Labstract – May 2016

Mycobacteriology and Tuberculosis (TB) Laboratory Update: Change to real time PCR for the direct detection of *M. tuberculosis* complex (MtbC) and *Mycobacterium avium* complex (MAC)

To Health Care Providers:

As of June 2016 the Public Health Ontario Laboratory (PHOL) will be using a real time polymerase chain reaction (PCR) assay[1,2] for the direct detection of *Mycobacterium tuberculosis* complex (MtbC) (including *M. tuberculosis, M. africanum, M. bovis* subsp. *bovis, M. bovis* subsp. *caprae, M. bovis* BCG, *M. microti, M. canettii,* and *M. pinnipedii*) and *Mycobacterium avium* complex (MAC) (including *M. avium, M. intracellulare, M. chimaera, M. arosiense, M. colombiense, M. marseillense, M. bouchedurhonense,* and *M. timonense)* in all specimens.

This assay detects the presence of *Mycobacterium tuberculosis* complex and *Mycobacterium avium* complex DNA in specimens. This real time PCR assay is replacing the AMTD (Amplified Mycobacterium Direct Test, Hologic) and the TB PCR assay (Seeplex® MTB Nested ACE Detection, Seegene).

Laboratory Test algorithm:

1. Acid-fast bacilli (AFB) smear-positive specimens will be automatically tested by the real time PCR assay in patients for whom there has been no previous smear-positive sample (i.e., “new smear positive”).

2. Acid-fast bacilli (AFB) smear-negative specimens will be tested only upon request and must be requested within 72 hours of the date and time the specimen was received to ensure specimen integrity. Requests should be considered for patients in whom tuberculosis is highly likely and have significant risk factors for tuberculosis. A negative result cannot be used to “rule out” tuberculosis. Please contact the PHOL Customer Service Centre to request the real time PCR assay.

3. While the optimum tissue specimen for this test is fresh tissue, real time PCR can be performed on formalin-fixed, paraffin embedded (FFPE) tissue samples from patients where there is a strong suspicion of tuberculosis disease clinically and epidemiologically, and there is histopathological evidence of possible tuberculosis (e.g. presence of caseating granulomata or acid fast bacilli). Requirements for submitting these samples may be found in the Test Directory on the PHO website under "Mycobacterium – Tissue Formalin Paraffin Block".

Testing Schedule:

This real time PCR testing will be conducted daily, Monday – Friday (with the exception of holidays) at PHOL-Toronto. Specimens received at other PHOL sites will be forwarded to Toronto for testing. Specimens must be received and processed by 9:00 am in order to be tested the same day. Specimens processed after 9:00 am will be tested the next day in the schedule.

Reporting:

The results will appear on the report as follows:

Mycobacterium tuberculosis complex DNA by real-time PCR	[Detected] or [Not Detected] or [Indeterminate]
Mycobacterium avium complex DNA by real-time PCR	[Detected] or [Not Detected] or [Indeterminate]

In addition, FFPE samples will have the following notes in the laboratory report:

Results must be interpreted in the context of the clinical history, histopathology and other laboratory findings.

Although the methods used for this analysis enable DNA extraction from formalinized and paraffin embedded tissue (FFPE), PCR sensitivity may be decreased resulting in a false negative. Due to possible environmental contamination by non-tuberculous mycobacteria, detection of MAC in FFPE specimens (positive result) must be interpreted in context with all clinical and laboratory information.

Optimal specimen is fresh tissue.

Turnaround Time:

The turnaround time from start of testing to reporting is one business day (24 hours).

Sensitivity and Specificity:

The original assay as developed (Wadsworth Laboratory, New York State Dept. of Health, Albany, NY) had the following performance characteristics:

	AFB POSITIVE (%)	AFB NEGATIVE (%)	ALL SPECIMENS (%)
MtbC: Sensitivity	99.6	75.4	95.5
Specificity	100.0	99.9	99.9
PPV	100.0	98.0	99.6
NPV	97.7	98.4	98.4
MAC: Sensitivity	83.8	52.7	71.1
Specificity	100.0	99.2	99.5
PPV	100.0	93.6	98.0
NPV	89.6	90.5	90.2

The performance of the assay will be monitored routinely at PHOL. Note that the performance of the assay may vary with TB and MAC disease prevalence, and the type of specimen.

Limitations:

- Nucleic acid amplification tests, such as this real time PCR assay, target nucleic acids from viable and non-viable bacteria, and cannot be used to monitor the progression or success of treatment of patients with anti-tuberculous therapy, or whether a patient may be infectious
- Results are qualitative (i.e., "detected" or "not detected") and cannot be used to determine bacillary load.
- This real time PCR assay contains an internal inhibition control. In the presence of inhibiting factors, an indeterminate result will specify that it is due to inhibition within the sample.
- The assay has a slightly lower sensitivity for detecting *M. avium* complex organisms, particularly with mixed cultures.

Determining the species within the Mycobacterial complexes will be performed, if possible, from the culture material.

References:

1. Halse TA et al. J Clin Microbiol, 2010;48(4) :1182-1188
2. Tran AC et al. Diagn Microbiol Infect Dis 2014;79:43-48

For further information:

- Contact the PHOL Customer Service Centre at 416-235-6556 or 1-877-604-4567 (toll-free), or by email at CustomerServiceCentre@oahpp.ca
- For PHOL specimen collection information and previous Labstracts, refer to http://www.publichealthontario.ca/Labs
- The current version of the PHOL General Test Requisition and other forms are available at http://www.publichealthontario.ca/Requisitions
- To subscribe to future Labstracts, email labstracts@oahpp.ca
- To register for Autofax and receive laboratory reports by fax directly from our laboratory information system as soon as they are released, contact the PHOL Customer Service Centre.