Take-Home Message
All smear-positive respiratory specimens from potential TB index patients are now tested by real-time PCR for *M. tuberculosis* complex at the DSM WRHA microbiology laboratory sites (Health Sciences Centre and St. Boniface Hospital). This test rapidly and accurately diagnoses *M. tuberculosis* complex from respiratory specimens. As of January 2nd 2014, PCR-based testing for confirmation of *M. tuberculosis* complex in sputum specimens is now offered at the Westman laboratory using the GeneXpert™ system.

Tuberculosis Background
Tuberculosis (TB) remains one of the most prevalent infectious diseases worldwide, and although declining in most areas, regions such as Africa suffer from substantial morbidity and mortality and a high incidence of TB co-infection with HIV. In Canada, there have been consecutive declines in both incidence and mortality rates of TB since the 1950s, however, this is not true for some population groups. Foreign-born Canadians now account for over half of all cases, and the overall incidence rate is highest amongst Canadian-born aboriginals, with some groups having endemic and epidemics of TB disease. This trend is markedly more pronounced within Manitoba and Nunavut, where both the rates and number of TB cases among aboriginals are the highest in the country.

Laboratory diagnosis of TB typically takes longer than other infectious diseases due to the slow-growing nature of this microbe. Specialized methods are required to isolate and test *Mycobacterium tuberculosis* (MTB). Commercial molecular tests are now available to rapidly and accurately detect MTB complex directly from processed respiratory specimens, and this significantly improves the turn-around-time (TAT) for MTB diagnosis.

Culture-Based Testing in Manitoba
All specimens collected in Manitoba that request MTB testing are ultimately processed at either the Health Sciences Centre (HSC) or St. Boniface Hospital (SBH) microbiology laboratories. Specimens are rapidly screened for Mycobacteria via microscopy using a Kinyoun stain for acid-fast bacilli (AFB, or ‘AFB smear’) following the concentration and decontamination of the specimen, as appropriate. MTB represents the most commonly encountered acid-fast microbe, however a positive AFB smear is only suggestive of MTB, and this result does not differentiate between the possible *Mycobacterium* species (such as MTB, *M. avium* or the other ‘non-tuberculous’ Mycobacteria, or so-called ‘NTMs’).

All specimen types sent for AFB testing (except blood cultures and in-patient follow-up specimens) are simultaneously cultured in antibiotic supplemented Middlebrook 7H9 broth (MP bottles) on the automated BacT/ALERT® system and on solid Lowenstein-Jenson (LJ) media. An additional liquid culture in a second Middlebrook medium is also initiated for tissues, swabs and clotted fluid specimens. Please note that blood cultures for Mycobacteria require bedside inoculation in special media that is available by contacting the microbiology laboratory.

If the culture grows an AFB, the AccuProbe™ (GEN-PROBE™) hybridization assay is then used to identify MTB complex, *M. avium* and *M. gordonae*. The MTB complex includes: *M. tuberculosis*, *M. africanum*, *M. bovis* BCG, *M. microti*, *M. canettii*, *M. caprae*, and *M. pinnipedii*. The final identification of MTB and the other NTMs is performed by the National Reference Centre for Mycobacteriology at the National Microbiology Laboratory using DNA sequencing methods. Culture-derived results could take upwards of 6 weeks to obtain due to the slow-growing nature of many Mycobacteria. For patients who are not known to have a TB infection, but for which they are now suspected, the long wait for growth by culture can have significant implications for their treatment and also infection prevention and control activities.

Current Molecular Testing in the WRHA
The DSM microbiology laboratories within the Winnipeg Regional Health Authority (WRHA; HSC & SBH) have now implemented a real-time polymerase chain reaction (PCR) test that allows for rapid detection of MTB complex directly from concentrated specimens, soon after they are submitted to the laboratory (the expected TAT is within 24 hours on weekdays). All members of the MTB complex (listed above) should result in a positive test result using the molecular assay available in WRHA DSM laboratories. In North America, MTB is the predominant member of this complex (other species rarely cause infections).
Table 1. Performance of the WRHA TB real-time PCR test for smear-positive and smear-negative specimens.

<table>
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<tr>
<th>Smear Result</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>AFB positive (n = 89)</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>AFB negative (n = 130; 14 index)</td>
<td>78.6%</td>
<td>100%</td>
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Due to the excellent sensitivity and specificity with AFB smear-positive results, our current approach is to perform the MTB real-time PCR test on all new patients who have an AFB smear-positive result. This test result will allow rapid rule-in or rule-out of TB for smear-positive patients, and the overall approach is in line with the recently released Canadian Tuberculosis Standards (7th Edition). Notably, the lower sensitivity of the assay for AFB smear-negative specimens presents the possibility of a falsely negative PCR result from specimens that do in fact contain MTB; therefore, AFB smear-negative specimens will not receive the PCR test routinely. Testing will however be possible for high-risk new patients who have a smear-negative result after consultation with the on-call microbiologist by the Infectious Diseases or Respiratory Medicine services. The high specificity of this test (absence of false positives) indicates it can be used to rule-in TB.

Since commencement of testing in the WRHA, the performance of the MTB real-time PCR test has been congruent with culture findings. To date, 43 smear-positive sputum specimens have been tested: 40% had a negative PCR result and were also negative for MTB by culture; 60% had a positive PCR result and were also MTB culture positive.

Further Expansion of Molecular Testing in Manitoba
In order to provide rapid TB diagnosis capabilities at sites that do not locally perform MTB culture, the GeneXpert™ automated, cartridge-based nucleic acid amplification test (NAAT) for the MTB complex was implemented at the Westman (WL; Brandon) laboratory in January 2014. The GeneXpert™ platform will be used for testing unconcentrated sputum specimens, using a highly user-friendly instrumentation and protocol. This test will provide a rapid confirmation of positive TB status. Note that this test is not intended to rule-out MTB because the sensitivity during DSM test validation was 66.7% in AFB smear-negative patients, providing a potential for falsely negative results. Meta-analyses likewise concluded that smear-negative specimens have a reduced sensitivity to detect MTB.

Despite the addition of rapid molecular tests, our culture procedures remain unchanged, as a positive culture result for MTB provides definitive confirmation and also the opportunity to perform antibiotic susceptibility testing. For public health and epidemiologic purposes, MTB cultures are fingerprinted in collaboration with the National Microbiology Laboratory.

Summary
Why implement new MTB tests?
To support the rapid diagnosis of MTB disease directly from patient specimens. Such testing approaches are supported by the recently released Canadian Tuberculosis Standards (7th Edition).

What are the new MTB tests?
Molecular diagnosis of MTB by real-time PCR for rapid rule-in and rule-out of MTB disease for smear-positive patients; GeneXpert™ automated MTB NAAT for rapid rule-in of MTB.

Where are the DSM mycobacterial tests performed?
AFB smear and culture (HSC, SBH); MTB real-time PCR (HSC, SBH); GeneXpert™ automated MTB NAAT (WL)

Who are the patients that will be tested?
In the WRHA, the first AFB smear-positive specimen from a potential new case will be tested by MTB real-time PCR (smear-negative high-risk patients tested upon consultation only). At Westman Laboratory, the GeneXpert™ MTB automated NAAT will be performed on one sputum for patients not known to have TB. Note: MTB molecular testing cannot be used for assessment of treatment response.

What specimen types are tested by the MTB PCR?
MTB real-time PCR: Respiratory only (e.g. sputum, induced sputum, bronchial specimens, endotracheal secretions). GeneXpert™ MTB automated NAAT: Sputum only.

What are the specimen collection and transportation requirements?
Timing of sputum collection has been recently detailed in a Clinical Practice change (see ref. 7; briefly, initial diagnostic specimens can be collected the same day but follow-up specimens must be collected a minimum of 8-24 hours apart).

For expectorated sputum, use a sterile screw-capped container to collect 5-10 mL of sputum (minimum 3 mL). Transport the specimen to your local laboratory within 2 hours at room temperature, or if using a courier, within 48 hours at 4°C (less than 24 hours is optimal). For full details and for other specimen types, please see:
http://www.dsmcanada.ca/professionals/files/Policy_120-10-05.pdf

References and Related DSM Physician Alerts
3. Clinical practice change relating to AFB smear testing only for follow-up specimens from in-patients with known positive TB status (no culture will be initiated): http://www.dsmcanada.ca/professionals/files/TFollowup.pdf