly from pre-processed clinical specimens.[11] AMPLIFIED MTD Test (Gen-Probe, Hologic)

detects *Mycobacterium tuberculosis* ribosomal ribonucleic acid (rRNA) directly and rapidly, with a sensitivity similar to that of culture techniques. TMA produces over 1 billion copies of RNA amplicon which are then detected through hybridization protection assays (HPA).[36] The nucleic acid amplification test for Gen-Probe's MTD Test involves a test kit, lysis of cells and release of the nucleic acid target.

The sensitivity of this test is of 96% and its specificity is 100% for M *tuberculosis* on specimens that are smear-positive for acid-fast bacilli.[28] False-negative or false-positive results can be either due to contamination or the small number of bacilli available for the test. One other disadvantage of the technique is that positive results are recorded for both viable and dead bacilli.[9,11]

Ligase chain reaction (LCR)

Ligase chain reaction is another DNA amplification technique which is based on the ligation of two adjacent synthetic oligonucleotide primers which uniquely hybridize to one of the strands of the target DNA.[37] A second pair of oligonucleotides is designed to hybridize to the complementary DNA, in the same region.[11] When the nucleotides are present, the DNA polymerase and the ligase create a gap between the adjacent primers, which will then be filled with the appropriate nucleotides leading to ligation of the primers.

Genotyping methods

New PCR-based genotyping techniques include spacer oligonucleotide typing (spoligotyping), IS6110-based restriction fragment length polymorphism (RFLP) and mycobacterial interspersed repetitive unit (MIRU) typing. Genotyping is useful in analyzing suspected outbreaks of tuberculosis in institutions such as hospitals, schools and prisons.[22]

When used together for strain typing, spoligotyping and MIRU can discriminate strains which are not part of the chain of transmission. In certain cases however, these tests can also return false-positive results, a disadvantage which decreased with the advent of DNA fingerprinting.[22,38]

Spoligotyping

Spoligotyping can be used for both detection and typing *of* M *tuberculosis*, through PCR amplification of a highly polymorphic direct repeat locus in the genome of M *tuberculosis*. Prior culturing of the bacteria is needed and results are available from culture within one day, with a sensitivity of 96% and a specificity of 98%.[39]

DNA fingerprinting

This type of test, using IS6110-based restriction fragment length polymorphism, has proven useful in phylogenetic studies of tuberculosis bacilli particularly since IS6110 is unique for the M *tuberculosis* complex.[40-42] RFLP DNA fingerprinting is the gold standard for strain typing in mycobacteriology and this method of genotyping has been standardized in order to increase the inter- and intra- laboratory comparability, so that it could be used for subspeciation of M *tuberculosis*.[40] Roughly one week is needed before the results can be interpreted.[22]

The disadvantages of RFLP genotyping are that a large cell mass is required and that comparison is difficult since the results are band patterns, hard to convert into digital formats.[22]

Mycobacterial interspersed repetitive unit typing

Mycobacterial interspersed repetitive unit typing is a technique based on variable numbers of tandem repeat at 12 loci in the genome of M *tuberculosis*.[11,43]

Antigen and antibody tests

Serology

In contrast to other infectious diseases for which detection of antibodies or antigens in blood are used, no serological first-line methods are currently used for tuberculosis[24,44] particularly due to variability in results and cross-reactivity with environmental mycobacteria which leads to false-positive results. The use of these techniques is currently limited to private sectors of countries without diagnostic regulating bodies.[24]

Serological tests are performed through application of blood sample to immuno-

chromatographic strips, the patient bars showing positive results in a mean time span of 15 minutes.[24]

The sensitivity of these tests is high in patients with smear-positive disease, but much lower in children, patients with extra-pulmonary disease, HIV infection or smear-negative cases. Moreover, these tests cannot reliably distinguish latent infection from active disease or different species of mycobacteria.[24]

ELISA

A study performed in India applied indirect ELISA tests with monoclonal antibodies against the purified Ag 85 complex. Serum samples were collected from 197 patients. The test showed 82% sensitivity and 86% specificity.[45]

Antigen detection

A number of affinity-purified antibodies have proven to be useful in the diagnosis of *M tuberculosis* infections. Among these, the excretory-secretory protein ES-31 was one of the first antigens to be detected. A study performed in India compared a sandwich ELISA test for ES-31, ES-43, and EST-6 with the monospecific anti-ES-31 antibody detection in 68 smear-positive cases of tuberculosis and showed a sensitivity of 91-97% for the three antigens compared to 79-91% for the monospecific test.[46]

A number of antigens which can be detected when present at a concentration of 3-20ng/mL include mycobacterial sonicates, tuberculin purified protein derivative (PPD) and antigens 5, A60, P32 and LAM, detected through sandwich or inhibition ELISA, latex agglutination or reverse passive hemagglutination (RPHA) tests.[11,47] The reported sensitivity of these tests is quite low, roughly between 40-50%, with slightly higher specificities, of 80-95%.[11]

Detection of lipoarabinomannan

An interesting tool relies on the detection of lipoarabinomannan (LAM), which is a cell wall lipopolysaccharide antigen of *M tuberculosis*. LAM-ELISA assays have demonstrated variable sensitivities in diagnosing tuberculosis. A recent study has demonstrated that urinary LAM appears to be related to host immune factors and that it declines steadily after two weeks of anti-tuberculous treatment.[48] LAM-ELISA may be a suitable option for the diagnosis of human immunodeficiency virus (HIV)-associated tuberculosis in urine specimens from patients with low CD4 cell counts.[49]

Another approach is that of using a dipstick test to detect LAM in urine and pulmonary specimens, a method which may be particularly useful in developing countries lacking biosafety level 3 facilities.[50] Preliminary reports showed a sensitivity of 93% and a specificity of 95%.[11,51]

Multi-antigen and antibody assays (SEVA TB ELISA)

A recent study used SEVA TB ELISA with a cocktail of ES-31 and EST-6 (containing ES-38 and ES-41) antigens and their IgG antibodies for the analysis of sera or pleural fluid specimens from patients with suspected tuberculous pleuritis. The test showed 83% sensitivity and 100% specificity in pleural fluid compared with 92% sensitivity and 78% specificity in serum samples.[52]

Cytokine detection assays. Interferon-gamma release assays (IGRAs)

Cytokine detection assays measure the cell-mediated immune response elicited against *M tuberculosis*. IGRAs measure the interferon (IFN)-gamma released by sensitized white blood cells. Four IGRAs which use different antigens to stimulate IFN-gamma release and different methods of measurement have been approved in the United States of America (USA). QuantiFERON-TB (Cellestis Limited, Carnegie, Victoria, Australia, 2001) was approved as an aid for diagnosing latent tuberculosis infection and is no longer commercially available since it was replaced by QuantiFERON-TB Gold (Cellestis Limited, Carnegie, Victoria, Australia, 2005), which is approved as an aid for diagnosing both latent infection and active disease. Newer tests include QuantiFERON-TB Gold In-Tube test (Cellestis Limited, Carnegie, Victoria, Australia, 2007) and the T-SPOT.TB test (Oxford Immunotec Limited, Abingdon, United Kingdom).[53]

QuantiFERON-TB Gold

QuantiFERON-TB Gold (Cellestis Limited, Carnegie, Victoria, Australia) is an ELISA test which detects the release of IFN-gamma in fresh heparinized whole blood from sensitized persons upon incubation with synthetic peptides simulating ESAT-6 and culture filtrate protein-10 (CFP-10).[53]

The test steps for QuantiFERON-TB Gold involve blood sample collection, addition of stimulating antigens, incubation for 16-24 hours at 37°C, harvesting of plasma and addition of conjugate solution. The samples are then incubated for two hours at room temperature, the plates are washed at least six times and then the substrate is added. The samples are then incubated for 30 minutes, adding stop solution, reading absorbance at 450 nm and calculating results using dedicated software. The patient only needs to visit once, for specimen collection, and results can be obtained in 48 hours.

QuantiFERON-TB Gold In-Tube

The QuantiFERON-TB Gold In-Tube (Cellestis Limited, Carnegie, Victoria, Australia) was developed to overcome the limitation of QuantiFERON-TB Gold, which could only be used in facilities where blood testing could begin within a few hours of its collection. This test uses a mixture of 14 peptides representing ESAT-6, CFP-10 and a part of TB7.7.[53]

T-SPOT.TB

T-SPOT.TB (Oxford Immunotec Limited, Abingdon, United Kingdom, 2008) incubates peripheral blood mononuclear cells with mixtures of peptides (ESAT-6, CFP-10) and uses an enzyme-linked immunospot assay (ELISpot) to detect increases in the number of cells that secrete IFN-gamma (spots in each test well).[53]

A recent study has compared QuantiFERON-TB Gold In-Tube, T-SPOT.TB and tuberculin skin test (TST) in 373 HIV-infected patients,[54,55] reporting that IGRAs were more sensitive than TST for the diagnosis of *M tuberculosis* infection in this category of patients. Ramos et al. propose dual sequential testing with both TST and IGRAs as the optimal approach for latent tuberculosis infection screening in this population.[56]

Monokine-amplified IFN-gamma release assays (MIGRAs)

Given that IFN release leads to subsequent release of IFN-responsive chemokines such as MIG and IP-10,[57] recent studies have investigated whether measurement of these chemokines might provide a sensitive tool for the detection of mycobacterial infection and antigen-specific T-cell responses.[58]

Other diagnostic approaches

Detection of anti-mycobacterial superoxide dismutase antibodies.

Superoxide dismutase (SOD) has been evaluated for its role in establishing mycobacterial phylogeny based on the immunological relatedness among mycobacteria.[59] Tests based on enzyme immunoassays have provided different results regarding the sensitivity and the specificity of this technique.

MPB 64 patch test

MPB 64 is a mycobacterial antigen specific for *M tuberculosis* complex. A study from the Philippines delivered MPB 64 in a transdermal patch as a diagnostic skin test reagent for detecting active tuberculosis. The test results can be interpreted three to four days after patch application, with a sensitivity of 87.8% and a specificity of 100%.[60] This test is particularly useful since it can discriminate between latent infection and active disease.[11]

FAST Plaque TB

FAST Plaque TB (BIOTEC Laboratories Ltd, FIND – Foundation for Innovative New Diagnostics) is a test that uses mycobacteriophages to detect *M tuberculosis* directly from sputum samples. The principle of this assay, as described by the manufacturing companies, is that a specific mycobacteriophage infects cells of the *M tuberculosis* complex present in the specimen. After infection, the sample is treated with a virucide to destroy phages not infecting a host cell and thus the remaining intracellular phages undergo a replication cycle with subsequent release of the phage from the host cells. During incubation, released phages are

allowed to infect a lawn of non-pathogenic organisms within an agar plate, where the phages undergo further replication and lyse the non-pathogenic organisms, producing zones of clearing (plaques) on the surface of the agar, which indicate a positive sample.[61]

This is a rapid test, with results available within 24 hours of sample preparation, it is safe, since there is no need for culturing of *M tuberculosis*, and it only detects live bacilli, thus reducing the possibility of false positives.[61]

The test has a sensitivity of 70.3-75.2% and a specificity of 98.7-99.0% when compared with smear microscopy, which has a sensitivity of 61.3-63.4% and a specificity of 97.3-97.4%.[9,11]

This test may prove useful in high burden countries. In a study conducted in South Africa, its sensitivity was 70.3% and its specificity, 99% in previously untreated patients with tuberculosis.[24,44] Its disadvantages include its low sensitivity in smear-negative culture-positive specimens, the technical expertise required, and the fact that is has been evaluated only on sputum specimens.[24]

FAST Plaque-Response

This assay is an extension of the technique used for FAST Plaque TB, which allows the early detection of rifampicin resistance through the use of mycobacteriophages. The smear-positive sputum specimen needs to be incubated with and without rifampicin overnight at 37°C. Interpretation of the test is based on the fact that rifampicin-resistant *M tuberculosis* strains survive drug treatment and are able to produce plaques, compared with susceptible strains which not produce plaques.[61]

ADA

Adenosine deaminase (ADA) has been proposed to be a useful surrogate marker for tuberculosis in pleural, pericardial and peritoneal fluids. The results of a study performed in India revealed a sensitivity of 100%, a specificity of 94.6% specificity, and a cutoff value of 40 U/L for ADA in pleural, peritoneal and pericardial fluids.[62]

Conclusion

In conclusion, the advantages and disadvantages of each available TB diagnostic method are evident and no test is yet available to meet target specification in terms of performance and ease of use.

Performance is a compilation of sensitivity, specificity and speed, while ease of use is a compilation of safety, number of steps, cost, robustness and training simplicity.[63] Serology and microscopy are easier to use than X-ray techniques, culture, or nucleic acid amplification tests (NAAT); nonetheless, culture and NAATs performs better than X-ray, microscopy and serological techniques.

Apart from these characteristics of current TB diagnostics, the quality of test results with the existing methods is dependent on the availability of sufficient human and financial resources, training of laboratory personnel and monitoring of performance.

New methods that manage to overcome limitations and respond to challenges posed by special populations (including patients with concomitant infections such as hepatitis B,[64] hepatitis C, HIV,[65] or with hematologic malignancies) will definitely be well received.

Conflicts of interest All authors – none to declare.

Author contributions All authors had equal contributions.

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