Mycobacteriology Questions Asked of Diagnostic Services Manitoba, 2011-2014

Introduction

The following document contains correspondence between Dr. Patricia Simner and public health over the years regarding Diagnostic Services Manitoba (http://www.dsmanitoba.ca/) practices and procedures. Dr. Simner reviewed the document June 13th, 2014. The questions have been edited for clarity and to reduce repetitiveness.

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For questions about samples or requests for additional testing of a sample contact the Microbiologist on call during working hours (8:00-16:00), depending on where the sample was sent:

Health Sciences Centre: (Paging) 204-787-2071
St. Boniface General Hospital: (Paging) 204-237-2053

Q1. What is the correct terminology to use when referencing TB 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. 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

Q3. Where should Microbiology specimens be dropped off in the Winnipeg Health Region?

A. Dr. Patricia Simner (Clinical Microbiologist, Section Lead for Mycobacteriology, Diagnostic Services Manitoba) and Dr. Paul Van Caeseele (Director Cadham Provincial Laboratory) both indicated dropping off specimens at HSC Microbiology is strongly preferred over dropping off specimens at Cadham Provincial Laboratory. Cadham Provincial Laboratory 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: 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 should not drop off their own specimens at the laboratory.
If specimens are dropped off at the Microbiology Laboratory at HSC they need to be brought to the lab by at least 11:15 PM so that they will be placed in the fridge. Usually after hours specimens go to central accessioning on the fifth floor at HSC and are stored appropriately until the next day.

Q4. 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 TB culture) directly to the St. Boniface General Hospital Microbiology Laboratory. Seven Oaks General Hospital sends all of their microbiology work directly to HSC. None of the community hospitals send TB specimens to Cadham Provincial Laboratory.

Q5. The following is a sputum specimen report that was received. What does the culture result mean?

A. This scenario occurs when the patient is already known to be positive for Mycobacterium tuberculosis (MTB), and subsequent cultures (not part of a batch of follow-up specimens, but rather 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 MTB. So under Culture the term "acid-fast bacilli" is intended to indicate what has grown and the text under it provides the Lab Number of the MTB specimen that has already been reported on this client. The following section of the Protocol Manual outlines this process:

Mycobacteria Positive Cultures – Subsequent Specimen Referral in the Lab

The criteria listed below are for referring subsequent positive cultures from the same patient.

MTB Complex:

If a patient has 3 consecutive respiratory samples submitted (i.e., one per day) and one is probe positive for Mycobacterium tuberculosis complex (MTBC) and the others have cording AFB, they can be referred to the first specimen for identification and sensitivities (AST). Note: Respiratory samples include; sputum, endotracheal, BAL, induced sputum.

If a patient has a sample that is probe positive for MTBC and subsequent samples are submitted and become positive, the following protocol applies:

- Within 3 months of previous positive → refer identification and AST to previous report. AST is performed ONLY on the first isolate. Note: It must be the same type of sample, i.e., respiratory
respiratory (samples may come from different health care facilities). Add comment: Refer to specimen number that was probe positive for MTBC for ID and AST.

- For different types of samples (e.g., respiratory versus sterile fluid) → MTBC probe must be done but sensitivities (AST) can be referred to the first isolate. Add comment: Refer to specimen number that was previously MTBC probe positive for AST.

- If >3 months since the previous positive → work-up as per usual (identification and sensitivities (AST)).

- If a sample is received from a different site (e.g., first from Garden Hills and subsequently from HSC), DNA probe will be performed on the second isolate but the AST will not be performed. A comment is added to later cultures to the probe result that is reported and the comment says “Susceptibility testing performed on isolate from Garden Hill Nursing station on (date)”.

Q6. A laboratory report states under culture: “Acid fast bacilli”. What does this mean?

A. If under Culture DSM reports “acid fast bacilli”, this means “acid fast bacilli grown, not MTB, MAC or M. gordonae”.

If there are AFB in the direct specimen smear, this is reported to Manitoba Health. When the corresponding culture grows AFB but they are probe negative for MTB, MAC and M. gordonae probes, the isolate is sent to NML for identification. The length of time it takes to get the final identification back depends on NML workload. They do their identification by sequencing so in normal circumstances identification should not take too long. DSM will start to include additional information on the preliminary report, for example: “Acid Fast bacilli grown, Not MTB, MAC or M. gordonae, sent to reference lab for identification.” In addition, DSM will send a copy of the final report to Manitoba Health on those specimens that were AFB smear positive, but grew an NTM.

Q7. What does it mean when a laboratory report indicates “un-identified organism”?

A. Generally speaking this represents some other non-mycobacterial organism that also grew in the culture.

Q8. When is genetic susceptibility testing done on an AFB isolated from a sputum sample?

A. The genetic resistance testing currently done on a culture that has grown an acid fast bacilli (AFB) that is probe positive for MTB complex. The broth culture (BacT/ALERT® bottle) that is growing the MTB complex
isolate is sent from the DSM laboratory to the National Reference Centre for Mycobacteriology (NRCM, http://www.nml-lnm.gc.ca/eb-be/myco-eng.htm) at the National Microbiology Laboratory (NML). Once received by the NML lab, the positive BacT/ALERT® culture is subcultured to their test media (Mycobacteria Growth Indicator Tube, or MGIT). Typically within three to seven days of NML receiving the isolate, genetic susceptibility testing is performed for isoniazid (INH), rifampin, and pyrazinamide (the ethambutol testing is done as well but these results are not reported, as they suffer from poor correlation with culture-based resistance results).

Q9. What is GeneXpert® testing?

A. The GeneXpert® MTB/RIF is a cartridge-based, automated diagnostic test that can identify Mycobacterium tuberculosis (MTB) and resistance to rifampin (RMP). The test detects DNA sequences specific for Mycobacterium tuberculosis and rifampicin resistance by polymerase chain reaction (PCR). The test purifies and concentrates MTB 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 very little technical training required to operate.

GeneXpert® testing includes rifampin (RMP) genetic resistance detection (this is the only anti-mycobacterial agent that is tested directly). We cannot do this with the current "Rotor-Gene" direct test that we are using for AFB smear positive specimens. The Rotor-Gene PCR method is an "in-house" validated method for detection of MTB complex (i.e., for the organism) and it does not include RIF resistance detection. In order to realistically offer RIF genetic resistance testing on the direct specimen, we need to implement GeneXpert® testing. Although we could theoretically in-house validate RIF genetic resistance, it would take us forever to do this as we don't get a lot of RIF resistance.

Q10. In the current system it may take several weeks from the time a specimen is first obtained to the time genetic susceptibility testing results are available. The GeneXpert® TB PCR test is able to provide rifampin genetic resistance testing, and when used on a sample collected from a client, results can be made available within hours. Can GeneXpert® be used in the Winnipeg Health Region to discover rifampin resistance soon after the sample is collected from the client?

A. Yes. Once the GeneXpert® TB PCR test is implemented in the Winnipeg Health Region, rapid genetic resistance testing for rifampin will be available. The first step will be setting up a GeneXpert® TB PCR unit at the Health Sciences Centre Microbiology Laboratory (there is one at St. Boniface General Hospital, and one at Westman Regional Laboratory in Brandon which were put in place for Influenza testing but HSC does not have a GeneXpert® unit at present).

Another issue to address with this testing relates to false positive rifampin resistance in low prevalence populations. Because of this the clinician receiving the genetic resistance testing for rifampin would need to appreciate this limitation; the positive predictive value of the test is lower when the prevalence of the condition is low. It may be wise to recommend that until we have more experience with rifampin genetic resistance results from GeneXpert®, the results should be treated in a similar fashion to genetic resistance on the isolate. That is to say, if rifampin resistance is detected by GeneXpert® on direct respiratory specimens, second line agent(s) can be added to the client's therapy, but medications currently being used should be continued until the culture based rifampin and INH resistance results are obtained.

NOTE: The new Canadian TB Guideline (2013) recommends treating the GeneXpert® positive result for rifampin resistance with caution.

Q11. 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 TB culture result is not required. What should be written on the specimen requisition? [May 2013]

A. Include the phrase "Follow-up sample, culture not needed" on the DSM requisition; this will instruct the laboratory that for that particular specimen a culture is not required. If such a phrase were not included on the requisition, the laboratory would automatically test using both smear and culture.
For clients admitted to WRHA facilities, if the client is known already to the laboratory as being TB positive, automatically the TB culture will be omitted on follow-up sputum samples. In this example it would still be possible to request culture on these specimens as there is a portion of the processed specimen that is kept frozen.

Q12. When did rapid TB PCR tests start?
A. The start date for the rapid TB PCR on AFB smear positive index patients was Feb 25, 2013 (please refer to the DSM Memo outlining this change).

Q13. Are rapid TB PCR tests being done only on respiratory samples?
A. The testing is only done on respiratory specimens from index patients (i.e., first time diagnosed with TB). 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, SBGH and Brandon Regional Hospital. Infection Prevention and Control have asked for caution regarding the need for isolation precautions if the direct TB PCR test is negative on patients who are AFB smear positive since [as of Feb 2013] the test has only been evaluated on about 50 AFB smear positive patient samples. However, the data showed the TB PCR to be 100% sensitive and specific for this group. This concept of ensuring the clinical features are considered in conjunction with the TB PCR results when deciding what to do regarding airborne isolation precautions is included in the memo and once DSM has collected more extensive data, this recommendation may be revised.

Q14. Can a PCR be requested on a sample that would not normally be tested at this time (e.g., a sputum sample submitted from a suspicious contact during an investigation)?
A. A physician can contact the Microbiologist on call at HSC or SBGH; the Microbiologist will handle the call as a “consult” and the test can be arranged. If it is not possible for the site contacted to do this testing then the site can contact NML and ask NML to do PCR testing on the specific patient specimen.

Q15. 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. A secure fax number must accompany the name, as this is the way the report is sent out through the computer. This option is available to Communicable Disease Coordinators and Public Health Nurses. However, the fax needs to be a secure fax 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 would get every report printed for the client (i.e., all the interim reports as well as the final report).

This approach will be used during “potential outbreaks” for TB; 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.

Q16. 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 TB culture kept in the fridge for a few days?
A. DSM recommends that each sputum sample should be submitted to the lab as soon as it is collected – this applies to WRHA as well as all rural health authorities and First Nations Inuit Health Nursing Stations. Within the WRHA (both community and healthcare facilities) DSM recommends all specimens reach the Microbiology lab within 24 hours as this ensures optimal recovery.

DSM States in its Clinical Microbiology Sample Collection Manual (May 2011): 
“Specimens transported locally should be received in the processing laboratory within 24 hours and those transported from distant locations should be received within 48 hours. Specimens
transported and received beyond 48 hours in transit are compromised but in some instances will be processed and a disclaimer will be included in those reported as negative (as outlined in the following table)."

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

For example, if a sample is collected on Tuesday, and another on Wednesday, and another on Thursday, it is better if each sample is sent out with the regular run of specimens the day it was collected. This will speed up 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 – as opposed to all three samples being sent on Thursday, arrive in the lab on Friday, and then AFB smear results on all three would not be available until Monday the following week. However, if all specimens are collected on the weekend they can all be kept in the fridge and sent together on Monday.

Even though the number of AFB will decrease over time, DSM concentrates the sample for culture; since mycobacteria are in general pretty hardy, they do not deteriorate as rapidly during transit as other more fastidious bacteria. However, as with all specimens, faster transit is better.

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 did not alter if samples were held even up to eight weeks. However, culture results deteriorate; they found that approximately 39% of sputum samples that were originally culture positive remained culture positive after four weeks at room temperature (if stored in the fridge there were about 67% of the same samples that remained culture positive after four weeks).

The viability of the MTB culture decreases over time, but very slowly. For example, after seven days there is no significant drop in culture viability, but by four weeks there is about a 40% reduction in viability, even for samples stored at four degrees Celsius. In practice, specimens are never delayed by four weeks. The laboratory will accept specimen for MTB 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, and a few days delay will not change the culture or AFB smear results
- A few days delay will change the turnaround time for the AFB smear results
- Specimens should not be held for several days if this will increase an already lengthy transit time
- DSM recommends that for all locations (hospital, community, or rural sites) as each specimen is collected it should be submitted to the Microbiology lab

Q17. 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 collected Friday, Saturday, or Sunday and dropped off at a community laboratory, Cadman Provincial Laboratory or the Health Sciences Centre will be kept in a fridge until Monday when they will be processed.

Q18. Recently three samples that individually were insufficient were pooled into one. When is this appropriate practice?

A. Only in special situations and in consultation with the Mycobacteriology Section Lead, Clinical Microbiology Discipline, DSM, can pooling of suboptimal sputum samples sometimes be arranged. For example, 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 where possible the multiple saliva samples would be pooled as one sample, and that an attempt would be made to get at least one induced
sputum sample from the contacts to ensure the screening was optimal. This was a one time response just to ensure that there was at least one specimen processed, as there were a fairly large number of people affected if all the specimens were rejected (as they were all less than 3 mL). Only where the combined volume was more than 3 mL was pooling of samples permitted (i.e., there were still some specimens that were rejected after pooling as the total volume of specimens received was not up to 3 mL).

Q19. 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.

Q20. 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).

Q21. 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 certainly valid and should be considered as Sample 1. On many other sample types the pooling of samples occurs, particularly for samples taken and received on the same day, e.g., stool samples, respiratory samples for mycobacteria, etc.

Q22. 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 taken should be considered if sputum collection is indeed required.

[Editor's note: In some situations Public health and staff communities will continue to ask some “asymptomatic” contacts to attempt spontaneous sputum; sometimes such contacts have coughs they do not admit to, sometimes they can produce sufficient samples, and of course sometimes they cannot. Aggressive sputum collection has been very useful in certain outbreak settings and high-incidence communities; using this approach staff have managed to find cases of TB who may not otherwise have found, or who would have been found after disease progression. Therefore some insufficient samples at times may be submitted to the lab. It is understood that some of these samples may need to be rejected by the laboratory. Sputum induction is not readily accessible in most First Nations communities but if a client requires sputum collection and the client is unable to produce an adequate specimen spontaneously, sputum induction will be arranged.]

Q23. 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 MTB (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 at DSM is the Mycobacteria Growth Indicator Tube (MGIT) liquid broth based system, and in that system the 0.4 mg/L is all that is 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. There has been no indication that any other test concentration in the broth systems are needed or are about to emerge as a new diagnostic tool.

Q24. Can you explain the two thresholds used to report resistance to INH? [April 2011]

A. The current DSM practice is to test 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 (Note: the MGIT is the system used for susceptibility testing for TB). If the strain is sensitive at both testing levels it is reported as “Sensitive”, and if resistant at both levels, then it is reported as “Resistant”. If it is resistant at 0.1 mg/L but sensitive at 0.4 mg/L, then both the results are reported.

This then 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.”

CLSI recommends the following comment be added to the report: “These test results indicate low-level resistance to INH. Some experts believe that patients infected with strains exhibiting this level of INH resistance may benefit from continuing therapy with INH. A specialist in the treatment of tuberculosis should be consulted concerning the appropriate therapeutic regimen and dosages.”

DSM Microbiology uses the following comments:

If sensitive 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 TB."

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 TB."

Q25. If a mycobacterial report was issued but later needs to be corrected, how is this handled?

A. DSM has indicated the following:

"AMENDED TB REPORTS: 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. For example if an error has been made after a drug susceptibility report has been issued the details listed in the original report cannot be altered. However a comment can be added to the bottom of the report that indicates that the reported value is incorrect and what the corrected value is. Because the originally reported values remain on the report, the new information added to the bottom of the report may not be obvious at a quick glance. Therefore when a report is sent please make sure you read every line in the report, particularly if a report is sent out again."

Q26. Can you comment on Manitoba's rates of laboratory mycobacterial cross-contamination?

A. In the past few years DSM has been much more pro-active about suspecting cross-contamination, and has implemented a standard pro-active critique of any MTB positive culture that occurs in the later stages of incubation. Previously this was not done unless the physician contacted DSM to say the laboratory result did not fit with the clinical picture. Laboratory staff are much more aggressive about suspecting possible cross-contamination and acting on it. If cross-contamination is suspected, DSM will contact the physician of record to review the clinical picture, and also expedite the Mycobacterium intespersed repetitive units (MIRU) genetic typing. Rates of Manitoba cross-contamination are likely less than compared to several years ago.
There may have been more TB cross-contamination events that happened in the past that were not recognized as such.

The rates of laboratory mycobacterial cross-contamination are likely no greater than at other sites that proactively review their culture results. Medical Microbiology at the Vancouver General Hospital did a retrospective review of their culture results combined with detailed clinical review and identified that there were a much larger number of likely TB cross-contaminations than they had realized. They subsequently implemented a pro-active review process for any MTB cultures that occur late in the incubation period. For centres that passively wait for physicians to contact the laboratory regarding results that do not match the clinical, the rates of cross-contamination are likely very much underreported.

Q27. A small child mistakenly had the BCG vaccine administered intramuscular in the Vastus lateralis; the site formed a pustule. The attending physician sent a swab for Mycobacterial culture (AFB); this came back as TB complex. The final culture came back as *Mycobacterium bovis*. Usually the lab will specify that it is the “BCG strain”; 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 will report this out. Future reports will indicate that the final determination is pending. [Nov 2011]

Q28. Why is there no smear result reported when bone marrow is tested for MTB?

A. Bone marrow is collected by a hematopathologist and is treated by the laboratory like a blood sample, in that 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 AFB smear. It is possible that with miliary (disseminated) TB the AFB load might be high enough to see organisms on an AFB smear.

Q29. 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.