Diagnosis and Management of Tuberculosis

Chapter 6

Diagnosis of Tuberculosis

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1. Introduction

Tuberculosis (TB) laboratories play a critical part in national TB programmes, providing clinicians with invaluable information that is used to diagnose and guide the care of patients. Because of the specialized nature of the different technical procedures needed to diagnose TB, and the need for quality assurance and effective laboratory management, TB control programmes require a tiered network of laboratories in which different tiers use complementary diagnostic tools and mechanisms for referring specimens [1].

Diagnostic capacity continues to be a major bottleneck in TB control, including scaling up management and control efforts to tackle drug resistant TB and TB associated with HIV. An unprecedented effort to improve and expand the capacity of TB laboratories is under way, coordinated by the World Health Organization's (WHO's) Global TB Programme and with the active involvement of the Global Laboratory Initiative (GLI), a working group of the Stop TB Partnership [1].

WHO's global strategy for TB prevention, care and control for 2015–2035 (known as the End TB Strategy) prioritizes the early diagnosis of TB, which should include the universal availability of DST, and systematic screening of contacts and high-risk groups [1,2]. Therefore, all national TB control programmes should prioritize the development of a robust network of TB laboratories that have adequate biosafety standards, use modern methods of diagnosis, use standard operating procedures (SOPs) and appropriate quality assurance processes, and that have qualified and sufficient human resources; these priorities should be comprehensively addressed in national strategic plans [1].

Overall, the development landscape for TB diagnostics is promising: many different organizations are developing products, and there is a robust pipeline of technologies. The

range of technologies that may replace sputum-smear microscopy continues to expand, and smaller, simpler and more robust products are expected to become available in the coming years. Several technologies aim to deliver results in less than 1 hour, including DST results; this should improve the time to treatment, enable point-of-care testing programmes and provide greater access to DST [1,3].

Until these new technologies become available, the use of existing WHO-recommended diagnostic techniques must be accelerated and strengthened. This will require ensuring that laboratories have adequate infrastructure and human-resources capacity. Additionally, there must be clear country-level policies on using these recommended tests in the most effective screening and diagnostic algorithms, depending on each country's specific epidemiology and resources [1].

2. Tuberculosis Diagnostic Tools/Methods

2.1. WHO's recommended techniques for diagnosing TB

2.1.1. Microscopy

Mycobacteria can be visually distinguished from other microorganisms by their thick lipid containing cell walls, which retain biochemical stains despite decolourization by acid-containing reagents (known as 'acid fastness'). Given that the examination of two sputum specimens is adequate to identify the majority (95-98%) of smear-positive TB patients, WHO's current policy on case-finding using microscopy recommends that in settings with appropriate external quality assessment and documented good-quality microscopy two specimens should be examined. In settings with appropriate quality assurance procedures, a case is defined as someone with one positive smear – that is, at least 1 acid-fast bacillus in at least 100 microscopic fields [4].

In 2010, WHO confirmed the diagnostic accuracy of examining two consecutive smears on the same day to diagnose TB, so that treatment can be started during the patient's first visit to a health-care facility [5].

Advantages

- Microscopy of sputum smears is simple and inexpensive, and allows rapid detection of the most infectious cases of pulmonary TB. Sputum specimens from patients with pulmonary TB, especially those with cavitary disease, often contain sufficiently large numbers of AFB to be detected by microscopy.
- Microscopy is suitable for peripheral-level and higher-level laboratories.
- Microscopy can be done safely in a laboratory that has implemented only a low level of

precautions to mitigate the risk of laboratory acquired TB infection.

- It is a simple, rapid and inexpensive test and is necessary for treatment follow up of patients with susceptible TB.

Disadvantages

- Direct sputum-smear microscopy is relatively insensitive: at least 5000 bacilli per ml of sputum are required for a positive result. The sensitivity is further reduced in patients with extrapulmonary TB, children and in those who are co infected with HIV.

- A comprehensive quality assurance programme is necessary; although this may be challenging to implement, it is necessary to ensure high-quality test results.

Limitations

- Microscopy for AFB cannot distinguish Mycobacterium tuberculosis complex from non tuberculous mycobacteria; it cannot distinguish viable from nonviable organisms; and it cannot distinguish drug-susceptible strains from drug-resistant strains.

- Smears that have been stained with auramine will need to be stained again if they are to be rechecked as part of an external quality assessment programme.

1. ***Conventional light microscopy***

Direct Ziehl–Neelsen staining of sputum specimens and examination using light microscopy is suitable for use at all levels of laboratory, including peripheral laboratories at primary health-care centres or district hospitals. There is insufficient evidence that processed sputum specimens (for example, those that are concentrated or chemically treated) give better results than direct smear microscopy. Therefore, the use of such methods is not recommended [6].

The number of Ziehl–Neelsen smears examined by 1 microscopist each day should not exceed

20–25 because visual fatigue can occur and lead to a deterioration in quality.

In general, it is sufficient for there to be 1 centre using Ziehl–Neelsen staining and light microscopy per 100 000 population;[7] however, if services are expanded, then it is important to consider the location of the centre, the workload of the technicians, the accessibility of the centre to the population as well as the effectiveness of specimen transportation.

2. *Light-emitting diode fluorescence microscopy*

Light-emitting diodes (LEDs) provide a relatively inexpensive light source for fluorescence microscopy. LED microscopes or attachments require less power than conventional fluorescence microscopes and can run on batteries. Also, the bulbs have a long half-life and do not release potentially toxic products if they are broken.

Evidence shows that the diagnostic accuracy of LED microscopy is comparable to that of conventional fluorescence microscopy and it surpasses that of conventional Ziehl–Neelsen microscopy (by an average of 10%). Therefore, WHO recommends replacing conventional fluorescence microscopy with LED microscopy, and that LED microscopy should be phased in as an alternative to conventional Ziehl–Neelsen light microscopy in all settings, prioritizing high-volume laboratories [8].

2.1.2. Culture and species identification

Mycobacteria can be cultured in specific solid or liquid media. Bacterial growth can be identified visually (that is, by identifying specific characteristics) or by automated detection of its metabolism. All positive mycobacterial cultures must be tested to confirm the identification of *M. tuberculosis* complex (MTBC).

The *M. tuberculosis* complex comprises eight distinct closely related organisms, the most common and important agent of human disease is *M. tuberculosis*. The complex includes *M. bovis* (the bovine tubercle bacillus—characteristically resistant to pyrazinamide, once an important cause of TB transmitted by unpasteurized milk, and currently the cause of a small percentage of human cases worldwide), *M. caprae* (related to M. bovis), *M. africanum* (isolated from cases in West, Central, and East Africa), *M. microti* (the "vole" bacillus, a less virulent and rarely encountered organism), *M. pinnipedii* (a bacillus infecting seals and sea lions in the Southern Hemisphere and recently isolated from humans), *M. mungi* (isolated from banded mongooses in southern Africa), *M. orygis* (described recently in oryxes and other Bovidae in Africa and Asia and a potential cause of infection in humans), and *M. canetti* (a rare isolate from East African cases that produces unusual smooth colonies on solid media and is considered closely related to a supposed progenitor type) [9].

Differentiation of the members of the MTBC is necessary for the treatment of individual patients and for epidemiological purposes, especially in areas of the world where tuberculosis has reached epidemic proportions or wherever the transmission of *M. bovis* between animals or animal products and humans is a problem. In addition, it can be important to rapidly identify isolates of *M. bovis* bacillus Calmette-Guérin (BCG) recovered from immune compromised patients. Differentiation of species with the MTBC can be achieved using either phenotypic [10] and/ or genotypic methods [11].

The use of rapid immune chromatographic assays (or strip tests for speciation) to identify cultured isolates is recommended because they provide definitive identification of all members of the MTBC (including *M. bovis*) in 15 minutes [12].

Much remains to be understood about the pathogenesis of non-tuberculosis mycobacteria (NTM) infection and disease in humans. There is no evidence to suggest either animal-to-human or human-to-human transmission of NTM and it is assumed that most persons are infected by NTM from the environment. NTM may cause both asymptomatic infection and symptomatic disease in humans. Several factors increase the likelihood of clinical significance of NTM isolates, including the recovery from multiple specimens or sites, recovery of the organism in large quantities (AFB smear–positive specimens), or recovery of an NTM isolate from a normally sterile site such as blood.

Awareness of the context from which an NTM isolate is obtained can be critically important in determining the need for speciation of that isolate.

Hence, communication between the clinician and laboratorian is essential for determining the importance and extent of identification and for drug susceptibility testing of an NTM isolate. Differentiation of NTM species can be achieved busing a variety of phenotypic or genotypic Methods [13]. Conventional solid or liquid culture is required to monitor the treatment of patients with MDR-TB.

Advantages

- Culture and identification of *M. tuberculosis* provide a definitive diagnosis of TB as well as significantly increasing in the number of cases identified when compared with microscopy: there is often an increase of 30–50%.

- Culture also provides the necessary isolates for conventional DST.

Disadvantages

- Culture is more complex and expensive than microscopy; it also takes longer, requiring facilities for preparing media, processing specimens and encouraging the growth of organisms.

- Culture also requires specific laboratory equipment, technicians with additional skills, and appropriate bio safety conditions.

Limitations

- Specimens must be decontaminated before culture to prevent overgrowth by other microorganisms. To some extent, all decontamination methods are also harmful to mycobacteria;

therefore, culture is not 100% sensitive.

- Good laboratory practices must maintain a delicate balance between the yield of mycobacteria and contamination by other microorganisms.

- Solid and liquid culture methods are suitable for central reference laboratories (regional laboratories in large countries) or intermediate level laboratories. Solid culture methods are less expensive than liquid, but the results are invariably delayed because of the slow growth of mycobacteria. Liquid culture increases the case yield by approximately 10% over solid media, and automated systems reduce the diagnostic delay to days rather than weeks. However, liquid systems are more prone to contamination, and the manipulation of large volumes of infectious material mandates appropriate additional bio safety measures [14].

2.1.3. Drug-susceptibility testing

DST determines whether a strain is susceptible to particular anti-TB agents: a result indicating that the strain is sensitive to particular agents means that treatment with those agents will most likely be successful; a result indicating that a strain is resistant means that there is a high possibility that treatment with those agents will fail and, therefore, other agents should be used. Thus, using standardized and reliable DST for *M. tuberculosis* provides guidance on treating a patient.

Techniques of drug susceptibility testing

- **Phenotypic methods** involve culturing *M. tuberculosis* in the presence of anti-TB agents to detect growth (which indicates resistance) or inhibition of growth (which indicates susceptibility).

- **Genotypic methods** target specific molecular mutations associated with resistance against individual anti-TB agents. Phenotypic DST methods are performed as direct or indirect tests in solid or liquid media.

Direct testing involves inoculating drug-containing and drug-free media directly with a concentrated specimen.

Indirect testing involves inoculating drug-containing media with a pure culture grown from the original specimen.

Indirect phenotypic tests have been extensively validated. Three methods are commonly used: the proportion, absolute concentration and resistance ratio methods. For first-line anti-TB agents, the results obtained do not differ significantly among the three methods. Liquid culture systems for DST reduce the time to result to as little as 10 days, compared with the 28–42 days needed for conventional solid media. Because liquid culture systems have increased

sensitivity and reduce delays in diagnosis, they may contribute significantly to improving patient management [14].

WHO recommends that formal links be established between the TB Supranational Reference Laboratory (SRL) Network and national reference laboratories to ensure that DST is available for both first-line and second-line anti-TB agents. Countries wishing to offer DST should seek advice from the TB Supranational Reference Laboratory Network to ensure they have continual, adequate expert input into the requirements for laboratory design, the transportation of specimens, processes, bio safety standards, SOPs, schedules for maintaining equipment, and processes for external quality assessment. The absence of capacity to treat patients with MDR-TB should not deter countries to build capacity for DST, as it is ethically justified [15].

Advantages

DST provides a definitive diagnosis of drug resistant TB.

Disadvantages

- Non-molecular DST methods take longer to provide results.

- These methods are suitable for use only at the central reference laboratory level, given the need for appropriate laboratory infrastructure (particularly bio safety precautions) and the technical complexity of the techniques and methods.

- Liquid DST fails to detect some clinically relevant "borderline rifampicin resistant strains" with *rpo*B mutations [16].

Limitations

- The accuracy of phenotypic DST varies according to the anti-TB agent being tested.

1) Drug-susceptibility testing for first-line anti-TB agents

DST is most accurate in detecting susceptibility to rifampicin and isoniazid; results are less reliable and reproducible for streptomycin, ethambutol and pyrazinamide.

At a minimum, national TB-control programmes should establish sufficient laboratory capacity to detect rifampicin-resistant TB (RR-TB) or MDR-TB (MDR-TB is TB that is resistant to at least isoniazid and rifampicin). In many settings and for many groups of patients, rifampicin resistance is a valid indicator of or proxy for MDR-TB. Persons at risk for MDR-TB should be targeted as a priority for rapid DST. Phenotypic culture-based DST methods, using the critical concentrations recommended by WHO in the updated table, are the current

reference standards for rifampicin resistance [17].

However, a number of recent studies have raised concerns about using phenotypic DST to detect rifampicin resistance, in particular the automated liquid system [18]. If rifampicin resistance has been detected, DST for resistance to isoniazid and second-line anti-TB agents should be performed, following WHO's recommendations [19]. WHO will be updating policy recommendations on DST in 2016.

2) Drug-susceptibility testing for second line anti-TB agents

Commercial liquid methods and the proportion method used on solid media have been studied; methods for the absolute concentration or resistance ratio methods on solid media for second line anti-TB agents have not been validated.

The recommended gold standard for DST for second-line anti-TB agents is the automated liquid system [20]. Routine DST for second-line agents is not recommended unless laboratory infrastructure and capacity have been established, rigorous quality assurance is in place and sustained proficiency has been demonstrated [21].

Phenotypic DST for second-line injectable agents (kanamycin, amikacin, capreomycin) and fluoroquinolones (ofloxacin, levofloxacin, moxifloxacin, gatifloxacin) is generally reliable and reproducible across various settings [21]. The susceptibility of *M. tuberculosis* to all fluoroquinolones used by a national TB programmes should be tested to guide the choice of the most appropriate agent for treatment.

Current molecular methods cannot replace phenotypic DST for second-line agents because there is incomplete cross-resistance among second-line injectable agents. Current molecular methods cannot identify resistance to specific second-line injectable agents; thus, they cannot be used to guide the choice of second-line agents included in individualized MDR-TB regimens [22].

Routine DST for other second-line agents (such as ethionamide, prothionamide, cycloserine, terizidone, *p*-aminosalicylic acid, clofazimine, amoxicillin/clavulanic acid, clarithromycin and linezolid) is not recommended because the reliability and reproducibility tests for these anti-TB agents cannot be guaranteed. The WHO SRL network is currently developing and validating DST methods for the new and re-purposed second-line agents (bedaquiline, delamanid, clofazimine, linezolid).

3) Non-commercial methods

Non-commercial methods of culture and DST are less expensive than commercial systems but are prone to errors due to a lack of standardization and to local variations in the methods.

The performance of these methods is highly operator-dependent; therefore, it is imperative that good laboratory practices are followed, good microbiological techniques are used, and there is adequate quality assurance, supported by adequate training. Similar to the conditions needed with commercial systems, noncommercial systems require the implementation and enforcement of stringent laboratory protocols, SOPs and internal quality controls.

The evidence base for selected non-commercial methods of culture and DST has been reviewed by WHO, and the performance of these methods has been found to be acceptable *in reference or national laboratories in selected settings only when stringent laboratory protocols are followed* [23].The methods evaluated include the microscopic observation drug-susceptibility (MODS) assay, colorimetric redox indicator (CRI) methods, and the nitrate reductase assay (NRA).

The recommendations for their use are listed below.

- **MODS** is a microcolony method that uses liquid culture. Drug-free and drug containing media are inoculated, and this is followed by microscopic examination of early growth. MODS is recommended as a direct or indirect test for rapid screening of patients suspected of having MDR-TB.

- **CRI** methods are indirect methods. A coloured indicator is added to liquid culture medium on microtitre plate after *M. tuberculosis* strains have been exposed to anti-TB agents in vitro. Resistance is detected by a change in the colour of the indicator, which is proportional to the number of viable mycobacteria in the medium. CRI methods are recommended for use as indirect tests on *M. tuberculosis* isolates from patients suspected of having MDRTB; however, the method is slower in detecting MDR-TB than conventional DST methods using commercial liquid culture and molecular LPAs, but it is less expensive.

- **NRAs** can be used as direct or indirect methods on solid culture. NRAs are based on the ability of *M. tuberculosis* to reduce nitrate, which is detected by a colour reaction. NRAs are recommended for use as direct or indirect tests to screen patients suspected of having MDR-TB; however, indirect NRA is not faster in detecting MDR-TB than conventional DST using solid culture.

- Both commercial and non-commercial culture and DST systems and methods are suitable for use only by central or regional reference laboratories. Non-commercial methods are recommended for use only as an interim option while capacity is being developed for rapid genotypic DST. Furthermore, non-commercial methods have not been validated for use with second line agents.

2.1.4. Molecular testing

Genotypic methods have considerable advantages when the programmatic management of drug-resistant TB is being scaled up, in particular with regard to their speed, the standardization of testing, their potentially high throughput and the reduced requirements for biosafety. The ultimate aim should be to use molecular assays – including LPAs, Xpert MTB/RIF, and any other molecular platform that may be recommended by WHO in the future – for rapid first-step identification of RRTB and MDR-TB.

Line-probe assays

Performing an LPA involves extracting DNA from *M. tuberculosis* isolates or directly from clinical specimens and using polymerase chain reaction **(PCR)** to amplify the resistance-determining region of the *rpo*B gene using biotinylated primers. Subsequently, labelled PCR products are hybridized with specific oligonucleotide probes immobilized on a strip. Colorimetric development of the captured and labelled hybrids enables the presence of *M. tuberculosis* complex to be detected as well as the presence of wildtype *M. tuberculosis*. It also detects mutations associated with drug resistance. If a mutation is present in one of the target regions, the amplicon will not hybridize with the relevant probe.

Therefore, mutations are detected by a lack of binding to wild-type probes as well as by binding to specific probes for the most commonly occurring mutations. The post-hybridization reaction leads to the development of coloured bands on the strip at the site of probe binding, and it can be read by the laboratory technician [23]. In 2015, WHO plans to update the policy recommendations on LPA for the detection of rifampicin resistance conferring mutations as well as utility of LPA in detection resistance to fluoroquinolones (FQ) and second-line injectable anti-TB agents.

Advantages

- Molecular LPAs enable rapid detection (in less than 48 hours) of resistance to rifampicin (alone or in combination with resistance to isoniazid); they were endorsed by WHO in 2008, and WHO has provided detailed policy guidance on introducing them at the country level [23].

- LPAs are a high throughput technology, allowing up to 48 specimens to be processed simultaneously and enabling several batches of tests to be done each day.

Disadvantages

- LPAs do not eliminate the need for conventional culture and DST.

- Available LPAs are recommended for use only on smear-positive sputum specimens and

isolates of *M. tuberculosis*.

• Current LPAs cannot replace phenotypic DST for second-line anti-TB agents. There is incomplete cross-resistance among second line injectable agents. LPAs cannot identify resistance to specific second-line injectable agents; thus, they cannot be used to guide the choice of second-line agents included in individualized MDR-TB regimens [20].

Limitations

• LPAs are suitable for use at the central or national reference laboratory level; they have the potential to be used at the regional level if the appropriate infrastructure can be ensured (three separate rooms are required).

• The sensitivity of LPAs to detect resistance to isoniazid is lower (approximately 85%) than that of culture methods.

Xpert MTB/RIF assay

The Xpert MTB/RIF assay is an automated, cartridge-based nucleic acid amplification test (NAAT) that uses the multi disease Gene Xpert platform. The Xpert MTB/RIF assay is performed directly on sputum, processed sputum sediment and selected extrapulmonary specimens from adults and children. GeneXpert instruments are modular, and options include systems with the capacity to have 1, 2, 4, 16, 48 or 80 independently functioning modules. The technology was first recommended by WHO in 2010, and a policy update was issued in 2013 following the meeting of an expert group to assess its use for detecting pulmonary and extrapulmonary TB and rifampicin resistance in adults and children [18, 24]. The "how to" Xpert MTB/RIF implementation manual was updated in 2014; it describes the operational aspects of and practical considerations associated with introducing and using the system.

Advantages

• The Xpert MTB/RIF assay simultaneously detects *M. tuberculosis* and rifampicin resistance in less than 2 hours.

• The sensitivity of the Xpert MTB/RIF assay for detecting TB is similar to that of to liquid culture (sensitivity, 88% when compared with liquid culture as a reference standard); the specificity is also high (99%).

• For smear-negative culture-positive TB, the pooled sensitivity of Xpert MTB/RIF has been found to be 68% [24]. The superior performance of Xpert MTB/RIF in detecting TB over that of microscopy makes it a particularly useful tool for case-finding among people living with HIV. As a tool for detecting rifampicin resistance, Xpert MTB/ RIF has a sensitivity of 95% and specificity of 98% when compared with phenotypic reference standards.

- The biosafety precautions required for Xpert MTB/RIF are similar to those for smear microscopy, and the training is minimal, which allows the technology to be used at relatively low levels in a laboratory network.

Disadvantages

- A stable uninterruptable electrical supply is needed; in settings where extended power outages may occur, uninterrupted power devices (UPS) and/or additional batteries may be needed to provide up to 2 hours of power.

- The ambient operating temperature of the instrument cannot exceed 30 °C, and cartridges must be stored at less than 28 °C.

- The shelf-life of the cartridges must be monitored to prevent them from expiring before they are used; thus, careful planning and management of supplies are essential.

- Security measures must be put in place to prevent the theft of the accompanying laptop or desktop computer.

- Limitations

- The modules require annual calibration; if modules fail the calibration test, using a specific calibration cartridge, they must be exchanged, which entails the importation of additional modules and exportation of the faulty modules.

- The use of Xpert MTB/RIF does not eliminate the need for conventional microscopy, culture and DST, which are required to monitor the progress of treatment and to detect resistance to anti-TB agents other than rifampicin.

- In patients who are not at risk for drug resistance but who initially test positive for rifampicin resistance by Xpert MTB/RIF, a second Xpert MTB/RIF test should be performed to control for preanalytical and postanalytical errors, and to improve the clinician's confidence in the diagnosis [19, 24].

- An increasing amount of evidence has shown that the infrequent occurrence of false-positive results may be linked to the detection by Xpert MTB/RIF of strains that are truly resistant to rifampicin but for which resistance is not detected by phenotypic culture-based DST, which is the present reference standard. Such strains appear to have clinically relevant mutations in the region conferring resistance to rifampicin, causing disease for which first-line treatment is likely to fail. In cases where discordant results are obtained from Xpert MTB/RIF and phenotypic DST or LPA, the culture isolate should be referred to a reference laboratory for DNA sequencing; while awaiting the results, a clinical decision should be made whether to continue the MDR-TB regimen [24].

2.1.5. Testing for latent TB infection

Persons with latent TB infection (LTBI) do not have active TB disease but may develop it in the near or remote future, a process called TB reactivation [25].

The lifetime risk of TB reactivation for a person with documented LTBI is estimated to be 5–10%, with the majority developing TB disease within the first five years after initial infection [26, 27]. A direct measurement tool for *M. tuberculosis* infection in humans is currently unavailable; hence, there is no gold standard for the diagnosis of LTBI. The tuberculin skin test (TST) and Interferon gamma release assays (IGRAs) indirectly measure

TB infection by detecting memory T-cell response signifying the presence of host sensitization to *M. tuberculosis* antigens. WHO recommends that either TST or IGRA can be used to test for LTBI in high-income and upper middle-income countries with estimated TB incidence less than 100 per 100 000 population. IGRA should not replace TST in low-income and other middle-income countries.

Advantages

- IGRAs require a single patient visit; results are available in 24-48 hours, and prior BCG vaccination does not cause false positive results.

- TST is widely used, not expensive, and does not require any special laboratory infrastructure or supplies.

Disadvantages

- TST requires two patient visits, results are available in 48-72 hours, and requires an injection into the skin, and adequately trained staff. Moreover, it has poor specificity in BCG-vaccinated populations, cross-reactivity with non-tuberculous mycobacteria and poor sensitivity in immune compromised persons.

- IGRAs are expensive, require blood to be drawn, special laboratory infrastructure and supplies, and adequately trained staff. Given comparable performance but increased cost, replacing TST by IGRAs as a public health intervention in resource constrained settings is not recommended.

Limitations

- IGRAs and the TST cannot accurately predict the risk of infected individuals developing active TB disease.

- Neither IGRAs nor the TST should be used for the diagnosis of active TB disease.

2.2. Techniques not recommended by WHO for the diagnosis of active TB

2.1.1. Commercial sero diagnostic tests for diagnosis of active TB disease

It is strongly recommended that commercial sero diagnostic tests not be used for the diagnosis of pulmonary and extra-pulmonary TB. Currently available commercial sero diagnostic tests (also referred to as serological tests) provide inconsistent and imprecise findings. There is no evidence that existing commercial serological assays improve patient outcomes, and high proportions of false positive and false-negative results may have an adverse impact on the health of patients [28].

2.1.2. IGRA for diagnosis of active TB disease

There is no consistent evidence that IGRAs are more sensitive than TST for diagnosis of active TB disease. Studies evaluating the incremental value of IGRAs to conventional microbiological tests show no meaningful contribution of IGRAs to the diagnosis of active TB. IGRAs are considered inadequate as rule-out or rule-in tests for active TB, especially in the context of HIV infection. IGRAs should not be used for the diagnosis of active TB disease.

2.3. CDC's tuberculosis testing and diagnosis

2.3.1. Testing

There are two kinds of tests that are used to detect TB bacteria in the body:

1. TB skin test (TST)

2. TB blood tests.

A positive TB skin test or TB blood test only tells that a person has been infected with TB bacteria. It does not tell whether the person has latent TB infection (LTBI) or has progressed to TB disease. Other tests, such as a chest x-ray and a sample of sputum, are needed to see whether the person has TB disease.

2.3.2. Diagnosis

If a person is found to be infected with TB bacteria, other tests are needed to see if the person has latent TB infection or TB disease.

Who should be tested

1. Certain people should be tested for TB infection because they are at higher risk for being infected with TB bacteria, including:

2. People who have spent time with someone who has TB disease

3. People from a country where TB disease is common (most countries in Latin America, the Caribbean, Africa, Asia, Eastern Europe, and Russia)

4. People who live or work in high-risk settings (for example: correctional facilities, long-term care facilities or nursing homes, and homeless shelters)

5. Health-care workers who care for patients at increased risk for TB disease

6. Infants, children and adolescents exposed to adults who are at increased risk for latent tuberculosis infection or TB disease

7. Many people who have latent TB infection never develop TB disease. But some people who have latent TB infection are more likely to develop TB disease than others. Those at high risk for developing TB disease include:

8. People with HIV infection

9. People who became infected with TB bacteria in the last 2 years

10. Babies and young children

11. People who inject illegal drugs

12. People who are sick with other diseases that weaken the immune system

13. Elderly people

14. People who were not treated correctly for TB in the past

15. TB tests are generally not needed for people with a low risk of infection with TB bacteria.

Testing for TB Infection

There are two types of tests for TB infection: the TB skin test and the TB blood test. A person's health care provider should choose which TB test to use. Factors in selecting which test to use include the reason for testing, test availability, and cost. Generally, it is not recommended to test a person with both a TB skin test and a TB blood test.

TB skin test

The TB skin test is also called the Mantoux tuberculin skin test (TST). A TB skin test requires two visits with a health care provider. On the first visit the test is placed; on the

second visit the health care provider reads the test. The TB skin test is performed by injecting a small amount of fluid (called tuberculin) into the skin on the lower part of the arm. A person given the tuberculin skin test must return within 48 to 72 hours to have a trained health care worker look for a reaction on the arm. The result depends on the size of the raised, hard area or swelling.

a) **Positive skin test:** This means the person's body was infected with TB bacteria. Additional tests are needed to determine if the person has latent TB infection or TB disease.

b) **Negative skin test:** This means the person's body did not react to the test, and that latent TB infection or TB disease is not likely.

There is no problem in repeating a TB skin test. If repeated, the additional test should be placed in a different location on the body (e.g., other arm). The TB skin test is the preferred TB test for children under the age of five.

TB blood tests

TB blood tests are also called interferon-gamma release assays or IGRAs. Two TB blood tests are approved by the U.S. Food and Drug Administration (FDA) and are available in the United States: the Quanti FERON®–TB Gold In-Tube test (QFT-GIT) and the T-SPOT®.TB test (T-Spot). **A health care provider will draw a patient's blood and send it to a laboratory for analysis and results.**

a) Positive TB blood test: This means that the person has been infected with TB bacteria. Additional tests are needed to determine if the person has latent TB infection or TB disease.

b) Negative TB blood test: This means that the person's blood did not react to the test and that latent TB infection or TB disease is not likely.

TB blood tests are the preferred TB test for:

1. People who have received the TB vaccine bacille Calmette–Guérin (BCG).

2. People who have a difficult time returning for a second appointment to look for a reaction to the TST.

Testing in BCG-Vaccinated Persons

Many people born outside of the United States have been given a vaccine called BCG. People who were previously vaccinated with BCG may receive a TB skin test to test for TB infection. Vaccination with BCG may cause a false positive reaction to a TB skin test. A positive reaction to a TB skin test may be due to the BCG vaccine itself or due to infection with

TB bacteria.

TB blood tests (IGRAs), unlike the TB skin test, are not affected by prior BCG vaccination and are not expected to give a false-positive result in people who have received BCG. TB blood tests are the preferred method of TB testing for people who have received the BCG vaccine.

Testing Health Care Workers

Tuberculosis (TB) transmission has been documented in health care settings where workers and patients come in contact with people who have TB disease. Periodic testing of health care workers is recommended as part of a TB Infection Control Plan and may be required by state regulations.

TB testing programs should include anyone working or volunteering in health-care settings. Persons (health care workers and non- health care workers) who have face to face contact or potential exposure to TB through shared air or space with infectious patient(s) should be part of a TB testing program.

There are two types of testing for TB in health care workers.

1. Initial baseline testing upon hire: Two-step testing with a TB skin test or a TB blood test

2. Annual or serial screening: determined by state regulations or risk assessment outcomes.

Frequency of TB testing

Health care facilities have different TB testing requirements. Facilities should conduct staff TB testing based on risk classification.

Risk classification	Frequency of testing
Low	Baseline; then test if TB exposure occurs
Medium	Baseline, then annually
Potential ongoing transmission	Baseline, then every 8–10 weeks until evidence of transmission has ceased

1. Baseline Testing

A baseline test should be given prior to employment. The result of this test can be compared with later tests (due to potential exposure or as part of annual testing) to help determine if recent TB transmission has occurred in the facility.

2. Annual or Serial Testing

You may need to test for TB on a regular basis. To standardize the interpretation of results, the same test should be used for the baseline and the later tests.

TB Skin Test: Two Step Testing

1. Baseline Testing: Two-Step Test

Two-step testing with the Mantoux tuberculin skin test (TST) should be used for baseline or initial testing. Some people with latent TB infection have a negative reaction when tested years after being infected. The first TST may stimulate or boost a reaction. Positive reactions to subsequent TSTs could be misinterpreted as a recent infection.

- Step 1

  Administer first TST following proper protocol

- Review result

  Positive — consider TB infected, no second TST needed; evaluate for TB disease.

  Negative — a second TST is needed. Retest in 1–3 weeks after first TST result is read.

- Document result

- Step 2

  Administer second TST 1-3 weeks after first test

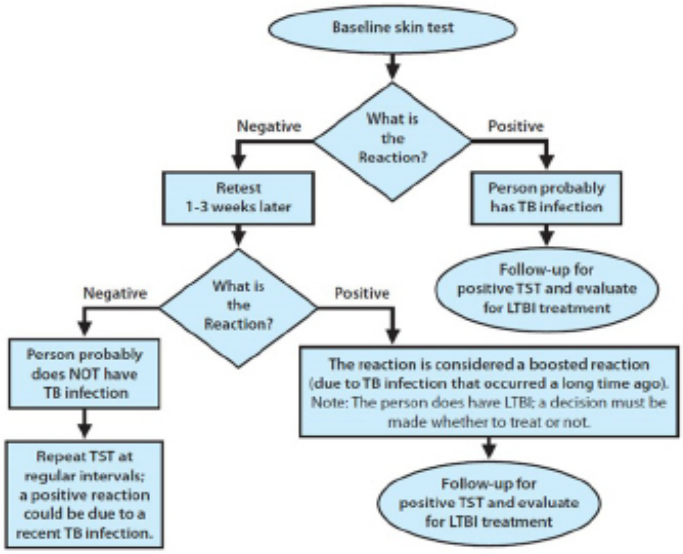
- Review results

  Positive — consider TB infected and evaluate for TB disease.

  Negative — consider person not infected.

- Document result

Two-Step TST Testing



2. Annual or Serial Testing

Once everyone in your facility has a baseline TB test, you may need to test on a regular basis. To standardize the interpretation of results, the same test should be used for the baseline and the later tests. There is no need for a two-step TST test process for annual TB testing. The process for annual testing with a TB skin test is as follows:

- Administer the TB skin test following proper protocol
- Review result — a change from a prior negative test result to a positive test result is evidence of recent TB infection
- Document result

TB blood Test

1. Baseline Testing

Using a TB blood test for initial or baseline testing does not require two-step testing and is not affected by BCG vaccination. The process for baseline testing using a TB blood test is as follows:

Administer TB blood test following usual protocol

Review result

Negative — consider not infected

Positive — consider TB infected and evaluate for TB disease

- Document result

2. Annual or Serial Testing

Once everyone your facility has an initial TB test result, you may need to test on a regular basis. To standardize the interpretation of results, the same test should be used for the baseline and the later tests. The process for annual testing for TB with a TB blood test is as follows:

- Administer the TB blood test following proper protocol
- Review result — a change from a prior negative test result to a positive test result is evidence of recent TB infection
- Document result

Testing During Pregnancy

There is a greater risk to a pregnant woman and her baby if TB disease is not diagnosed and treated. TB skin testing is considered both valid and safe throughout pregnancy. TB blood tests also are safe to use during pregnancy, but have not been evaluated for diagnosing TB infection in pregnant women. Other tests are needed to show if a person has TB disease.

Diagnosis of Latent TB Infection

A diagnosis of latent TB infection is made if a person has a positive TB test result and a medical evaluation does not indicate TB disease. The decision about treatment for latent TB infection will be based on a person's chances of developing TB disease by considering their risk factor [29].

3. Effectiveness of diagnosis of Tuberculosis

Diagnosis represents only one aspect of tuberculosis (TB) control but is perhaps one of the most challenging. The drawbacks of current tools highlight several unmet needs in TB diagnosis, that is, necessity for accuracy, rapidity of diagnosis, affordability, simplicity and the ability to generate same-day results at point-of-care (POC). When a return visit is required to access test results, time to treatment is prolonged, and default rates are significant. However, a good diagnostic tool is also critically dependent on obtaining an adequate biological sample [30].

3.1. Obtaining a biological sample and other considerations

Approximately 85% of the burden of TB is due to pulmonary TB. Diagnosis of pulmonary TB, particularly at primary care level, depends on obtaining an adequate expectorated sputum sample. However, in up to a third of TB cases, an adequate biological sample is not readily available or has a very low concentration of TB bacilli rendering the sample smear negative (cases of extrapulmonary TB requiring sampling at secondary care level, sputum scarce (unable to produce sputum), and smear-negative patients]. The latter is particularly relevant in children TB-HIV co-infection where up to 50% of persons are smear-negative [31]. Thus, alternative techniques such as sputum induction, gastric aspiration, bronchoscopy, and organ aspiration or biopsy may be required to obtain an adequate sample. However, the availability of these techniques is severely limited in high TB burden settings. Attention has therefore been focused on alternative biological samples, such as exhaled breath and urine, which are more readily available even in children.

Urine as a biological fluid for diagnostic testing is particularly attractive because it is sterile, less complex than other fluids such as sputum and serum, is readily available and TB-specific proteins and DNA may be found in the urine of patients with TB [32, 33]. Even

though a biological sample may be successfully obtained, other characteristics including sample volume (e.g. Xpert MTB/RIF requires _1 mL) and time-to-testing have the potential to impact results. It should be borne in mind that the reference standard for TB, that is, culture, is a suboptimal gold standard (prone to bacterial overgrowth, excessive decontamination, cross-contamination, etc.) and appropriate analytical strategies and methods may have to be employed to deal with this when evaluating new POC tests. Finally, more consideration should be given in combining tests and developing testing algorithms to rule-in TB [34], and to screening tests, including chest X-ray and computer-assisted diagnosis, to rule-out TB. These measures would help decrease the number of patients that require more expensive and complex tests, thus reducing burden on the patients as well as cost.

4. Effectiveness of Smear Microscopy

4.1. Direct Ziehl–Neelsen microscopy

Direct microscopy of Ziehl–Neelsen-stained sputum smears remains the mainstay of POC diagnosis in most TB endemic countries. The method is relatively rapid, inexpensive and has high specificity. However, direct Ziehl–Neelsen microscopy has low sensitivity (~50–60%) and is less sensitive in children, in HIV co-infected patients and in patients with extrapulmonary TB [35, 36]. Decontamination using chemicals, including bleach and NaOH and concentration of acid fast bacilli by centrifugation slightly improves the sensitivity [37, 38].

4.2. Fluorescence microscopy

An alternative to Ziehl–Neelsen -based direct microscopy is staining with a fluorescent molecule such as auramine O and visualization using a microscope with a mercury vapour bulb. This method is faster and improves sensitivity by ~10% without a compromise in specificity but its use has been limited by its higher cost, maintenance and darkroom requirements [39, 40].

4.3. LED microscopy

More recently, light-emitting diode (LED) microscopy was introduced. This low-cost method offers the benefit of fluorescence microscopy without the associated operational requirements, including a dark room and special microscope. LED has a lifespan of up to 50 000 h and may even be battery-operated. LED microscopy is endorsed by the WHO and also for use in resource-limited settings [41].

However, there are limited data about performance of LED microscopy in HIV-infected persons. A recent large study using samples from TB-HIV co-infected persons, LED microscopy was cheaper, faster and performed, as well as Ziehl–Neelsen and fluorescence microscopy independent of the staining and processing methods used [42].

4.4. Front-loaded microscopy

Another recent WHO-endorsed approach to smear based diagnostic work-up is front-loaded microscopy. Front-loaded microscopy addresses the problem of high dropout rates with focused collection of two or more sputum specimens during one clinic visit, and immediate referral and treatment of patients with positive smears [43, 44]. Front-loaded microscopy leads to a minor reduction in diagnostic sensitivity for the individual patient but is expected to improve case findings through enhanced quality of service and reduced dropout rates [45, 46].

5. Filtration techniques and magnetic beads

Other novel approaches to smear microscopy include filtration techniques and magnetic beads [47] to concentrate samples, and an automated slide-reading prototype that captures images and uses computerized algorithms to count acid fast bacilli [48].

6. Nucleic Acid Amplification Tests

A key advantage of near-patient rapid testing is that it may allow for the initiation of treatment within a very short time frame. New phenotypic methods such as commercial liquid culture drug susceptibility testing [49, 50] microscopic observation drug susceptibility [50, 51], colorimetric redox indicator methods [52] and the nitrate reductase assay [53], although approved by the WHO cannot provide results within a single clinic visit and also require extensive operator training, infrastructure needs and standardization before implementation [54]. By contrast, nucleic acid amplification tests (NAAT), which can rapidly detect small quantities of DNA through several different amplification methods, including the polymerase chain reaction, represent one of the most accurate known methods of detecting TB. With their improved simplification and automation in recent years, NAAT is becoming increasingly attractive candidate for use at the POC.

7. The Xpert MTB/RIF assay

Xpert MTB/RIF is a largely automated real-time polymerase chain reaction assay able to detect *M. tuberculosis* complex DNA and resistance to rifampicin [55]. It performs optimally on expectorated sputum specimens, using a disposable single-use cartridge and the test may be completed within 2 h, including a 15-min sample preparation step where sputum is homogenized using sterilizing sample buffer [56].

Several large-scale trials have assessed the accuracy of Xpert MTB/RIF [56, 57 58-61], where its sensitivity for TB detection in smear-positive and smear-negative patients was found to be ~98% and ~75%, respectively, [63] although some studies from high HIV prevalent settings have reported sensitivities in latter group to be as low as ~50% [57, 59, 62]. The

specificity of the assay for TB detection is ~98%. For the detection of rifampicin resistance in regions with high disease prevalence, the sensitivity and specificity are ~94% and ~97%, respectively [63]. Importantly, a recent large study [58] has shown that the improved accuracy of Xpert MTB/RIF over that of smear microscopy (the most widespread diagnostic test for TB, including at the POC) can translate into an improvement in the time-specific proportion of TB patients initiating TB treatment, where about 90% of TB patients could initiate treatment based on their Xpert MTB/RIF result on the same day they provided a sample. In contrast, only about 67% of TB patients were diagnosed by smear microscopy and able to initiate treatment rapidly, as this usually happened the day after a sample was provided for testing. However, whether this advantage is sustained and whether earlier diagnosis translates into reduction in morbidity and mortality remains unclear. It is critical that MDR-TB treatment capacity be scaled up in parallel to the rollout of the MTB/RIF assay.

8. Antigen Detection-Based Tests For Active Tb

The search for suitable TB-specific diagnostic antigens is ongoing and has been extensively studied in a variety of biological samples (e.g. sputum, blood, body cavity fluids and urine) [64]. A recent meta-analysis evaluated 47 studies using 12 single or combinations of TB antigens in different clinical specimens for pulmonary and extrapulmonary TB [64]. With the exception of LAM, TB antigen test sensitivity was as low as 2%, and specificity was suboptimal. However, antigen detection tests appear to offer a number of advantages over conventional diagnostics and have great potential for use as simple bedside tools. Antigen, as compared with whole *M. tuberculosis* organisms or TB-specific genetic material, is more likely to be detectable remote from the disease site in easily accessible biological fluids like urine. Antigen detection platforms, such as the lateral flow immune chromatographic assay (otherwise known as a strip test), require little or no sample processing to yield a rapid result. Unfortunately, despite the promise that antigen detection holds for POC diagnosis, available technologies have not yet delivered clinically useful results. Diagnostic accuracy measures vary widely, and with the exception of LAM, there are limited data about antigen-specific tests and none are currently in routine clinical use [64].

LAM, a 17.3-kDa immunogenic glycolipid component of the mycobacterial cell wall, has been the most extensively studied antigen and offers potential clinical utility in HIV-infected patients with advanced Immune suppression in both inpatient or outpatient (antiretroviral clinic) setting [65,66]. In HIV-infected patients, urinary LAM using an enzyme-linked immunosorbent assay kit had an overall sensitivity of ~50%, increasing to 67% and 85% in HIV-infected patients with CD4 count <50 cells/mL from outpatient and inpatient settings, respectively [65,66] and an overall specificity of 83–100% [65, 67, 68]. In addition, urine LAM correlated with bacterial burden [69] and may have prognostic utility by identifying TB HIV co-infected patients with the highest mortality [70]. Performance of the TB LAM enzyme-linked immunosorbent

assay using sputum or induced sputum samples has also been evaluated. Although sensitivity improved to over 80%, the specificity dropped to under 50% likely due to cross-reactivity with *Candida* spp and normal oral flora containing LAM-like molecules [65, 71]. The enzyme linked immunosorbent assay kit has now been superseded by the POC determine TB LAM Ag strip test (Alere), which is the first bedside TB test, provides a result within 25 min and will have a likely landing cost under USD 3.5 in the first quarter of 2013 [65]. Two initial evaluations in HIV-infected outpatients and inpatients showed similar diagnostic accuracy to the preceding TB LAM enzyme-linked immunosorbent assay and improved sensitivity (over the LAM strip test) when combined with sputum smear microscopy [72, 33]. However, specificity and inter reader agreement decreased when using the manufacturer's suggested grade-1 cut point. Thus, we recommend the grade 2 cut point at the expense of a lower sensitivity but with a higher specificity. In addition, the test either alone or combined with urine-based Xpert MTB/RIF testing was useful in sputum-scarce diagnostically challenging patients [32].

Further study is ongoing to clarify cut-point selection and the impact on patient-important outcomes, for example mortality, when LAM is used to guide the early initiation of treatment.

A number of antigens have also been evaluated in various compartments using non-sputum or urine samples, for example pleural fluid, cerebrospinal fluid, etc [64]. Urinary LAM had poor sensitivity and specificity in pleural and pericardial fluid, and in cerebrospinal fluid [64, 73, 74]. A limited number of studies with small numbers of patients have evaluated alternative diagnostic antigens using mainly 'in-house' assays with widely variable diagnostic accuracy [64]. These data reflect that antigen concentration in different body compartments are modulated by several factors including molecular weight, structure, and host degradation and processing. Thus, antigen performance may be highly variable between body compartments and sample specific. A possible solution may be to use combinations of antigens to improve overall diagnostic accuracy [64].

The availability, low cost, rapid format and modest performance of urine LAM in HIV-infected patients, although not ideal, gives us hope that antigen detection may still provide a broadly applicable and effective POC test. For this to be successful, candidate antigens or combinations of antigens will need to be specific for *M. tuberculosis*, be produced in abundance, be excreted into the extracellular environment and be resistant to rapid degradation associated with the host inflammatory response.

9. Antibody Detection and Microfluidic Technologies

Antibody detection tests based on lateral flow or other immune chromatic formats are attractive candidates. These tests monitor the humoral antibody immune responses to antigens and have proven to be rapid and accurate in the context of HIV diagnosis [75]. A number of

commercial antibody-based rapid TB tests are on sale [76] but significant clinical validation is absent and diagnostic accuracy is, at best, poor. In a recent updated meta-analysis including 67 studies of commercial serological tests, Steingart *et al*. showed that study quality was generally poor, and estimates of sensitivity and specificity were inconsistent and imprecise [40]. These findings lead the WHO to proclaim a negative recommendation (its first) against the use of TB serological tests [77].

The failure to develop antibody-based TB tests that meet clinical needs does not imply that such an approach should be abandoned. However, the heterogeneity in antibody responses from patient to patient suggests that a more complex multiplex approach is required [78-81]. Several novel promising antigenic targets have been identified [78, 82, 83]. A POC platform targeting several antigens, and co-developed by FIND (Geneva, Switzerland) and M Bio Diagnostics Inc. (Boulder, CO, USA), using multiplex serology on dot matrix readout will soon enter field evaluation studies. Microfluidics technology permits manipulation of fluids on a sub-millimetre scale enabling portability, affordability, easy disposal, user-friendliness, rapidity, multiplexing and feasibility with limited sample [84, 85]. A microfluidic platform seems well suited to a future multiplexed serological test.

10. POC Approaches for the Diagnosis of LTBI

In presumed LTBI, mycobacteria are not directly detectable, and therefore, diagnostic tests rely on measuring the presence of an adaptive immune response against *M. tuberculosis* [86].The major drawback of this approach is that a detectable response may represent exposure without infection or infection that has been cleared. The tuberculin skin test has been main stay of LTBI diagnosis for a century. This test is cheap and simple to apply, but there are several drawbacks including the need for a second test reading visit, subjective interpretation, cross reactivity in persons BCG vaccinated after birth and no assessment of immune energy. The discovery of regions of differentiation, that is, parts of the *M. tuberculosis* genome absent from most non-TB mycobacteria and BCG [87, 88], facilitated the development of specific immunodiagnostic tests—the interferon-g release assays (IGRA). IGRA are *in vitro* assays detecting interferon-g secretion from RD1 (ESAT6 and CFP10)-specific T cells. Two IGRA are commercially available—the Quanti FERON-TB Gold In-Tube assay (QFT, Qiagen, Hilden, Germany) and the T-SPOT.TB (Oxford Immuno tec, Oxford, UK). IGRA addresses several of the limitations of the tuberculin skin test; they have excellent specificity, but sensitivity (assessed in cases with active TB) is only 80%, and they are expensive and require specialized equipment and overnight incubation [89, 90]. Although data are variable [91, 92], a recent meta-analysis showed that ability to predict short-term progression to active TB was similar to the tuberculin skin test ~1–2% [89].

Although several biomarkers have been studied [93-97], the most promising interferon-g alternative is the chemokine inducible protein-10 (IP-10), which has comparable diagnostic accuracy and higher sensitivity in HIV-infected persons [97, 98, 99]. An IP-10 lateral flow platform can deliver quantitative results within minutes [100] and is stable in dried blood spots on filter paper allowing for letter based sample transport for centralized analysis [98].

Another interesting approach to LTBI diagnosis, similar to that of measuring interferon-g messenger RNA levels and transcriptional profiles [101], is IP-10 and MIG detection at messenger RNA level [102]. Advances in microfluidics and lab-on-a-chip technology could enable a novel generation IGRA-like test devices that combine incubation of small volumes blood (e.g. from a finger prick) and detection in a disposable device [103, 104]. A novel skin test using recombinant ESAT6 and CFP10 antigens, C-Tb (Statens Serum Institute, Copenhagen, Denmark) recently entered phase III clinical trials in South Africa and elsewhere, and performs comparably with the QFT in unexposed volunteers as well as in HIV-positive and -negative adults with confirmed TB. Diaskintest (Pharm standard, Ufa, Russia) is a similar product, but accuracy data are unavailable at the time of publication.

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