ISSN: 2536-7153 SJMLS

Sokoto Journal of Medical Laboratory Science 2022; 7(3): 60 - 70

SJMLS - 7(3) - 006

Current Innovations in Medical Laboratory Diagnosis of Tuberculosis

Obadire, S.O.* [1], Mitsan, O. [2], Ige, I.P. [3], Oke, O.C. [4], Odewusi, F.O. [5]

Medical Laboratory Department, Federal Medical Centre Birnin Kudu, Jigawa State [1], Department of Medical Laboratory Science, College of Medicine and Health Sciences, Igbinedion University Okada, Edo State, Nigeria [2], Medical Laboratory Department, General Hospital Ile Oluji, Ondo State [3], Department of Microbiology, Ladoke Akintola University Ogbomosho, Oyo State [4], Department of Medical Laboratory Science, College of Medicine and Health Sciences Afe Babalola University, Ado Ekiti, Ekiti State, Nigeria [5].

Author for Correspondence *: obadire@gmail.com/+234-0803-693-9559.

https://dx.doi.org/10.4314/sokjmls.v7i3.7

Summary

Tuberculosis (TB) is a major global health problem, ranking as the second highest cause of death from an infectious disease globally, after the human immunodeficiency virus (HIV). Nigeria ranked first in Africa with burden of TB. Tuberculosis is a major public health problem in Nigeria with about 407,000 people infected. The World Health Organization estimates that 120,000 people developed TB in 2019, with 154,000 death of whom, 13% were HIV positive individuals. Among the incident cases, 56% were from the South-East Asia and Western Pacific Regions and one quarter were from Africa. The African continent accounts for the highest rates of cases and deaths relative to population. Traditionally, tuberculosis is mostly being diagnosed by a combination of chest X-rays, the staining of sputum with special dyes followed by microscopy, the growth of *Mycobacterium tuberculosis* in culture and the Mantoux test. The sputum smear microscopy is easy to do and is very cheap and combined with chest X-rays has been used for a long time by TB control agencies worldwide. However, the sputum smear microscopy (sputum AFB) test has some problems in HIV-positive patients and children also in patients with low bacterial load and in latent TB infections. TB continues to be a complex disease to diagnose and manage due to the chronicity of the disease, the nature of the host-pathogen relationship, and the resulting diversity in its clinical manifestations. Therefore, this review considers recent innovations in TB laboratory diagnosis in conventional (traditional), non- conventional (molecular), serological (immunological) methods and the latest methods of TB diagnosis.

Keywords: Tuberculosis (TB), *Mycobacterium tuberculosis*, sputum AFB, GeneXpert.

Introduction

Tuberculosis (TB) is a communicable disease caused by infection with *M. tuberculosis* complex organisms (*M. tuberculosis*, *M. bovis*, *M. africanum*), which typically spreads to new hosts via airborne droplet nuclei from patients with respiratory tuberculosis disease (Hopewell, 1992). A newly infected individual can become ill from tuberculosis within weeks to months, but most infected individuals remain well. Latent tuberculosis infection (LTBI), a non-communicable condition, persists in some, who might develop tuberculosis disease months or years later (Hopewell, 1992).

Tuberculosis (TB) stands as a major global health problem, ranking as the second highest cause of death from an infectious disease globally, after the human immunodeficiency virus (HIV). Nigeria ranked first in Africa with burden of TB. Tuberculosis is a major public health problem in Nigeria with about 407,000 people infected. The World Health Organization estimates that 120,000 people developed TB in 2019, with 154,000 death of whom, 13% were HIV positive individuals, (WHO, 2020). Among the incident cases, 56% were from the South-East Asia and Western Pacific Regions and one quarter were from Africa. The African continent accounts for the highest rates of cases and deaths relative to population (WHO, 2020). Traditionally, tuberculosis is mostly being diagnosed by a combination of chest X-rays, the staining of sputum with special dyes followed by

microscopy, the growth of *Mycobacterium tuberculosis* in culture and the Mantoux test. The sputum smear microscopy is easy to do and is very cheap and combined with chest X-rays has been used for a long time by TB control agencies worldwide. However, the sputum smear microscopy (sputum AFB) test has some problems in HIV-positive patients, children as well as in patients with low bacterial load and in latent TB infections (Diane and Barne, 2010). TB continues to be a complex disease to diagnose and manage due to the chronicity of the disease, the nature of the host-pathogen relationship, and the resulting diversity in its clinical manifestations. Therefore, this review considers recent innovations in TB laboratory diagnosis in conventional (traditional), non-conventional (molecular), serological (immunological) methods and the latest methods of TB diagnosis.

Conventional Method:

AFB Smear Microscopy

An estimated 80% of all TB cases worldwide are from 22 high-burden countries, and in the majority of these countries, the diagnosis of TB relies primarily on the identification of AFB in sputum smears using a conventional light microscope. The sputum specimens are smeared directly onto the slides (direct smears) and subjected to Ziehl-Neelsen (ZN) staining. Although all mycobacterial species are acid fast, this assay is highly specific for *M. tuberculosis* in countries where TB is endemic because of the high burdens of this disease (Steingart *et al.,* 2007). In spite of the high specificity, the sensitivity of the test has been reported to vary from 20 to 80%, and its usefulness is questionable for patients with reduced pulmonary cavity formation or reduced sputum bacillary load, such as children and HIV-coinfected patients. The sensitivity of the direct smear assay has been found to be dependent on staff that has been well trained so that sufficient time is spent on preparing, staining, and reading each smear, with a well-functioning external quality assurance (EQA) program in place. Although new technologies are under development, microscopy will likely remain the primary tool for the laboratory diagnosis of TB in resource-poor countries for the foreseeable future.

Culture method

Culture is the gold standard method and drug sensitivity testing (DST) can be done but it is costly and therefore unavailable in most sites. There is also the risk of contamination and prolonged turnaround time due to the slow growth rate of the TB bacilli (Chihota *et al.,* 2010). Available newer and faster methods like MODS (Microscopic Observation Drug Susceptibility Assay), MGIT (*Mycobacterium tuberculosis* Growth Indicator Tube) and colorimetric assay all require specialized skills and bio-safety laboratories that are often unavailable in the regions where these methods are mostly needed. Acid-fast microscopy is easy and quick, but it does not confirm a diagnosis of TB because some acid-fast bacilli are not *M. tuberculosis*. Therefore, a culture is done on all initial samples to confirm the diagnosis. A positive culture for *M. tuberculosis* confirms the diagnosis of TB disease. In an ideal situation, it is recommended that culture examinations be completed on all specimens, regardless of AFB smear results. Mycobacterial culture is more sensitive, but the growth of TB bacilli on traditional solid medium requires 4–8 weeks, which delays appropriate treatment in the absence of a confirmed diagnosis. Culturing mycobacteria is mainly done on solid media, the Lowenstein-Jensen slope, or in broth media. These methods are slow, with cultures from microscopy-positive specimen ranging from 2–4 weeks and for a microscopy-negative specimen from 4–8 weeks. Liquid media is significantly faster (between 10 and 14 days) and is better for isolation, compared to solid media. For drug susceptibility testing (DST), the delay may be reduced to as little as 10 days compared to 4–6 weeks with conventional solid media. Liquid systems are more sensitive for detecting mycobacteria and may increase the case yield by 10% compared to solid media (Bhargava *et al*., 2007).

Non-conventional method

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. (Scarparo *et al*., 2004) An Indian study showed that the BACTEC 460TB

radiometric method obtained 87% of the positive results within seven days and 96% within 14 days (Venkataraman *et al*., 1998). Therefore, by facilitating early diagnosis, the BACTEC 460TB method is considered cost-effective in countries endemic for tuberculosis (Ramachandran and Paramasivan, 2003). 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 (Seth and Kabra, 2006). 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 (Ranjan and Sharma, 2010).

Nucleic acid amplification (NAAT)

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 (WHO,2006). 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, 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 (EDMA, 2012). 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 (Ramachandran and Paramasivan, 2003).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%) (Catanzaro *et al.,* 2000) and sensitivity (higher than 95% acid-fast bacilli positive sputum, and between 60-70% in smear-negative, culture-positive specimens) (Clarridge *et al.,* 1993). Newer amplification tests may display better sensitivity in smear-negative specimens while retaining high specificity (Roggenkamp *et al.,* 1999). 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. The disadvantages include the high costs and a possibly lower specificity in clinical conditions (Pai *et al*., 2004).

GeneXpert 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 (Boehme *et al.,* 2010). The gene Xpert machine (XpertMTB/RIF Assay method) has the potential to revolutionize the diagnosis of TB based on its speed, sensitivity and specificity. It is a cartridge-based automated diagnostic test that can, in less than 2 hours, simultaneously detects *Mycobacterium tuberculosis* organisms as well as rifampicin resistance by using three specific primers and five unique molecular probes which ensure a high degree of specificity. The use of sealed and disposable cartridges apparently overcomes the problem of cross contamination. There is minimal bio-hazard so that bio-safety cabinets are not compulsorily needed. The Xpert MDR/Rif assay method which is a nucleic acid amplification test (NAAT) based on the principle of polymerase chain reaction (PCR) has opened a new era of widespread molecular diagnosis of TB. Its hands-on operation is easy and requires minimal technical expertise (WHO, 2012). It was developed by the laboratory of Professor David All of the University of Medicine and Dentistry of New Jersey together with Cepheid incorporation and FIND (Foundation of Innovative New Diagnostics) with additional financial support from the United States National Institute of Health (NIH) and Bill and Melinda Gates Foundation (Papaventsis *et al.,*

2014). The gene Xpert system was launched in 2004 while the first clinical validation studies were carried out in 2009 in some countries including South Africa and India, after which the machine was endorsed by WHO in 2010 for use in TB endemic countries (WHO,2010).

Immuno-diagnosis of Tuberculosis

Serology.

In the last decade, serology-based tests using formats well suited for resource-poor countries (performed without specialized equipment and with minimal training) have been successfully developed for many infectious diseases (e.g., HIV and malaria) (Steingart *et al.,* 2007). In these assays, antigens are typically precoated in lines across a nitrocellulose membrane to which serum or whole blood samples are applied. Antigen-antibody reactions are visualized on the lines using anti-human antibody bound to substances such as colloidal gold. The tests take only minutes to perform. These technologies are very attractive candidates for the simple, accurate, inexpensive, and, ideally, point-of-care (POC) diagnosis of TB. However, attempts to successfully develop sensitive and specific serological tests for the diagnosis of TB have been ongoing for decades, without a major breakthrough. There are currently over 40 rapid serologic TB tests (Kang, 2007) (that use various antigenic compositions to detect patients' antibodies) available in many low- and middle-income countries. These tests differ in a number of 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). The performance and reproducibility of 19 of these commercially available rapid *M. tuberculosis*-specific antibody detection tests were recently compared in a laboratory-based evaluation with 355 well-characterized archived serum samples aimed at identifying promising candidates for rapid TB diagnostics (WHO,2007). In these studies, the sensitivity of the tests ranged from 1% to 60% (mean, 27%), and the specificity ranged from 53% to 98.7%, compared against a combined reference standard of mycobacterial culture and clinical follow-up. In general, tests with a high specificity (>95%) had a very low sensitivity (0.97 to 21%). The test performance was poorer for patients with sputum smear-negative TB (P = 0.0006 for sensitivity and specificity) and for HIV-positive patients (P = <0.0001 for sensitivity; P = 0.44 for specificity). Some products showed a high variability in lot-to-lot, run-to-run, operator-to-operator, and inter-reader comparisons. Twelve of the tests (63%) were rated as easy to use and therefore appropriate for use in primary health care settings in developing countries; however, none of the assays performed well enough to replace AFB smear microscopy. A subsequent review of commercial serological tests for the diagnosis of TB currently sold and used in countries where the disease is endemic also concluded that there was a lack of evidence of effectiveness (Steingart *et al.,* 2007). Overall, commercial tests varied widely in sensitivity (10 to 90%) and specificity (47 to 100%). Accuracy was higher in smear-positive than in smear-negative specimens, and specificity was higher in healthy populations than in patients for whom TB disease was initially suspected and subsequently ruled out. There were insufficient data to determine the accuracy of most commercial tests in smear-negative patients, and none of the assays performed well enough to replace AFB smear microscopy. Also, there were no studies of commercial tests of sufficient quality to enable their evaluation for HIV-positive patients or for children, the groups for which these tests could be most useful. Thus, it was concluded that these tests must be considered to have little to no role in the diagnosis of TB at this time. The choice of immune biomarkers for the diagnosis of TB has possibly been biased for decades by the panels of antigens and antibodies preselected for analysis. Progress in antibody detection has also been limited by the heterogeneity of host immunological responses to TB antigens. Furthermore, the profile of antigenic proteins of *M. tuberculosis* recognized by antibodies differs at different stages of infection and disease progression. Current research on new biomarkers is exploiting the availability of advanced technological platforms that allow an interrogation of all proteins that can be synthesized by viable TB organisms. It is

expected that these new approaches can provide the basis for novel diagnostic biomarkers to more reliably diagnose active TB. Almost certainly, accurate diagnostic tests for TB will need to be based on a combination of biomarkers to increase their predictive value (Stürenburg and Junker, 2009).

Gamma interferon release assays. (IGRAs)

One-third of the world's population is estimated to be infected with latent tuberculosis (LTBI). This infection will lead to active disease in 10% of these individuals during their lifetimes; however, if the infected individuals are immunocompromised (e.g., HIV infected), 8 to 10% of them will develop tuberculosis disease within a year (Dye *et al.,* 1999). Therefore, it is imperative to accurately diagnose and treat patients with LTBI and also to predict who among the infected will develop the disease. The currently used tuberculin skin test (TST) is quite inexpensive and has been used worldwide for many years. The TST measures a delayed-type hypersensitivity response to purified protein derivative (PPD), a crude mixture of antigens from the members of the MTBC (and also NTM). Unfortunately, the TST has a low sensitivity (e.g., in patients with either immune suppression or very advanced disease) and a low specificity (e.g., in BCG-vaccinated individuals or in NTM-exposed populations) (Iwamoto *et al*., 2003). Also, the administration and reading of the TST require a certain amount of expertise that, when lacking, may result in erroneous interpretations. The recent introduction of gamma interferon (IFN-γ) release assays (IGRAs) has provided an alternative test for the diagnosis of LTBI. Currently, two commercial assays are available, the Quantiferon-TB assay (Cellestis Ltd., Carnegie, Victoria, Australia) and the T SPOT-TB assay (Oxford Immunotec, Oxford, United Kingdom) (Lalvani *et al.,* 2005). These tests measure the IFN-γ release from T cells after stimulation by *M. tuberculosis*-specific antigens via an enzyme-linked immunosorbent assay (ELISA) or an enzyme-linked immunospot (ELISPOT) assay. The first-generation Quantiferon-TB assay is a whole-blood test that measures IFN-γ release to PPD with an ELISA; this test was approved by the FDA (Mazurek and Villarino, 2003). The Quantiferon-TB Gold test is the enhanced form of the assay, which uses the *M. tuberculosis*-specific ESAT6 and CFP10 antigens instead of PPD. Subsequently, this test and the T SPOT-TB assay also received FDA approval (Mazurek, *et al.* 2005). An even newer version of the test is the Quantiferon-TB Gold in Tube assay, which entails simpler sample preparation and is further enhanced by the addition of the TB7.7 (p4) antigen. The T SPOT-TB assay, which requires the separation of peripheral blood mononuclear cells, detects IFN-γ release after exposure to ESAT6 and CFP10 with an ELISPOT assay. A major drawback of either method is that the incubation with the antigens must be initiated within 8 to 16 h following blood collection. According to a recent systematic review of the performance of these commercially available assays as well as a significant number of in-house assays, IGRAs that use a cocktail of *M. tuberculosis*-specific antigens may offer several advantages over conventional TST. These advantages have higher sensitivity and specificity, better correlation with exposure to the MTBC organism, lower cross-reactivity with BCG and NTM, and the potential to identify individuals with LTBI who are at an elevated risk of developing active disease (Pai *et al.,* 2004). However, in the absence of a true gold standard, the reliable determination of sensitivity and specificity is very difficult to predict. In addition, there is not yet adequate evidence for the accuracy of the IFN-γ tests for specific populations, including HIV-infected or other immunocompromised patients, children, extrapulmonary tuberculosis cases, MDR tuberculosis cases, or patients with NTM infections, or for monitoring patient responses to treatment (Richeldi, 2006). Their cost-benefit is not well proven, although two recent studies indicated that IGRAs may be cost-effective and positively affect the control of the disease (Wrighton-Smith and Zellweger, 2006). Therefore, the potential use of these assays in the clinical routine awaits further confirmatory studies, especially in high-incidence, resource-poor settings. It is also likely that IGRAs may be more appropriately performed in the routine clinical laboratory than in a TB laboratory. Interferon gamma release assays detect cellular immune responses to proteins that are specific for latent TB infection. If a patient is infected with TB, their immune cells will release IFN-gamma in response to stimulation by TB antigens. The results of interferon gamma release assays are based on the amount of IFN-gamma that is released in response to these antigens (Diel *et al*.,2006).

Advantages:
Very high specificity and much less likely than the tuberculin skin tests (TST) to be confounded by exposure to environmental mycobacteria or by prior BCG vaccination. Does not boost responses that will be measured by subsequent tests, as happens with TST. Interferon gamma release assays do not require a second clinical contact to evaluate the test result, thus potentially reducing costs to the patient. Results can be available within 24 hours. Preliminary studies indicate that at least one test (the T-SPOT.TBR test) may have better overall sensitivity than the TST and better sensitivity for HIV-infected people and patients with extrapulmonary TB than the TST. Interferon gamma release assays are frequently packaged in easy-to-use kits.

Detection of lipoarabinomannan (LF-LAM)
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 (Wood *et al.,* 2012). 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 (Lawn, 2012). 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. (Hamasur *et al.,* 2001). Preliminary reports showed a sensitivity of 93% and a specificity of 95%.TB LAM Ag test (LF-LAM) is a urine test for the detection of LAM antigen, a lipopolysaccharide present in mycobacterial cell walls, which is released from metabolically active or degenerating bacterial cells. LAM appears to be present predominately in people with active TB disease. The test is performed manually by applying 60 μL of urine to the Determine™ TB LAM Ag test strip and incubating at room temperature for 25 minutes. The strip is then inspected by eye. The intensity of any visible band on the test strip is graded by comparing it with the intensities of the bands on a manufacturer-supplied reference card. The reference card includes four bands (grade 1 representing a very low intensity band to grade 4 representing a high/dark intensity band) (Ramachandran and Paramasivan, 2003).

Advantages of LF-LAM
Urine-based testing has advantages over sputum-based testing because urine is easy to collect and store, and lacks the infection control risks associated with sputum collection. Presence of LAM in urine is indirectly related to human immune response, and its detection process is amenable to inexpensive point of care platforms. Owing to suboptimal sensitivity and specificity, current urinary LF-LAM assays are deemed unsuitable as a general screening or diagnostic test for TB. Unlike traditional TB diagnostic methods, however, LF-LAM demonstrates improved sensitivity in seriously ill HIV infected individuals, especially in those with low CD4 counts.

Adenosine deaminase (ADA)
Adenosine deaminase (ADA) has been proposed to be a useful surrogate marker for tuberculosis in pleural, pericardial and peritoneal fluids (Mathur *et al.,* 2006). The results of a study performed in India revealed a sensitivity of 100%, a specificity of 94.6% and a cutoff value of 40 U/L for ADA in pleural, peritoneal and pericardial fluids (Mathur *et al.,* 2006).

Detection of MTB in pediatric stool samples using TruTip technology
The World Health Organization estimates that one million new pediatric tuberculosis (TB) cases and 194,000 childhood TB -related deaths occurred in 2017 (WHO, 2018). Rapid case detection and treatment initiation is critical to minimizing TB morbidity and mortality in children but is hampered by the absence of a rapid, accurate diagnostic tool for this group. Bacteriologic confirmation of Mycobacterium tuberculosis (Mtb) in children is often difficult to achieve because they are frequently unable to expectorate sputum for bacteriologic testing and often have paucibacillary disease that cannot be detected using sputum smear microscopy, culture, and/or molecular testing [e.g. Xpert (Cepheid, Sunnyvale CA, USA)]. Sputum induction and gastric

aspiration can be used to obtain respiratory specimens from children unable to expectorate sputum; however, gastric aspiration is invasive and neither procedure is widely implemented in resource-constrained settings. Due to these diagnostic challenges, bacteriologic confirmation of TB is obtained in only a small minority of children diagnosed with TB (Banada *et al*., 2016, Starke *et al*., 2003). Stool can be easily obtained from most children and Mtb can be detected in stool using Xpert or other laboratory-developed PCR assays (Dinardo *et al.,* 2018). Tru- Tip workstation in combination with real-time IS6110 PCR can be used for detection of Mtb in pediatric stool samples. The TruTip workstation is an automated platform including lysis and homogenization with TruTip nucleic acid extraction and purification (Akonni Biosystems, Frederick, MD, USA). TruTip has been used for nucleic acid isolation from a variety of pathogens and sample types and has demonstrated efficient Mtb DNA recovery from raw sputum (Thakore *et al.,* 2018). The platform can be connected to a closed amplicon system for amplification and microarray-based detection of Mtb as well as a number of drug resistance associated mutations (Chandler *et al.,* 2012). Akonni TruTip is not yet commercially produced. The TruTip workstation had a number of attractive attributes for Mtb DNA extraction from stool. Extraction procedures required limited pipetting steps compared to commercially available kits. Additional future advantages include that the extracted DNA eluate can be isolated for other purposes, such as sequencing. Most importantly, the TruTip workstation can be integrated with a microarray-based detection of Mtb as well as a number of drug resistance-associated mutations (Chandler *et al*., 2012).

MTB urease test

MTB urease is a bacterial virulence factor. Isotopically labelled urea as substrate, Urea tracer has detected in exhaled breath using portable infrared spectrophotometer. Signal correlated with bacteria load (Jassal *et al.,* 2010).

Biophotonic detection platform

Biophotonic detection platform has been developed that utilizes reporter enzyme fluorescence to detect β-lactamase produced by MTB. This innovative new technology is now being adapted for point of care (POC) use (Arafa *et al.,* 2021).

Immunological Biomarkers

Cytokines and chemokines that have been extensively studied in relation with their potential as diagnostic and prognostic biomarker of tuberculosis includes; IL-2, IL-6, and IL-10, IL-4, IL-12 in active TB diagnosis. IP-10 (inducible protein -10) in immunocompromised individuals and children. IFNγ/IL-4 ratio, IL-17 and TNF in latent and active TB diagnosis. These are presently been deployed as point of care (POC) for management of TB patient (Walzl *et al.,* 2011). However, these biomarkers are expensive for routine diagnosis and management of TB patients, lacks standardization, inconsistent across laboratories and cross reactions across array of infections (Goletti *et al.,* 2010).

Rapid whole-genome sequencing

Whole-genome sequencing (WGS) has been shown to provide a rapid and comprehensive view of the genotype of *M. tuberculosis*. WGS can be successfully performed directly from uncultured sputum. Allows simultaneous identification of all known resistance-associated loci with high concordance to culture-based drug susceptibility testing (DST). With an average resistance-predictive value of 93% and susceptible-predictive value of 96% for 8 drugs (RIF, INH, FLQ, PZA, KAN, EMB, STR, ETH). High costs associated with the test, high technical skill required, complex bioinformatic procedures and the unavailability of sequencing facilities are the major drawback. There are currently no plans for routine implementation of WSG in resource-limited, high-TB burden countries (Nimmo *et al.,* 2017).

Conclusion

Building capacity and enhancing universal access to rapid and accurate laboratory diagnostics are necessary to control TB and HIV-TB infection. This paper has described several new and established methods as well as some of the issues associated with implementing quality TB laboratory services. These proven methods have already shown their potential to significantly improve case detection and management of patients, including drug-resistant TB cases, and enhance the identification of the disease in HIV-positive individuals. However, it is important to realize

that at present, there is no stand-alone test for the rapid detection of tuberculosis in all patients. While some new techniques are simple, others have complex requirements, and therefore, it is vital to carefully determine how to link the new laboratory tests together and incorporate them within a country's national TB diagnostic algorithm, taking into account factors such as the capacity of different levels of the tiered health care system and the opportunity of decentralizing laboratory services that is possible through the recent improvements in integrated molecular testing.

Recommendations

Because TB is a complex disease to diagnose and manage due to the chronicity of the disease, the nature of the host-pathogen relationship, and the resulting diversity in its clinical manifestations, we therefore, recommend the followings:

1. TB clinical diagnosis should be based on at least two methods of laboratory analysis.
2. The appropriate molecular method of laboratory diagnosis should be used for treatment management and follow-up of patients.
3. Rapid diagnostic test should be used in peripheral or resource limited health centres as screening test only. Then, sophisticated and confirmatory analysis should follow in a referral laboratory.
4. There is the urgent need for government at all levels to collaborate in scaling up diagnosis of TB in order to reduce the burden of the disease and or eradicate it.

References

Arafa, H.A., Doaa, M. and Zaky, A. Z. (2021). Novel Biosensor Detection of Tuberculosis Based on Photonic Band Gap Materials January 2021 *Materials Research* **24(3)** DOI:10.1590/1980-5373-mr-2020-0483.

Kanayeva D, Bekniyazov I, Ashikbayeva Z. Detection of tuberculosis using biosensors: recent progress and future trends. Sensors & Transducers. 2013;149:166.

21. WHO: World Health Organization. Global tuberculosis report. Geneva: WHO; 201

Kanayeva D, Bekniyazov I, Ashikbayeva Z. Detection of tuberculosis using biosensors: recent progress and future trends. Sensors & Transducers. 2013;149:166.

21. WHO: World Health Organization. Global tuberculosis report. Geneva: WHO; 201

Srivastava SK, Van Rijn CJ, Jongsma MA. Biosensor-based detection of tuberculosis. RSC Advances. 2016;6:17759-71.

Srivastava SK, Van Rijn CJ, Jongsma MA. Biosensor-based detection of tuberculosis. RSC Advances. 2016;6:17759-71.

Banada, P.P., Naidoo, U., Deshpande, S., Karim, F., Flynn, J.L., O'Malley, M. (2016). A novel sample processing method for rapid detection of tuberculosis in the stool of pediatric patients using the Xpert MTB/RIF assay. *PLoS One*; **11(3)**:1–8.

Bhargava, A., Jain, A., & Agrawal, S.K. (2007). A comparison of Liquid and Solid Culture media with Radiometric system for detection of Mycobacteria in Clinical specimens. *Indian Journal of Tuberculosis;* **14:1024**—1031.

Boehme, C.C., Nabeta, P., Hillemann, D., Nicol, M.P., Shenai, S., & Krapp, F. (2010). Rapid molecular detection of tuberculosis and rifampin resistance. *New England Journal of Medicine;* **363(11)**:1005–1015.

Bradley, S.P., Reed, S.L., & Catanzaro, A. (1996). Clinical efficacy of the amplified *Mycobacterium tuberculosis* direct test for the diagnosis of pulmonary tuberculosis. *American Journal of Respiratory and Critical Care Medicine;* **153(5)**:1606–1610.

Catanzaro, A., Perry, S., Clarridge, J.E., Dunbar, S., Goodnight-White, S. & LoBue, P.A. (2000). The role of clinical suspicion in evaluating a new diagnostic test for active tuberculosis: results of a multicenter prospective trial. *Journal of American Medical Association*; **283(5)**:639–645.

Cegielski, J.P., & McMurray, D.N. (2004). The relationship between malnutrition and tuberculosis: evidence from studies in humans and experimental animals. *International Journal of Tuberculosis and Lung Diseases;* **8**:286–292.

Chandler, D., Bryant, L., Griesemer, S., Gu, R., Knickerbocker, C., & Kukhtin, A., (2012). Integrated amplification microarrays for infectious disease diagnostics. *Microarrays*; **1(3)**:107–124.

Chihota, A. D., Grant, K., Fielding, B., Ndibongo, A., van Zyl, D., & Muirhead, G.

J., (2010). Liquid vs. solid culture for tuberculosis: performance and cost in a resource constrained setting. *International Journal of Tuberculosis and Lung Diseases;* **14:**1024 1031.

Clarridge, J.E., Shawar, R.M., Shinnick, T.M., & Plikaytis, B.B., (1993). Large-scale use of polymerase chain reaction for detection of *Mycobacterium tuberculosis* in a routine mycobacteriology laboratory. *Journal of Clinical Microbiology;***31(8)**:2049–2056.

Diane, H.V., & Barnes, P.F., (1999). Tuberculosis in Patients with Human Immunodeficiency Virus Infection. *The New England Journal of Medicine;* **340**:367-373.

DiNardo, A.R., Kay, A.W., Maphalala, G., Harris, N.M., Fung, C., & Mtetwa, G., (2018). Diagnostic and treatment monitoring potential of a stool-based quantitative polymerase chain reaction assay for pulmonary tuberculosis. *American Journal of Tropical Medicine and Hygiene*;**99(2)**:310–316.

Diel, R., Nienhaus, A., Lange., C., & Schaberg, T., (2006). Cost-optimization of screening for latent tuberculosis in close contacts. *European Respiratory* Journal; **28**:35–44.

Dye, C., Scheele, S., Dolin, P., Pathania, V., & Raviglione, M. C., (1999). Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. *Journal of American Medical Association*; ***2*82:**677–686.

EDMA. Tuberculosis Fact Sheet. (2007) In Vitro Diagnostics. Making a real difference in health & life quality. European Diagnostic Manufacturers Association. 2007. Available at: http://www.vdgh.de/media/file/ 201.6_anlage-1f-tuberculosis-fact-sheet-jul07.

Goletti, D., Raja, A., Syed, A., & Kabeer, B., (2010). IP-10 as accurate marker for detecting M. tuberculosis specific response in HIV-infected person. *Plos One:* e12577.

Hamasur, B., Bruchfeld, J., Haile, M., Pawlowski, A., Bjorvatn, B., & Kallenius, G. (2001). Rapid diagnosis of tuberculosis by detection of mycobacterial lipoarabinomannan in urine. *Journal of Microbiological Methods*;**45(1)**:41–52.

Hopewell, P.C. (1992). Impact of Human Immunodeficiency Virus infection on the clinical features, management and control of Tuberculosis. *Journal of Clinical Infectious Diseases;* **15:** 540-547.

Huebner, R. E., Schein, M. F., & Bass, J. B. (1993). The tuberculin skin test. *Clinical Infectious Diseases;* **17:**968–975.

Jassal, M, Nedeltchev, G, Lee, J.H, Choi, S.W, Timmins, G.S, and Bishai, W. (2010). ^{13}[C]-urea breath test as a novel point-of-care biomarker for tuberculosis treatment and diagnosis. *PLoS One*;**5**: e12451.

Karp, C.L., & Auwaerter, P.G. (2007). Coinfection with HIV and tropical infectious diseases. I. Protozoal pathogens. *Clinical Infectious Diseases;* **45**:1208–1213.

Lalvani, A., Richeldi, L., & Kunst, H. (2005). Interferon gamma assays for tuberculosis. *Lancet Infectious Diseases;* **5**:322–324.

Lawn, S.D. (2012). Point-of-care detection of lipoarabinomannan (LAM) in urine for diagnosis of HIV-associated tuberculosis: a state-of-the-art review. *BMC Infectious Diseases*;**12**: 103- 106.

Linger, Y., Kukhtin, A., Golova, J., Perov, A., Lambarqui, A., & Bryant, L., (2014). Simplified microarray system for simultaneously detecting rifampin, isoniazid, ethambutol, and streptomycin resistance markers in mycobacterium tuberculosis. *Journal of Clinical Microbiology*;**52(6)**:2100–2107.

Mathur, P., Tiwari, K., Trikha, S., & Tiwari, D. (2006). Diagnostic value of adenosine deaminase (ADA) activity in tubercular serositis. *Indian Journal of Tuberculosis*;**53**: 92–95.

Mazurek, G.H. (2005). Guidelines for using the QuantiFERON-TB Gold test for detecting Mycobacterium tuberculosis infection, United States. MMWR Recommend. *Reports;* **54(RR15)**:49–55.

Mazurek, G.H., & Villarino, M.E. (2003). The Centers for Disease Control and Prevention. Guidelines for using the QuantiFERON-TB test for diagnosing latent Mycobacterium tuberculosis infection. MMWR Recommend. *Reports;* **52(RR2)**:15–18.

Newton, S., Brent, A., Andersonm S., Whittaker, E., & Kampmann, B. (2008). *Paediatric Tuberculosis. Lancet Infectious Diseases;**8(8)**:498–510.*

Nimmo, C., Doyle, R., Burgess, C., Williams, R., Gorton, R., & McHugh, T.D. (2017). Rapid identification of a *Mycobacterium tuberculosis* full genetic drug resistance profile through whole genome sequencing directly from sputum. *International Journal of Infectious Diseases*;**62**: 44–46.

Oberhelman, R.A., Soto-Castellares, G., Caviedes, L., Castillo, M.E., Kissinger, P., & Moore, D.A. (2006). Improved recovery of mycobacterium tuberculosis from children using the microscopic observation drug susceptibility method. *Pediatrics*;**118(1)**: e100–106.

Ogbaini-Emovon, E. (2009). Current trends in the laboratory diagnosis of tuberculosis. *Benin Journal of Postgraduate Medicine;* **11:79**–90.

Pai, M., Flores, L.L., Hubbard, A., Riley, L.W., & Colford, J.M., (2004). Nucleic acid amplification tests in the diagnosis of tuberculous pleuritis: a systematic review and meta-analysis. *BMC Infectious Diseases;***4:** 6-10.

Pai, M., Flores, L.L., Pai, N, Hubbard, A., Riley, L.W., & Colford, J.M Jr., (2003). Diagnostic accuracy of nucleic acid amplification tests for tuberculous meningitis: a systematic review and meta-analysis. *Lancet Infectious Diseases*; **3(10)**:633–643.

Pai, M., Riley, L. W., & Colford, J. M. (2004). Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. *Lancet Infectious Diseases*; **4**:761–776.

Ramachandran, R., & Paramasivan, C. (2003). What is new in the diagnosis of tuberculosis? Part 1: Techniques for diagnosis of tuberculosis. *Indian Journal of Tuberculosis;* **(50)**:133–141.

Ranjan, K., & Sharma, M. (2010). An approach to the detection of mycobacteria in clinically suspected cases of urinary tract infection in immunocompromised patients. *Webmed Central Bacteriology*; **9(1)**: WMC00616.

Richeldi, L. (2006). An update on the diagnosis of tuberculosis infection. *American Journal of Respiratory and Critical Care Medicine; **174**:736–742.*

Roggenkamp, A., Hornef, M.W., Masch, A., Aigner, B., Autenrieth, I.B., & Heesemann, J. (1999). Comparison of MB/BacT and BACTEC 460 TB systems for recovery of mycobacteria in a routine diagnostic laboratory. *Journal of Clinical Microbiology;* **37(11)**:3711–3712.

Starke, J.R. (2003). Pediatric tuberculosis time for a new approach. *Tuberculosis*. **83(1–3)**:208-212.

Steadham, J.E. (1979). Reliable urease test for identification of mycobacteria. *Journal of Clinical Microbiology*; **10**:134–137.

Scarparo, C., Ricordi, P., Ruggiero, G., & Piccoli, P. (2004). Evaluation of the fully automated BACTEC MGIT 960 system for testing susceptibility of *Mycobacterium tuberculosis* to pyrazinamide, streptomycin, isoniazid, rifampin, and ethambutol and comparison with the radiometric BACTEC 460TB method. *Journal of Clinical Microbiology;* **42(3)**:1109–1114.

Seth, V., & Kabra, S. (2006). Essentials of Tuberculosis in Children. Jaypee Brothers Medical Publishers; New Delhi: 132-135.

Steingart, K. R., & Ramsay, A., (2007). Commercial serological antibody detection tests for the diagnosis of pulmonary tuberculosis: *a systematic review. PLoS Medical;* **4**: e202.

Steingart, K.R., Ramsay, A., & Pai, M., (2007). Optimizing sputum smear microscopy for the diagnosis of pulmonary tuberculosis. *Expert Review in Anti-Infectious Therapy;* **5**:327–331.

Stürenburg, E., & Junker, R., (2009). Point-of-care testing in microbiology: the advantages and disadvantages of immunochromatographic test strips. *Dtsch Arztebl International;* **106**:48–54.

Thakore, N., Norville, R., Franke, M., Calderon, R., Lecca, L., & Villanueva, M. (2018). Automated TruTip nucleic acid extraction and purification from raw sputum. *PLoS One;***13(7)**: e0199869.

UNAIDS 2009. AIDS epidemic update. UNAIDS, Geneva, Switzerland: http://www.unaids.org/en/dataanalysis/epidemiology/2009aidsepidemicupdate/

Venkataraman, P., Herbert, D., & Paramasivan, C.N. (1998). Evaluation of the BACTEC radiometric method in the early diagnosis of tuberculosis. *Indian Journal of Medical Research*; **108**:120–127.

Walzl, G., Ronacher, K., & Hanekom, W. (2011). Immunological biomarkers of tuberculosis. *Nature Review in Immunology*; **11:343**-354.

Wood, R., Racow, K., Bekker, L.G., Middelkoop, K., Vogt, M., & Kreiswirth, B.N. (2012). Lipoarabinomannan in urine during tuberculosis treatment: association with host and pathogen factors and mycobacteriuria. *BMC Infectious Diseases*; **12**:47-54.

WHO (2006). Diagnostics for Tuberculosis: Global Demand and Market Potential. Special Programme for Research and Training in Tropical Diseases (TDR. 2006).

World Health Organization, (2010). WHO endorses new rapid tuberculosis test.

World Health Organization, (2018). Laboratory-based evaluation of 19 commercially available rapid diagnostic tests for tuberculosis. Special Program for Research and Training in Tropical Diseases. Diagnostics evaluation series, 2. World Health Organization, Geneva, Switzerland.

World Health Organization, (2019). Global tuberculosis control: a short update to the 2009 report. WHO/HTM/TB/2009.426. World Health Organization, Geneva, Switzerland.

WHO (2013). Monitoring of Xpert MTB/RIF rollout" World Health Organization. **48:** 9-12.

World Health Organization, (2020). Global Tuberculosis Report.

Wolf, H., Mendez, M., Gilman, R.H., Sheen, P., Soto, G., & Velarde, A.K. (2008). Diagnosis of pediatric pulmonary tuberculosis by stool PCR. *American Journal of Tropical Medicine and Hygiene*; **79(6)**:893–898.

Wrighton-Smith, P., & Zellweger, J.P. (2006). Direct costs of three models for the screening of latent tuberculosis infection. *European Respiratory Journal;* **28**:45–50.

Citation: Obadire, S.O., Mitsan, O., Ige, I.P., Oke, O.C., Odewusi, F.O. Current Innovations in Medical Laboratory Diagnosis of Tuberculosis. ***Sokoto Journal of Medical Laboratory Science*; 7(3):60 - 70.**
https://dx.doi.org/10.4314/sokjmls.v7i3.7