2024 Mycobacteriology Laboratory Testing Summary

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Mycobacteriology, Diagnostic Services, Shared Health

Shared health Soins communs Manitoba

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Purpose

This summary presents information on specimens tested for *Mycobacterium* sp. by the Mycobacteriology laboratory, Diagnostic Services, Shared Health from January 1, 2024 to December 31, 2024. This summary aims to describe the laboratory testing volumes, test performance criteria and result turnaround times. Turnaround times are evaluated on a 7 day a week, 24 hour a day schedule; however, the Mycobacteriology laboratory is only operational Monday to Friday, 0745 to 1600.

Testing Overview

All patient samples in Manitoba for which acid-fast bacilli (AFB)/Mycobacteria testing is requested are sent to the Health Sciences Centre for processing by the Mycobacteriology laboratory. Every AFB sample (except CSF, blood and bone marrow) is processed by concentration and decontamination, evaluated by microscopy for the presence of AFB and is inoculated into liquid media and onto solid media for culture. The standard inoculated media include BD BACTEC™ mycobacterial growth indicator tubes (MGIT™) and Löwenstein–Jensen (LJ) agar. MGIT™ tubes are continuously monitored for growth on the BD BACTEC™ MGIT™ automated mycobacterial detection system and LJ agar slants are inspected manually for growth. CSF samples are evaluated for AFB by direct culture of patient samples (i.e., without concentration and decontamination) using MGIT™ tubes and LJ agar slants. Blood and bone marrow samples are inoculated into BD BACTEC™ Myco/F Lytic culture tubes and evaluated for growth on the continuous monitoring BD BACTEC™ FX blood culture system.

AFB smears are reported semi-quantitatively (Negative or 1+ to 4+ AFB) following sample processing. The majority of mycobacterial cultures are incubated for 7 weeks. As soon as growth is detected in the MGIT™ tube or on the LJ agar slant, organism identification is attempted. Positive *M. tuberculosis* complex isolates are identified using an antigen-based assay (Bioline™ TB Ag MPT64). Nontuberculous mycobacteria (NTM) are identified by MALDI-ToF.

M. tuberculosis complex are routinely referred to the National Reference Centre for Mycobacteriology-National Microbiology Laboratory (NRCM-NML) for antimicrobial susceptibility testing and mycobacterial interspersed repetitive unit (MIRU) typing. Antimicrobial susceptibility testing is performed using growth-based phenotypic methods, providing susceptible and resistant results, and by molecular methods, which identify mutations that are associated with antimicrobial resistance and predict susceptible and resistant phenotypic results. Antimicrobial susceptibility testing for NTM isolates may be requested by consultation with the on-call Shared Health Microbiologist.

All microscopy smear-positive AFB samples receive a nucleic acid amplification test (NAAT), excluding blood, stool, mucosal and skin samples for which the NAAT is not validated. AFB smear-negative samples require a microbiologist consult if a NAAT is requested. The in-house NAAT detects *M. tuberculosis* and *Mycobacterium* sp. DNA. For patients with risk factors for rifampin-resistant/MDR tuberculosis, NAAT by GeneXpert® may be requested. The Xpert® MTB/RIF assay detects *M. tuberculosis* and rifampin-resistance associated mutations.

Sample collection instructions are detailed in the Laboratory Information Manual: https://apps.sbgh.mb.ca/labmanual/test/findTestPrepare

2024 Testing Results

The Mycobacteriology laboratory, Diagnostic Services, Shared Health received 16127 samples for AFB testing from January 1, 2024 to December 31, 2024. The number of samples by specimen type was as follows: 94 blood cultures, 46 bone marrow samples, 249 cutaneous samples, 130 stool samples, 2418 fluid samples, 1526 tissue samples and 11664 respiratory samples. The average transport time from collection to receipt in the laboratory was 1.5 days, with 55.1% and 73.3% of samples received within 1 and 2 days, respectively. The average turnaround time from sample receipt to AFB smear report was 1.0 days, with 63.4% and 83.6% completed within 1 and 2 days, respectively.

Of the 16127 samples, 1153 were positive cultures collected from 492 patients. 629 of the 1153 samples were positive for *M. tuberculosis* complex and 524 grew a NTM. The 629 *M. tuberculosis* complex-positive samples were identified from 169 patients. The average time from sample receipt to the identification of *M. tuberculosis* complex isolates from initial diagnostic samples was 15.3 days (range: 2 - 46 days), with 79.7% identified within 21 days. For smear-positive index samples, the average time to detection of *M. tuberculosis* was 8.9 days (range: 4 - 17 days). For smear-negative index samples, the average time to detection of *M. tuberculosis* was 20.7 days (range: 9 - 47 days). NTM alone were identified from 323 patients. The NTM identified were comprised primarily of *Mycobacterium avium-intracellulare* complex (MAC) (grown from 242 patient samples), *Mycobacterium gordonae/paragordonae/vicigordonae* (58 patients, alone or in combination with other NTMs) and lower numbers of *Mycobacterium abscessus* (10 patients), *Mycobacterium lentiflavum* (4 patients), *Mycobacterium fortuitum* group (3 patients) and other species (1 or 2 patients each).

NAAT was completed on 247 samples with an overall test sensitivity of 89.5% and specificity of 100%. The overall sensitivity and specificity of the in-house NAAT (90.4% and 100%, n= 183) and Xpert® MTB/RIF assay (87.5% and 100%, n=64) were similar. The sensitivity and specificity of the smear-positive samples (n=97) was 100%. The sensitivity and specificity of the smear-negative samples (n=149) was 64.5% and 100%, respectively. Accordingly, it is important to

emphasize that NAAT cannot be used to rule-out *M. tuberculosis*, particularly in a smear-negative sample. The average time from specimen receipt to NAAT report for on-protocol samples (ie. smear-positive) was 2.7 days (range: 1 – 5 days), with 55.4% completed within 2 days.

Of the 177 *M. tuberculosis* complex-positive patients' samples (index and reactivation) referred to the NRCM-NML for antimicrobial susceptibility testing and MIRU typing in 2024, 162 (91.5%) were susceptible to the first-line agents, 7 (4.0%) demonstrated mono-resistance to isoniazid (5 low-level and 2 high-level resistance), 3 (1.7%) demonstrated mono-resistance to the fluoroquinolones, ofloxacin and moxifloxacin, and 1 (0.6%) was pre-extensively drug resistant (resistant to rifampin, isoniazid, moxifloxacin and ofloxacin, as well as ethambutol, ethionamide, rifabutin and streptomycin). Four isolates were reported as resistant to pyrazinamide; however, pyrazinamide testing could not be evaluated as a phenotypic testing recall was issued by the manufacturer in 2024. The molecular prediction of resistance for isoniazid (n=8) and rifampin (n=1) demonstrated 100% correlation with the phenotypic resistance results. The correlation of susceptibility predictions by the absence of mutations correlated with phenotypic susceptibility results 99.4% of the time. One isolate with no resistance mutations was resistant to moxifloxacin and ofloxacin. The average turnaround time from the identification of *M. tuberculosis* complex to receipt of the molecular susceptibility results was 16.7 days (range: 6 – 64 days). The average turnaround time from the identification of *M. tuberculosis* complex to receipt of the phenotypic susceptibility results was 22.0 days (range 9 – 95 days), with 41.2% completed within 17 days.

2024 MTB Testing Results Summary

Sample volume	16127
Positive cultures	1153
Positive MTB cultures	629
Positive MTB patients	169
MTB Susceptible to 1st line agents	91.5%
MTB Monoresistance	7.9%
MTB Pre-XDR	0.6%
Average TAT collection to receipt	1.5 days
Average TAT receipt to AFB smear	1.0 days
Average TAT receipt to NAAT	2.7 days
Average TAT receipt to MTB Identification	15.3 days
Average TAT genotypic susceptibility testing	16.7 days
Average TAT phenotypic susceptibility testing	22.0 days
NAAT sensitivity/specificity overall	89.5%/100%
NAAT sensitivity/specificity smear-positive samples	100%/100%
NAAT sensitivity/specificity smear-negative samples	64.5%/100%

Pre-XDR, Pre-extensively drug-resistant TAT, turnaround time; NAAT, nucleic acid amplification test

For questions related to the Mycobacteriology laboratory, please contact
Dr. Heather Adam (hadam@sharedhealthmb.ca; 204-787-8678) or
Dr. James Karlowsky (jkarlowsky@sharedhealthmb.ca; 204-237-2105)