Mycobacteriology Questions Asked of Diagnostic Services Manitoba, 2011-2015

Introduction

The following document contains a summary of some of the correspondence between the Mycobacteriology section lead microbiologists with Diagnostic Services Manitoba (DSM) and individuals working with Manitoba Public Health between 2011 and 2015 regarding DSM’s mycobacteriology practices and procedures (http://www.dsmanitoba.ca/). The questions have been edited for clarity. This documented was last updated on July 20, 2015.

This document is for general information only; questions pertaining to specific samples should be directed to the Microbiologist-on-call via paging (204-787-2071).

Please contact Dr. Heather Adam, Mycobacteriology section lead microbiologist, with any additional questions. Dr. Adam can be contacted by phone: 204-787-8678 or by email: hadam@dsmanitoba.ca
Specimen Collection Questions:

Q1. Where can I obtain a DSM Microbiology requisition?
A. Requisitions are available online under Clinical Microbiology at:
https://apps.sbgh.mb.ca/labmanualviewer/findRequisitions.action

Q2. Where should Microbiology specimens be dropped off in the Winnipeg Health Region?
A. It is recommended by DSM and Cadham Provincial Laboratory (CPL) that specimens be submitted to Microbiology Laboratory at the Health Sciences Centre (HSC). This process is preferable to dropping off specimens at CPL as CPL has to forward any specimens they receive to HSC.

Specimens can be delivered to:

Department of Clinical Microbiology
Health Sciences Centre
MS6 - 820 Sherbrook Street
Winnipeg, Manitoba R3A 1R9
Phone: 204-787-1273

Hours of operation: 8:00 am - 11:30 pm (seven days a week)

Medical couriers or healthcare workers can drop off samples. Individual clients cannot drop off their own specimens at the laboratory.

Specimens should be delivered at the HSC Microbiology Laboratory prior to 11:15 PM so that they can be stored appropriately. After hours, specimens should be submitted to central accessioning on the fifth floor at HSC and will be stored appropriately until the next day.

Q3. Where do other community hospitals submit their specimens?
A. All the community hospitals (other than Seven Oaks) submit all of their microbiology work (including specimens for Mycobacterium tuberculosis culture) directly to the St. Boniface Hospital (SBH) Microbiology Laboratory. Seven Oaks General Hospital sends all of their microbiology work directly to HSC.

Q4. On the DSM "Clinical Microbiology Laboratory Test Requisition" there is a section on the left hand side that says "Copy to". Does this need to be a physician or could the name of another healthcare worker (e.g., a Communicable Disease Coordinator or Public Health Nurse) be included?
A. In the "Copy to" section of the requisition, the contact information for any healthcare professional can be entered. This option is available to Communicable Disease Coordinators and Public Health Nurses. However, a secure fax number must be included and it must be in the computer system (all the physician faxes are already in the system). Staff should contact the Microbiology laboratory to ensure their secure fax number is in the system. Staff should be aware that they will get every report generated for the client (i.e., all the interim reports as well as the final report) if they have been included as a "copy to".

This approach will be used during potential outbreaks for M. tuberculosis; the name and secure fax of the "point person" (e.g., Communicable Disease Coordinator) can be used in the "Copy to" section of the requisitions.
Q5. When three morning sputum samples are collected from a client in the community on consecutive days, do the samples need to be brought into the lab right away, or can the samples be placed in a client's home fridge for several days? How significant an issue is this? Roughly how much of a drop in sensitivity is there for a *M. tuberculosis* culture kept in the fridge for a few days?

A. DSM recommends that each sputum sample be submitted to the lab as soon as it is collected – this applies to the WRHA as well as all rural health authorities and First Nations Inuit Health Nursing Stations. Within the WRHA, DSM recommends all specimens be submitted to the Microbiology lab within 24 hours as this ensures optimal organism recovery. Specimens transported from distant locations should be received within 48 hours.

Unfortunately for rural sites, the transit times are usually significantly longer than 24 hrs. The remote rural nursing stations often hold the sputum samples until they receive all three and then send them all together. It is better if the samples are sent as they are received (i.e., with the regular daily runs).

For example, if one sample is collected on Tuesday, another on Wednesday, and another on Thursday, it is better if each sample is sent out with the regular run of specimens on the day it was collected. This will improve the turnaround time for AFB smear results. The sample sent on Tuesday should arrive in the lab on Wednesday and the AFB smear will subsequently be available on Thursday. If all three samples were sent on Thursday, they wouldn’t arrive in the lab until Friday and then AFB smear results for all three samples would not be available until Monday the following week. However, if all specimens are collected on the weekend, they can be kept in the fridge and sent together on Monday.

Although the number of AFB will decrease over time, DSM concentrates the sample for culture and mycobacteria are hardy organisms that do not deteriorate as rapidly during transit as other more fastidious bacteria. Accordingly, small transit delays should have little impact on specimen quality.

An article that provides data on the decrease in AFB smear and viability results over time is: Banda HT et al. "Viability of stored sputum specimens for smear microscopy and culture." Int J Tuberc Lung Dis 2000;4:272-274.

The authors found that the AFB smear results were not affected for samples that were held up to eight weeks; however, culture results deteriorated after 7 days of transit/storage. The viability of the *M. tuberculosis* culture decreases over time, but very slowly. Approximately 39% of sputum samples that were originally culture positive remained culture positive after four weeks at room temperature while 67% of the same samples remained culture positive after four weeks when stored in the fridge.

Accordingly, the DSM laboratory will accept specimens for *M. tuberculosis* culture even after seven days of transit, but a comment is added indicating that the prolonged transit may affect results.

In summary:
- AFB microscopy is stable for long periods of time
- Transit delays of a few days will not change the culture or AFB smear results
- Transit delays will increase the turnaround time for the AFB smear results
- DSM recommends that each specimen should be submitted to the Microbiology lab as soon as it is collected

Q6. If a sputum sample is collected on Friday or the weekend and dropped off at a laboratory, is the sample processed in any way or is it simply kept in the fridge until Monday?

A. Specimens received by the HSC laboratory after Friday morning or on Saturday and Sunday will be kept in a fridge until Monday when they will be processed.
**Culture Questions:**

**Q1.** What is the correct terminology to use when referencing *M. tuberculosis* specimens on guideline documents?

A. The correct term is “Mycobacterial culture (AFB)”, which is the same terminology that is used on the DSM Microbiology Requisition. The term “AFB” alone (e.g., “...collect AFB x 3...”) is not proper terminology. Alternatively, the guideline could include a statement such as "Mycobacterial culture (AFB)’ as indicated on the test requisition is defined as ‘AFB culture’ in this document".

**Q2.** Recently, three samples that all had insufficient volume were pooled into one. When is this appropriate practice?

A. Pooling samples is highly discouraged and would be performed only in exceptionally rare circumstances. Only in special situations and in consultation with the Mycobacteriology Section Lead, Clinical Microbiology Discipline, DSM, can pooling of suboptimal sputum samples be arranged. In the most recent situation, several contacts in an outbreak community were asked to provide sputum samples. The contacts submitted several sample containers with about 1-2 mL (or less) of what looked like saliva. After further correspondence with the First Nations Inuit Health Branch Manager, a decision was made that the multiple saliva samples would be pooled as one sample and an attempt would be made to get at least one induced sputum sample from the contacts to ensure that screening was optimal. This was a one-time response to ensure that the maximum number of contacts was screened with at least one specimen. Pooling of samples was only permitted when the combined volume was more than 3 mL. This was a unique situation that should not be considered routine and does not ensure optimal testing.

**Q2a.** Do we treat the pooled sample as a completely unique "fourth sample" (even though it has the same lab number as one of the other samples)?

A. This pooled sample should be considered sample number 1. The rejected sample 2 and 3 should not be included in the count of specimens submitted. If necessary, additional samples should be submitted on these clients. If additional samples are required, sputum induction should be considered.

**Q2b.** How should healthcare workers handle documenting the original three insufficient sample reports, should they be completely ignored?

A. Sample 2 and 3 should be referred to sample 1 and considered part of the first sample (i.e., ignore these as they do not count as samples that were individually cultured).

**Q2c.** Is the result from the sample created through pooling valid and to be considered a single authentic sample?

A. The result from the pooled sample is valid and should be considered as Sample 1.

**Q2d.** In general what does DSM think of this method? Should it be used in the future by the lab if a similar situation were to arise?

A. The laboratory could have justified rejecting all of the insufficient samples in question. The pooling was a one-time effort to ensure there was at least one sample cultured for certain high-risk contacts. However, it does raise the bigger issue of how samples are acquired from asymptomatic contacts. If asymptomatic contacts are given sample containers but are not able to produce specimens or are submitting inadequate specimens, induced sputum samples should be sought if sputum collection is indeed required. The submission and processing of suboptimal samples risks reporting false-negative results and missing active cases of tuberculosis.
Q3. The following is a sputum specimen report that was received. What does the culture result mean?

![Sputum Specimen Report]

A. This scenario occurs when the patient is already known to be positive for *M. tuberculosis* and subsequent cultures (not part of a batch of follow-up specimens but from the original set) grows an acid-fast bacilli. Under certain conditions, the second or third positive cultures that are growing an acid-fast bacilli are referred to the original one that has already been confirmed as *M. tuberculosis*. Accordingly, the culture result is reported as "acid-fast bacilli", indicating that the organism has grown on this culture and providing the reference culture in which the *M. tuberculosis* work-up was completed and the identification was first reported.

*M. tuberculosis* identification and susceptibility results are referred to a previous culture if the new specimen is AFB positive, is the same type of specimen and was collected within 3 months of the culture for which full work-up was performed.

Complete identification of AFB positive specimens must be performed, but susceptibility results are referred to a previous culture, for *M. tuberculosis* from specimens received from a different healthcare location or different specimen source (e.g., respiratory versus lymph node) within 3 months of the culture for which the original identification and susceptibility testing was performed.

Q4. A laboratory report states “Non-tuberculous mycobacteria” as the culture result. What does this mean?

A. If the culture report says “Non-tuberculous mycobacteria”, an acid-fast bacilli has been isolated that is not *M. tuberculosis*, *Mycobacterium avium* complex or *Mycobacterium gordonae*. The final identification of this organism will be performed by the NML and the DSM report will be updated as soon as that information is available. Final identifications are usually reported within 1 week of the non-tuberculcous mycobacteria report.

Q5. An AFB smear-positive client is on home isolation in the community. Follow-up sputum samples are collected to determine if the client has now converted to being AFB smear-negative. In this situation only an AFB smear result is required, a *M. tuberculosis* culture result is not required. What should be written on the specimen requisition?

A. Include the phrase "Follow-up sample, culture not needed" on the DSM requisition. This will instruct the laboratory that a culture is not required for that particular specimen. If such a phrase were not included on the requisition, the
laboratory would automatically perform both smear and culture.

For clients admitted to WRHA facilities, if the client is already known to the laboratory as being *M. tuberculosis* positive, the *M. tuberculosis* culture will be omitted on follow-up sputum samples. Ideally, the wards will also include the phrase “follow-up sample” on the requisition. In this setting, it would still be possible to request culture on these specimens as a portion of the processed specimen is kept frozen for further testing.

**Q6. If a mycobacterial report was issued but later needs to be corrected, how is this handled?**

A. To ensure adequate traceability for Medico-legal reasons, the DSM Microbiology lab cannot completely delete any portion of a patient report after it has been sent; however, reports can be updated or corrected as necessary. The revised or updated information will be noted in addition to the original report. A comment will be added to the report to indicate that the report has been revised. Please note that this will generate a new copy of a report. Thus, it is essential that all reports be reviewed thoroughly even if it appears to be a “duplicate” report.

**Q7. Can you comment on Manitoba’s rates of laboratory mycobacterial cross-contamination?**

A. In the past few years, DSM has been much more proactive about suspecting cross-contamination and has implemented a standard proactive critique of any *M. tuberculosis* positive culture that occurs in the later stages of incubation. If cross-contamination is suspected, DSM will contact the physician of record to review the clinical picture and also expedite the Mycobacterium Interspersed Repetitive Units (MIRU) genetic typing. Rates of Manitoba cross-contamination are likely less than they were several years ago prior to the establishment of a formal proactive process. Rates of cross-contamination have not been consistently evaluated to provide a specific numerical answer.

**Q8. A small child mistakenly had the BCG vaccine administered intramuscularly in the Vastus lateralis and the site formed a pustule. The attending physician sent a swab for Mycobacterial culture (AFB) which came back as *M. tuberculosis* complex. The final culture came back as *Mycobacterium bovis*. Usually the lab will specify that it is the “BCG strain’ but this report does not do that. Will the culture be confirmed as “BCG strain’?**

A. NML does differentiate between *M. bovis* and BCG strains. NML will complete the final identification on this isolate and DSM will provide this report as soon as it is available. Future reports will indicate that the final identification is pending.

**Q9. Why is there no smear result reported when bone marrow is tested for *M. tuberculosis*?**

A. Bone marrow is collected by a Hematopathologist and is treated by the laboratory like a blood sample; the entire specimen is used to inoculate the broth culture media as well as the Löwenstein–Jensen (LJ) agar slants for culture for mycobacteria. Because the load of acid-fast bacilli (AFB) would be very low, a direct AFB smear is not done on these samples. The interim and final reports will not show any results for an AFB smear.

**Q10. Are there any other specimen types that cannot have microscopy for AFB done?**

A. For blood samples, an AFB smear would not be done due to the low load of AFB in such samples.
Antimicrobial Susceptibility Questions:

Q1. When is genetic susceptibility testing done on an AFB isolated from a sputum sample?

A. The genetic resistance testing is performed on a culture that has grown an acid-fast bacilli that is probe positive for *M. tuberculosis* complex. The culture that is growing the *M. tuberculosis* complex isolate is sent from the DSM laboratory to the NML. Typically, within three to seven days of the NML receiving the isolate, genetic susceptibility testing is performed for isoniazid (INH), rifampin, pyrazinamide, and ethambutol.

Q2. INH susceptibility is tested at the 0.1 mg/L and 0.4 mg/L levels. Is testing ever conducted at the 1.0 mg/L threshold, a level that is reported in some literature?

A. The 1.0 mg/L (or μg/mL) test cut-off listed in some literature is based on solid agar media testing for *M. tuberculosis* (e.g., Löwenstein–Jensen agar media) and this concentration represents the critical concentration for high-level INH resistance (for solid media). As stated in the Clinical and Laboratory Standards Institute (CLSI) guidance document, the 1.0 mg/L in agar media (reference method) is equivalent to the 0.4 mg/L in the liquid media. The method currently in use for DSM is the Mycobacteria Growth Indicator Tube (MGIT) liquid broth based system. In that system, the 0.4 mg/L is the level needed to predict the higher level of resistance. As such, resistance using the MGIT broth system at 0.4 mg/L is equivalent to resistance at 1.0 mg/L in the agar dilution method.

Although the agar dilution method is the reference method, it is very slow and the CLSI recommends that diagnostic laboratories should use the more rapid broth methods.

Q3. Can you explain the two thresholds used to report resistance to INH?

A. The current practice is to test INH at 0.1 mg/L and 0.4 mg/L to detect low and high level resistance of the isolate, respectively, as per the CLSI guidelines and the MGIT manufacturer’s guidelines. If the strain is susceptible at both testing levels, it is reported as “Susceptible” and if it is resistant at both levels, it is reported as “Resistant”. If it is resistant at 0.1 mg/L but susceptible at 0.4 mg/L, both results are reported. This allows the clinician to determine if treatment of these low-level resistant strains can be continued. This is reflected in the Canadian Tuberculosis Standards 6th edition: Chapter 2, page 27 which states: “When resistance is encountered to the critical concentration of INH by the methodology used, the CLSI recommends testing at a higher concentration. If the isolate is found to be susceptible at a higher concentration, this could indicate low-level or emerging resistance.”

In the instance of low and high-level resistance, DSM Microbiology adds the following comments to the reports:

If susceptible to 0.4 mg/L but resistant to 0.1 mg/L:

"The INH results indicate low-level resistance to INH. Patients infected with strains exhibiting this level of INH resistance may still benefit from continuing therapy with INH. A specialist in the treatment of tuberculosis should be consulted concerning the appropriate therapeutic regimen and dosages. Follow the Infection Prevention & Control Guidelines for *M. tuberculosis*.”

If resistant to both 0.1 mg/L and 0.4 mg/L:

"These test results indicate high-level resistance to INH. A specialist in the treatment of tuberculosis should be consulted concerning the appropriate therapeutic regimen and dosages. Follow the Infection Prevention & Control Guidelines for *M. tuberculosis*.”
Molecular Questions:

Q1. What is GeneXpert® testing?

A. The GeneXpert® MTB/RIF is a cartridge-based, automated diagnostic test that can identify *M. tuberculosis* and resistance to rifampin. The test detects DNA sequences specific for *M. tuberculosis* and rifampicin resistance by polymerase chain reaction (PCR). The test purifies and concentrates *M. tuberculosis* from sputum samples, isolates genomic material from the captured bacteria by sonication and subsequently amplifies the genomic DNA by PCR, identifying all the clinically relevant rifampin resistance-inducing mutations. Results are obtained from unprocessed sputum samples in 90 minutes with minimal biohazard and use of the instrument requires very little technical training. Although the test evaluates rifampin resistance, DSM does not report these results as they have not been validated by DSM and the test has an unacceptable number of false positives in low prevalence populations. Our population has a low prevalence of antimicrobial resistance.

Q2. When did rapid *M. tuberculosis* PCR tests start?

A. The start date for the rapid *M. tuberculosis* PCR on AFB smear positive index patients was Feb 25, 2013 (please refer to the DSM Memo outlining this change).

Q3. Are rapid *M. tuberculosis* PCR tests being done only on respiratory samples?

A. The testing is only done on AFB smear positive respiratory specimens from index patients (i.e., first time the patient is diagnosed with *M. tuberculosis*). The test has not been validated for any other type of specimen. The test has been cleared for use and added to the site test menus at HSC, SBH and Brandon Regional Hospital. The test has very high sensitivity and specificity (≥ 95%); however, Infection Prevention and Control have asked that decisions to discontinue airborne precautions when the direct *M. tuberculosis* PCR is negative be made cautiously. The concept of ensuring that the clinical features are considered in conjunction with the *M. tuberculosis* PCR results when deciding what to do regarding airborne isolation precautions was included in the DSM memo when this test was introduced into practice.

Q4. Can a PCR be requested on a sample that would not normally be tested at this time (e.g., an AFB smear negative sputum sample submitted from a suspicious contact during an investigation)?

A. A physician can contact the Microbiologist-on-call. The Microbiologist will arrange testing if it is warranted based on the particular situation.

Q5. Can the rapid *M. tuberculosis* PCR performed directly on specimens be used to rule out *M. tuberculosis*?

A. No. Due to the low load of mycobacteria in most specimens and other limitations of molecular testing, a negative *M. tuberculosis* PCR result from a direct specimen does not definitively rule out *M. tuberculosis*.

Q6. What is the better test culture or PCR?

A. Culture is always the gold standard for Mycobacterial testing. We need to grow the organism to perform susceptibility testing. As culture is the gold standard, DSM will only approve molecular testing if culture-based testing is also performed.