

# Canadian Tuberculosis Standards

7<sup>th</sup> Edition

## Chapter 3: Diagnosis of Active Tuberculosis and Drug Resistance



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## TABLE OF CONTENTS

<b>Diagnosis of Active Tuberculosis and Drug Resistance .....</b>	<b>2</b>
<b>Key Messages/Points .....</b>	<b>2</b>
<b>Diagnosis of Respiratory TB Disease .....</b>	<b>3</b>
<b>Clinical Picture of Pulmonary TB .....</b>	<b>3</b>
Epidemiologic Risk Groups .....	3
Symptoms.....	3
Signs .....	4
<b>Active TB Testing Algorithm for TB Suspects .....</b>	<b>4</b>
Chest Radiography.....	4
Limitations of Chest Radiography .....	5
<b>Microbiology.....</b>	<b>6</b>
Collection of Respiratory Specimens for Microbiology.....	6
Diagnosis of Drug Resistance .....	13
<b>References.....</b>	<b>15</b>

## CHAPTER 3

# DIAGNOSIS OF ACTIVE TUBERCULOSIS AND DRUG RESISTANCE

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### KEY MESSAGES/POINTS

- Testing for active tuberculosis (TB) is indicated in everyone with signs and symptoms of TB or considered to be at high risk of TB disease.
- Every effort should be made to obtain a microbiological diagnosis, which requires demonstration of acid-fast bacilli on smear microscopy and/or culture of *Mycobacterium tuberculosis*, or requires amplification and detection of *M. tuberculosis* complex (MTBC) nucleic acids using nucleic acid amplification tests (NAATs).
- Chest radiography is an integral part of the TB diagnosis algorithm but is not specific for the diagnosis of pulmonary TB. Chest radiography cannot provide a conclusive diagnosis on its own and should be followed by microbiological tests for TB disease.
- At least three sputum specimens should be collected and tested with microscopy as well as culture.
- Where feasible, three sputum specimens (either spontaneous or induced) can be collected on the same day, a minimum of 1 hour apart.
- Everyone with suspected TB should undergo testing with at least three concentrated fluorescent smears.
- Every specimen that is sent for smear microscopy should be set-up for culture in one solid medium and one liquid medium.
- At least one respiratory sample should be tested with a Health Canada approved or validated in-house NAAT in all new, smear-positive cases. In addition, NAA testing may be performed in smear-negative patients upon request by the physician or the TB control program. NAAT results are not recommended for monitoring TB treatment response.

- In settings where there is currently no on-site capacity for routine smear microscopy and culture, an automated cartridge-based NAA test can be used to make rapid decisions on TB treatment and isolation while routine smear and culture results are awaited. All such NAAT results should be confirmed by routine smears and cultures. In particular, all positive rifampin (RMP) resistance results should be interpreted cautiously, given the very low prevalence of multidrug-resistant (MDR)-TB in Canada and the likely low positive predictive value of RMP resistance results from the automated cartridge-based NAA assay.
- The use of serologic, antibody-based TB tests is not recommended for TB diagnosis.
- The use of tuberculin skin test (TST) or interferon gamma release assay (IGRA) for the diagnosis of active TB in adults is not recommended.
- Phenotypic drug susceptibility testing (DST) should be routinely performed for all first positive culture isolates obtained from each new TB case. While the agar proportion method is considered the gold standard for DST, a broth method is the recommended standard of practice in North America.
- Rapid molecular tests for DST should be reserved for patients with a high pretest probability of MDR-TB. **The use of these tests does not eliminate the need for conventional culture and DST**, which are recommended to confirm initial results and also detect resistance to drugs other than RMP and isoniazid (INH).

## DIAGNOSIS OF RESPIRATORY TB DISEASE

In Canada, respiratory TB includes primary TB, pulmonary TB, tuberculous pleurisy (nonprimary) and TB of the intrathoracic lymph nodes, mediastinum, nasopharynx, nose (septum) and sinus (any nasal). Pulmonary TB refers to TB of the lungs and conducting airways, which includes tuberculous fibrosis of the lung, tuberculous bronchiectasis, tuberculous pneumonia, tuberculous pneumo-thorax, isolated tracheal or bronchial TB and tuberculous laryngitis. The diagnosis of nonrespiratory TB is described in Chapter 7, Nonrespiratory Tuberculosis; the diagnosis of nontuberculous mycobacterial infections is described in Chapter 11, Nontuberculous Mycobacteria.

## CLINICAL PICTURE OF PULMONARY TB

### EPIDEMIOLOGIC RISK GROUPS

As summarized in Chapter 1, Epidemiology of Tuberculosis in Canada, foreign-born individuals, particularly those from countries with high TB incidence, Aboriginal Canadians, the elderly (particularly elderly males) and close contacts of infectious TB cases are at increased risk of TB disease.<sup>1</sup>

### SYMPTOMS

The classic symptom of pulmonary TB disease is a chronic cough of at least 2-3 weeks' duration. This cough is initially dry but after several weeks to months will become productive. Cough of 2 weeks duration is a more sensitive criterion, but cough of 3 weeks duration will be more specific. Selection of 2 or 3 weeks as the criterion depends on the local experience and epidemiology of TB. Fever and night sweats are common but may be absent in the very young and the elderly. Hemoptysis, anorexia, weight loss, chest pain and other symptoms are generally manifestations of more advanced disease.<sup>1,2</sup>

## SIGNS

The most common physical finding in pulmonary TB is a totally normal examination, even in relatively advanced cases. Bronchial breathing, rales or crepitations will be found in more advanced cases. It is important to examine for signs of extrapulmonary disease, such as lymphadenopathy, pleural effusion and abdominal or bone and joint involvement, as these may be present concomitantly, particularly in HIV-infected individuals.<sup>1,2</sup>

### RECOMMENDATIONS

- **Testing for active TB is indicated in everyone with signs and symptoms of TB or considered to be at high risk of TB.**  
*(Strong recommendation, based on moderate evidence)*
- **Every effort should be made to obtain a microbiological diagnosis, which requires demonstration of acid-fast bacilli on smear microscopy and/or culture of *Mycobacterium tuberculosis*, or requires amplification and detection of MTBC nucleic acids using NAATs. It is important to note that NAAT results are not confirmatory; they are presumptive, and confirmation by culture is recommended.**  
*(Strong recommendation, based on strong evidence)*

## ACTIVE TB TESTING ALGORITHM FOR TB SUSPECTS

In Canada, the standard testing algorithm for active TB includes the following tests:<sup>1</sup>

- chest radiography;
- sputum smear microscopy;
- mycobacterial culture and phenotypic DST;
- NAATs.

### CHEST RADIOGRAPHY

Chest radiography (posterior-anterior and lateral views) is the usual first step in evaluation of an individual with pulmonary symptoms.<sup>1</sup> However, it is important to be aware that chest radiography has substantial limitations in the diagnosis of pulmonary TB disease.<sup>1</sup>

**1. Typical findings:** a triad of classic findings is seen in immunocompetent adults.<sup>3</sup>

- Position – infiltrates in the apical-posterior segments of upper lobes or superior segment of lower lobes in 90%.
- Volume loss – this is a hallmark of TB disease as a result of its destructive and fibrotic nature.
- Cavitation – this is seen at a later stage and depends upon a vigorous immune response. Therefore, it often is not seen in immunocompromised individuals.

## 2. Atypical features

These will be seen in patients with immunocompromising conditions such as HIV infection, diabetes, renal failure or long-term use of corticosteroids and other immunosuppressive agents.<sup>3</sup>

- Hilar and mediastinal lymphadenopathy, particularly in HIV-infected individuals
- Non-cavitary infiltrates and lower lobe involvement.

## 3. Radiographic signs of complications<sup>3</sup>

- Endobronchial spread of disease. TB may spread via the airways to the ipsilateral and contralateral lower lobes. This results in irregular, poorly defined, small nodular shadows, which represent acinar shadows. These will slowly enlarge and coalesce to form TB pneumonia, formerly known as “galloping consumption.”
- Pleural effusion can be seen concomitant with pulmonary disease and may represent TB empyema.
- Pneumothorax can rarely occur as a result of erosion of a caseous focus into a bronchus and simultaneously into the pleural space, causing a bronchopleural fistula.

## LIMITATIONS OF CHEST RADIOGRAPHY

### 1. Sensitivity:

Chest radiography will have a sensitivity of only 70% to 80% for the diagnosis of active TB based on the abnormalities listed above. If any abnormality is considered, it will have more than 95% sensitivity.<sup>4</sup> Approximately 10% of HIV-positive people or close contacts with active culture-confirmed pulmonary disease will have normal x-rays.<sup>4</sup>

### 2. Specificity:

This is relatively poor, in the range of 60% to 70%. If the sensitivity were improved (any abnormality considered possible TB), then the specificity would be much lower.<sup>4</sup>

### 3. Inter-reader variability:

One of the greatest problems of chest x-ray reading is that the interpretation is highly variable.<sup>4</sup> There is very poor agreement among readers regarding the presence of cavitation, hilar lymphadenopathy and the likelihood of active disease.<sup>4</sup>

## RECOMMENDATIONS

**Chest radiography is an integral part of the TB diagnosis algorithm but is not specific for the diagnosis of pulmonary TB. Chest radiography cannot provide a conclusive diagnosis on its own and should be followed by microbiological tests for TB disease (described below).**

*(Strong recommendation, based on strong evidence)*

## MICROBIOLOGY

The role of the mycobacteriology laboratory is to detect, isolate, identify and perform susceptibility tests on clinically significant mycobacteria from clinical specimens. Mycobacterial culture, using both solid and liquid media, is considered the gold standard for diagnosis, and the use of broth-based culture methods for DST is the standard of practice in North America.<sup>2,5,6</sup> The most widely used rapid test is the examination of smears of sputum or other respiratory specimens after staining for acid-fast organisms (AFB smear). However, molecular-based techniques (NAATs) for the detection and identification of mycobacterial species are now widely available, enabling rapid identification of individuals with disease due to MTBC. Appendix D provides more information on TB laboratory standards.

### COLLECTION OF RESPIRATORY SPECIMENS FOR MICROBIOLOGY

Given the critical importance of microbiology for TB diagnosis, it is important to ensure that respiratory specimens are correctly collected and processed to achieve valid results. All specimens should be collected in sterile, leak-proof, laboratory-approved containers and accompanied by a carefully completed requisition form providing the patient's demographic data, the physician's name, the date and time of collection, and the specimen type and site. As much as possible, specimens collected for initial diagnosis should be obtained before the initiation of anti-TB therapy.<sup>1,2</sup>

Once collected, specimens should be transported to the laboratory promptly. If processing within 1 hour is not possible, samples should be refrigerated at 4 °C (not frozen) and protected from light. Clinical specimens should be handled, processed and transported in an environment in which biosafety procedures are in place. Appendix D on TB laboratory standards provides more details on biosafety and the transportation of samples.

### Sputum

At least three sputum specimens of 5-10 mL each should be collected and tested with microscopy as well as culture. While available evidence shows that the yield of the third sputum smear is only about 2%-5%,<sup>7</sup> the yield of the third culture may be as high as 5%-10%, especially in HIV-infected people.<sup>8,9</sup> Thus, it is important to collect at least three specimens for smears and cultures, especially in a low-incidence setting such as Canada, where smear-negative TB is the most common presentation.<sup>1</sup>

While it is conventional to collect sputum specimens using the standard spot-morning-spot (SMS) scheme, it is well known that this scheme is inconvenient to patients, and drop-outs during diagnosis are common. Recently published research has focused on the “same-day” or “frontloaded” diagnosis of TB using specimens collected on the same day in order to reduce patient drop-out, which is likely to happen if patients are asked to come back daily for sample collection.<sup>10</sup>

A multicentre clinical trial of 6,627 adults with cough of  $\geq 2$  weeks' duration compared the sensitivity/specificity of two sputum samples collected “on the spot” (one hour apart) during the first visit plus one sputum sample collected the following morning (spot-spot-morning [SSM]) versus the standard SMS scheme.<sup>11</sup> The centres participating in the study were randomly assigned each week for a year to use either the SMS or the SSM sample collection scheme. Compared with mycobacterial culture, the sensitivities of the SSM and SMS schemes were 70.2% and 65.9% respectively. Similarly, the specificity of SSM (96.9%) was not inferior to that of

SMS (97.6%). Importantly, the sensitivity of diagnosis using just the first two samples collected in the SSM scheme was also noninferior to the sensitivity of diagnosis using the first two samples collected in the SMS scheme (63.6% versus 64.8%). Finally, patients tested using the SSM scheme were more likely to provide the first two samples than patients tested using the SMS scheme (98% versus 94.2%).<sup>11</sup> This large trial informed a recent World Health Organization (WHO) policy on same-day diagnosis.<sup>10</sup>

The findings of this trial were confirmed by a meta-analysis published in 2012 of eight research studies (involving 7,771 patients) comparing the accuracy of same-day microscopy and standard sputum smear microscopy for the diagnosis of culture-confirmed pulmonary TB.<sup>12</sup> Compared with the standard approach of examination of two smears with Ziehl-Neelsen microscopy over 2 days, examination of two smears taken on the same day had much the same sensitivity (64% [95% confidence interval 60% to 69%] for standard microscopy vs 63% [58% to 68%] for same-day microscopy) and specificity (98% [97% to 99%] vs 98% [97% to 99%]).

Thus, same-day sample collection with an interval of as little as 1 hour between sample collection may be especially helpful to reduce patient drop-out and make faster decisions about TB infection control and discharge from respiratory isolation (please see Chapter 15, Prevention and Control of Tuberculosis Transmission in Healthcare and Other Settings).

## RECOMMENDATIONS

- **At least three sputum specimens should be collected and tested with microscopy as well as culture.**  
*(Conditional recommendation, based on moderate evidence)*
- **Where feasible, three sputum specimens (either spontaneous or induced) can be collected on the same day, at least 1 hour apart.**  
*(Conditional recommendation, based on moderate evidence)*

## Induced sputum

A recent meta-analysis of 17 studies evaluated the sensitivity of sputum induction and found that this procedure detects approximately 75% of culture-positive TB cases under study conditions among children and adults, regardless of the HIV prevalence, although the estimates varied across studies.<sup>13</sup> Another recent systematic review of 23 studies reported that the overall success of sputum induction was high, ranging from 76.4% to 100%, while adverse events associated with sputum induction were infrequent and mild. The sensitivity of microscopy compared with culture on induced sputum samples ranged from 0% to 100%. Yield was generally higher for sputum induction than nasopharyngeal aspiration and gastric lavage.<sup>14</sup>

It is important that sputum induction be performed with large volumes of 3% hypertonic saline. For best results, an ultrasonic nebulizer should be used that can administer 5 to 6 mL per minute over 15 minutes.<sup>1</sup> With the use of this, virtually all patients will produce sputum, and a single sputum induction will have equivalent or better yield than fiberoptic bronchoscopy.<sup>15</sup> Sputum induction has been performed successfully in very young children<sup>16</sup> (please see Chapter 9, Pediatric Tuberculosis). It is important to indicate on the requisition that the sputum was induced, because the resulting specimen often appears watery. However, it can be handled in the laboratory in the same way as spontaneously expectorated sputum.<sup>2,17</sup>

## Bronchoscopy

Bronchoscopy may be used to facilitate the diagnosis of TB when spontaneous sputum and induced sputum are unavailable, or all samples are smear-negative.<sup>17</sup> Bronchoscopy is very useful if other pulmonary diseases, such as lung cancer, are also suspected. However, for the diagnosis of active TB it entails risk and discomfort for the patient, is expensive and can contribute to nosocomial spread of TB if not performed in an appropriate environment with protection of staff. In addition, the overall yield of bronchoscopy in prospective series of patients is only 77%.<sup>18-21</sup> If bronchoscopy is done, post-bronchoscopy sputum should be sent for AFB testing, as this has a yield similar to that of bronchial washings and lavage.<sup>2,17</sup>

## Gastric aspirate

This technique was introduced more than 70 years ago and is still used in some centres.<sup>22</sup> The primary indications are investigation of possible TB in children who cannot expectorate sputum or, for the same reason, elderly demented patients. A recent systematic review of the accuracy of gastric aspiration (GA) and gastric lavage (GL) for TB diagnosis in children reported that GA/GL microscopy was positive in 0%-21% (median 7%), and culture was positive in 0%-75% (median 20%) of children with a clinical diagnosis of likely TB.<sup>23</sup> Culture isolation rates depended on the clinical criteria used to define TB.

The technique is relatively simple and is described in Chapter 9, Pediatric Tuberculosis. However, it is uncomfortable and unpleasant for patients, and may be difficult to implement because it needs to be performed immediately upon the patient awakening.<sup>24</sup> This often means that he or she has to be kept overnight in hospital, although it can be done for outpatients.<sup>24</sup>

## Smear Microscopy

Sputum smear microscopy is the most widely used test for TB disease.<sup>2,17</sup> Two stains are widely used: 1) the traditional Ziehl-Neelsen or Kinyoun staining, which requires a light or bright field microscopy and 2) the auramine stain, which requires fluorescence microscopy. In most high-income countries (including Canada), fluorescence microscopy is standard practice (see Appendix D, Tuberculosis and Mycobacteriology Laboratory Standards: Services and Policies). Everyone with suspected TB should undergo testing with at least three concentrated auramine smears. Spontaneous or induced sputum specimens can be used.

Smear microscopy is rapid, inexpensive and identifies the most infectious TB patients.<sup>1,2</sup> However, the test has well known limitations:

- Sensitivity is modest and variable (20%-80%) depending upon the type of specimen, patient population, stain used and the experience of the microscopist.<sup>25-27</sup> Thus, multiple sputum smears are recommended to increase the overall sensitivity and yield. Sensitivity is higher for respiratory than for nonrespiratory specimens, particularly body fluids.
- In low TB incidence settings, smear microscopy has lower specificity – a positive smear could be due to nontuberculous mycobacteria (NTM).
- Smear microscopy has lower sensitivity in childhood TB and extrapulmonary disease, especially in HIV-infected people.
- Smear microscopy cannot be used to determine drug resistance.

Specimens need to be homogenized and then concentrated. The fluorochrome stain auramine is the most widely used staining method for initial acid-fast bacilli (AFB) smears because it can be read at a lower magnification than the classical Ziehl-Neelsen or Kinyoun stain, and thus slides

can be read more quickly.<sup>5</sup> Fluorescence microscopy can be performed by conventional mercury vapour fluorescence microscopes or newer, light-emitting diode microscopes, which have many practical advantages<sup>28</sup> and have been endorsed by the WHO.<sup>29</sup> The sensitivity of all staining methods is inferior to that of culture. The threshold of detection of AFB in concentrated specimens using a fluorochrome stain is 5,000-10,000 bacteria/mL of sputum and is 100,000 bacteria/mL using the Ziehl-Neelsen stain. The threshold of detection in unconcentrated smears is 10-fold higher, resulting in much lower sensitivity. This is important to remember, since often “Stat” smears are unconcentrated. In contrast, as few as 10-100 viable bacteria can be detected by culture.<sup>2,5</sup>

The specificity of the AFB smear is high for mycobacteria, but it is important to remember that all NTM will be AFB-positive. Other organisms, such as *Nocardia* and other actinomycetes, can be weakly acid-fast, but these are less common. Therefore, a positive AFB smear almost always indicates the presence of mycobacteria, but not necessarily *M. tuberculosis*.<sup>5</sup>

When acid-fast organisms are seen, the number of bacteria is reported semi-quantitatively, as shown in Table 1. Although there are different scales in use, North American laboratories use the Association of Public Health Laboratories recommended semi-quantitative system<sup>5</sup> (Table 1).

**Table 1. Number of bacteria seen on microscopy and laboratory interpretation<sup>5</sup>**

Number of AFB seen by staining methods		
Fuchsin stain (Ziehl-Neelsen) (1,000-fold magnification)	Fluorochrome (250-fold magnification)	Semi-quantitative grading system
0 in 300 fields	0 in 30 fields	Negative
1-2 per 300 fields	1-2 per 30 fields	Inconclusive, repeat
1-9 per 100 fields	1-9 per 10 fields	1+ (rare)
1-9 per 10 fields	1-9 per field	2+ (few)
1-9 per field	10-90 per field	3+ (moderate)
>9 per field	>90 per field	4+ (numerous)

#### RECOMMENDATION

- **Everyone with suspected TB should undergo testing with at least three concentrated fluorescent smears. Spontaneous or induced sputum specimens can be used. Smear microscopy is performed routinely on all specimens submitted to the mycobacteria laboratory for testing.**

*(Conditional recommendation, based on moderate evidence)*

## Mycobacterial Culture

Mycobacterial culture is the most sensitive and the current gold standard method for the detection of active TB disease.<sup>2,5,17</sup> In Canada, every specimen that is sent for smear microscopy is submitted for culture on one solid medium and one liquid medium. The use of culture remains necessary for the definitive diagnosis of smear-negative TB. The benefits of culture include identification, DST and further use of culture isolates for molecular epidemiology using DNA fingerprinting.<sup>17</sup> Culture can be performed on all specimen types, but typically sputum is used for the diagnosis of pulmonary TB.<sup>17</sup> Standards for TB culture are described in Appendix D.

Culture results typically take 2-8 weeks, depending on the culture method used and the number of MTBC bacteria in the inoculum. Solid culture typically uses either Lowenstein–Jensen media or Middlebrook 7H10 or 7H11 agar media for the isolation of MTBC and DST is performed using either Middlebrook 7H10 or 7H11 agar media. MTBC typically has a faster growth rate in liquid media than on solid agar. Also, liquid cultures are about 10% more sensitive than solid cultures, although more prone to contamination.<sup>30</sup> Three automated liquid culture systems are approved by Health Canada: Beckton-Dickinson (Bactec960 MGIT [mycobacterial growth indicator tube]), bioMérieux (BacT/ALERT) and Trek Diagnostic Systems Inc. (Myco-ESP culture System II). These are fully automated systems that use either fluorometric or colorimetric detection of mycobacterial growth and can be used for the isolation of MTBC and for DST.<sup>31</sup> Automated systems permit a higher throughput of specimens for testing, and DST results are often available within 7 days from the time of DST set-up.<sup>5</sup>

Culture for *M. tuberculosis* is considered the gold standard in diagnosis.<sup>2,5,17</sup> For pulmonary TB, the sensitivity of three sputum cultures exceeds 90%, although six specimens are required to achieve 100% sensitivity.<sup>4</sup> Three sputum cultures are recommended, as this represents the best balance between high sensitivity and efficiency.<sup>5</sup> A single positive culture for *M. tuberculosis*, in general, is considered definitive for active disease. However, it is important to remember that cultures occasionally can be falsely positive, which may be due to cross-contamination within the laboratory. A report of a single positive culture, especially with a long detection time and/or few colonies, when clinical suspicion is low should raise the possibility of a false-positive result. The laboratory reporting this culture should investigate and review all positive cultures initially processed on the same day or within proximity to the culture, ideally performing DNA fingerprinting on the isolate.<sup>5</sup>

All culture isolates should be subject to species identification using methods recommended in TB laboratory standards (Appendix D).

### RECOMMENDATION

- **Every specimen that is sent for smear microscopy should be submitted for culture on one solid medium and one liquid medium.**

*(Strong recommendation, based on strong evidence)*

## Nucleic Acid Amplification Tests (NAAT)

The amplification of nucleic acids for the diagnosis of TB or to detect drug resistance is a sensitive method and produces a much faster result than conventional culture methods.<sup>32,33</sup> Polymerase chain reaction (PCR) is the most common method of amplification. In addition to commercial assays, there are many protocols for so-called “home brew” or in-house molecular

assays. Unlike standardized, commercial NAATs, in-house NAATs can produce inconsistent results.<sup>33</sup> It is therefore recommended that validation studies be conducted before implementation and that the tests be used in accredited laboratories with quality assurance systems in place. Please see Appendix D for reporting standards for in-house NAAT results.

The sensitivity of commercial NAATs to detect TB is high (>95%) in sputum smear-positive samples.<sup>32,34</sup> The sensitivity of NAATs is lower (50%-70%) when smear-negative/culture-positive specimens are tested.<sup>32-35</sup> The sensitivity of NAATs is also lower in extrapulmonary specimens.<sup>36-38</sup> Thus, it is recommended that a negative NAAT result should not be used to rule out TB, especially in paucibacillary forms of TB (i.e. smear-negative and extrapulmonary TB). However, the specificity of the commercial NAAT is very high in all specimens (90%-100%).<sup>32,34</sup>

In general, NAATs require sophisticated laboratory infrastructure and highly skilled technicians. The risk of contaminating the test site with amplified DNA also requires stringent quality control procedures and a specific infrastructure to limit contamination. Please see Appendix D.

The following assays are commercially available and Health Canada approved: Roche (COBAS<sup>®</sup>Taqman<sup>®</sup> MTB; real-time-PCR), Becton Dickson (BD ProbeTec<sup>®</sup>, strand displacement amplification [SDA]), Gen-Probe (Amplified *Mycobacterium tuberculosis* Direct [AMTD], transcription mediated amplification [TMA]), Hain Lifescience (GenoType<sup>®</sup> Mycobacteria Direct, PCR) and Cepheid (Xpert MTB/RIF<sup>®</sup>, automated cartridge-based nested PCR). The COBAS<sup>®</sup> Taqman<sup>®</sup> MTB, AMTD, and Xpert MTB/RIF tests are approved for direct testing on sputum specimens.

In 2010, the WHO published a policy on a new NAAT – the Xpert MTB/RIF<sup>®</sup> test (Cepheid Inc, Sunnyvale, CA), a cartridge-based, automated, nested, real-time PCR test utilizing the GeneXpert<sup>®</sup> platform, which can detect MTBC for diagnosis and can detect RMP resistance, a marker of MDR-TB, in less than 2 hours with minimal hands-on technical time.<sup>39,40</sup> This assay was approved by Health Canada in 2012 as a laboratory-based technology.

Unlike conventional NAATs, the Xpert MTB/RIF test is completely automated and self-contained, and is not dependent on reference laboratories or a high degree of technical expertise.<sup>41</sup> Sample processing steps are minimized to less than 5 minutes of hands-on time, and the use of a sample preparation reagent effectively inactivates the specimen with more than an 8-log decrease in viability, posing virtually no biosafety risk.<sup>42</sup> Currently, Xpert MTB/RIF is the only fully automated, cartridge-based NAAT on the market and the only product in its class.<sup>40</sup>

A recently completed Cochrane systematic review on the accuracy of Xpert MTB/RIF identified 18 published studies.<sup>43</sup> The majority were performed in low/middle-income countries. In 17 of the 18 studies, Xpert was performed by trained technicians in reference laboratories. In the meta-analyses for MTBC detection, pooled median sensitivity and specificity estimates (95% confidence intervals) were as follows: overall (15 studies, 7,517 participants), pooled median sensitivity and specificity were 88% (83%, 92%) and 98% (97%, 99%) respectively; in direct comparisons (15 studies), pooled median sensitivity was 98% (97%, 99%) for smear-positive, culture-positive TB and 68% (59%, 75%) for smear-negative, culture-positive TB; in direct comparisons (four studies), pooled median sensitivity was 80% (67%, 88%) in people living with HIV and 89% (81%, 94%) in people without HIV infection. In the meta-analysis for RMP resistance detection (11 studies, 2,340 participants), pooled median sensitivity and specificity were 94% (87%, 97%) and 98% (97%, 99%) respectively. When used as an add-on test following smear microscopy (15 studies), Xpert yielded a 25% higher sensitivity over smear. Xpert could

distinguish between MTBC and NTM in clinical samples with high accuracy (of 139 specimens with NTM, cross-reactivity was observed in only one specimen).<sup>43</sup>

Overall, the available evidence shows high accuracy for TB detection, but this evidence is mostly from high-burden countries and involves the use of spontaneous sputum samples. Similar data from low-incidence settings and with the use of induced sputum samples are lacking. The data, although limited, also suggest that Xpert MTB/RIF can significantly reduce the time to diagnosis and treatment.<sup>44</sup> The predictive value for RMP resistance will depend on the prevalence of drug-resistant TB in a given setting. In a low MDR-TB prevalence setting such as Canada, false-positive RMP results are a major concern. Thus, all Xpert results should be confirmed by conventional culture methods.

Because the Xpert technology is simple and can be implemented in peripheral laboratories, this test may be potentially useful in remote settings (for example, hospitals in northern regions of Canada serving Aboriginal populations) where there is currently limited on-site capacity for routine smear microscopy and cultures, and where smear and culture results may sometimes be delayed. In such settings, if the Xpert test is performed by trained laboratory technicians the results could be available within hours and used to inform rapid decisions on TB treatment and isolation pending routine smear and culture results. This could potentially help reduce diagnostic delays, especially in the context of the ongoing high rates of TB in Nunavut and Nunavik (Northern Quebec) (See Chapter 14, Tuberculosis Prevention and Care in First Nations, Inuit and Métis Peoples). **However, it is important to note that the use of Xpert in these settings should not replace conventional smears and cultures.** All Xpert MTB/RIF results should be confirmed by routine smears and cultures. In particular, all positive RMP resistance results should be interpreted cautiously, given the very low prevalence of MDR-TB in Canada<sup>45</sup> and the expected low positive predictive value of RMP resistance results from the Xpert MTB/RIF assay in this setting.<sup>43</sup> Because NAATs can amplify nonviable AFB, the Xpert result, as with any other NAAT, is not recommended for use in monitoring TB treatment response.<sup>2</sup>

## RECOMMENDATIONS

- At least one respiratory sample should be tested with a Health Canada approved or validated in-house NAAT in all new, smear-positive cases. In addition, NAA testing may be performed in smear-negative patients upon request by the physician or the TB control program. NAAT results are not recommended for monitoring TB treatment response.  
*(Conditional recommendation, based on moderate evidence)*
- In settings where there is currently no on-site capacity for routine smear microscopy and culture (for example, hospitals in the North serving Aboriginal populations), an automated, cartridge-based NAA test can be used to make rapid decisions on TB treatment and isolation pending routine smear and culture results. All Xpert MTB/RIF results should be confirmed by routine smears and cultures. In particular, all positive RMP resistance results have to be interpreted cautiously, given the very low prevalence of MDR-TB in Canada and the low positive predictive value of RMP resistance results from the Xpert MTB/RIF assay in this setting. The Xpert result is not recommended for monitoring TB treatment response.  
*(Conditional recommendation, based on moderate evidence)*

### Role of Immune-based Methods (Serology, TST and IGRAs)

For decades, researchers and the industry had pinned their hopes on serologic antibody-detection methods for point-of-care test development. Indeed, dozens of serologic rapid (lateral flow assays) and ELISA (enzyme-linked immunosorbent assay) tests were commercialized, even though no international guideline recommended their use.<sup>46,47</sup> Today, these tests are on the market in at least 17 of the 22 countries with the highest TB burden, and millions of patients in the private sector undergo serologic testing.<sup>48,49</sup> Unfortunately, TB serologic tests are neither accurate nor cost-effective,<sup>47,50</sup> prompting the WHO to issue a strong negative recommendation against their use.<sup>51</sup>

As described in Chapter 4 (Diagnosis of Latent Tuberculosis Infection), neither the TST nor IGRA can separate latent TB infection from active TB disease and therefore have no value for active TB detection in adults.<sup>52</sup> A recent WHO policy on IGRAs has discouraged their use for active TB diagnosis.<sup>53</sup> In children, TST and/or IGRAs are useful as an indicator of TB infection and can be used to support a diagnosis of TB disease, along with clinical data, radiologic and microbiological investigations (refer to Chapter 9, Pediatric Tuberculosis).

#### RECOMMENDATIONS

- The use of serologic, antibody-based TB tests is not recommended for TB diagnosis.  
*(Strong recommendation, based on strong evidence)*
- The use of TST or IGRA for the diagnosis of active TB in adults is not recommended.  
*(Strong recommendation, based on strong evidence)*

### DIAGNOSIS OF DRUG RESISTANCE

The diagnosis of drug-resistant TB can be made using two approaches: 1) phenotypic and 2) genotypic (molecular) methods (although molecular methods should be used in conjunction with phenotypic testing).<sup>2,5,6,31</sup>

Phenotypic DST should be routinely performed for all first positive culture isolates obtained from each new TB case.<sup>5,6</sup> While the agar proportion method is considered the gold standard for DST, a broth method is the recommended standard of practice in North America for DST<sup>6</sup> (See Appendix D). Set-up of first-line DST should provide phenotypic results within 4 to 14 days for first-line drugs and 4 to 21 days for second-line drugs.<sup>5,6</sup>

Genotypic methods involve NAATs, which amplify and detect mutations that confer drug resistance. Two genotypic methods are endorsed by the WHO for rapid diagnosis of drug-resistant TB: 1) line-probe assays (LPAs) and 2) the Xpert MTB/RIF test. In addition, other validated in-house NAATs may be used, as described earlier.

In patients with a high pretest probability of MDR-TB, rapid molecular tests can help make quick decisions on appropriate second-line TB therapy, while conventional DST results are awaited.<sup>54,55</sup> In patients with a low pretest probability of MDR-TB, rapid molecular tests will have low positive predictive value and should be interpreted very cautiously.<sup>43</sup>

LPAs have been developed and evaluated to perform DST from smear-positive sputum samples directly or to perform rapid DST on culture isolates. Two LPA tests are commercially available:

the Inno-LiPARif.TB line probe assay (Innogenetics, Belgium) and the GenoType MTBDR<sub>plus</sub> assay (Hain Lifescience, Germany). The GenoTypeMTBDR<sub>plus</sub> assay is approved by Health Canada. It is performed in reference level facilities with dedicated rooms for preparation and a containment level 2 (CL2) laboratory for processing sputum, or a containment level 3 (CL-3) laboratory if manipulation of MTBC culture is needed.

A meta-analysis showed that the GenoType MTBDR<sub>plus</sub> assay has a pooled sensitivity of 98.1% and specificity of 98.7%.<sup>56</sup> The accuracy for INH was variable, with lower and inconsistent sensitivity (84.3%) and high specificity (99.5%).<sup>56</sup> LPAs are endorsed by the WHO for rapid diagnosis of INH and RMP resistance from sputum smear-positive samples.<sup>57</sup> However, the use of LPAs does not eliminate the need for conventional culture and DST capability; culture is recommended for definitive diagnosis of TB in smear-negative patients, while conventional DST is recommended to diagnose extensively drug-resistant TB.<sup>57</sup>

As previously described, the Xpert MTB/RIF assay can rapidly diagnose RMP resistance in under 2 hours with a sensitivity of about 94% and a specificity of 98%.<sup>43</sup> However, these estimates are from high-burden settings. The predictive value for RMP resistance will depend on the prevalence of drug-resistant TB in a given setting. Thus, all positive RMP resistance results on the Xpert assay should be interpreted cautiously, given the very low prevalence of MDR-TB in Canada<sup>45</sup> and the expected low positive predictive value of RMP resistance results from the Xpert MTB/RIF assay in this setting.

## RECOMMENDATIONS

- Phenotypic DST should be routinely performed for all first positive culture isolates obtained from each new TB case. While the agar proportion method is considered the gold standard for DST, a broth method is the recommended standard of practice in North America for DST.

*(Strong recommendation, based on moderate evidence)*

- Rapid molecular tests for DST should be reserved for patients with a high pretest probability of MDR-TB. The use of these tests should not eliminate the need for conventional culture and DST, which are recommended to confirm initial results and also detect resistance to drugs other than RMP and INH.

*(Strong recommendation, based on moderate evidence)*

■ ■ ■

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