Labstract – August 2016

Tuberculosis and Mycobacteriology: Identification of *M. abscessus* subspecies and molecular detection of macrolide resistance

To Health Care Providers:

Effective August 29, 2016, the Public Health Ontario Laboratory (PHOL) will identify the subspecies of all new patient isolates of *Mycobacterium abscessus* (*M. abscessus ssp abscessus, M. abscessus spp bolletii,* and *M. abscessus ssp. massilense*) using a new molecular testing algorithm, and will identify mutations in two targets that are predictive of macrolide resistance.

M. abscessus is a rapidly growing mycobacterium that is present in the environment, including water and vegetation. It is a recognized human pathogen that can cause significant disease, particularly in patients with underlying lung disease. Effective therapy for *M. abscessus* usually includes a macrolide such as clarithromycin, but resistance to macrolides can occur. Knowledge of macrolide resistance is critical to clinical decision making.

Acquired resistance to macrolides will be detected by sequencing for the presence of mutations in *rrl* (23S rRNA gene).

Inducible macrolide resistance will be performed using sequence-based testing to detect the presence of a functional intact *erm*(41) gene in *M. abscessus subsp abscessus* and *M. abscessus subsp bolletii*. The *erm*(41) gene in *M. abscessus ssp. massiliense* contains a deletion rendering it non-functional and will not be subject to sequence analysis.

Test Algorithm:

- All new culture-positive non-tuberculous mycobacteria isolates will be identified by the line-probe assay, the GenoType Mycobacterium CM or AS assays (HAIN Lifescience).
- Where the isolate cannot be identified by the line-probe assay, 16S rRNA sequencing will be used for identification.
- Isolates identified as *M. abscessus* by either line-probe assay or 16S rRNA will be further subspeciated by DNA sequencing methods.
- Additional isolates from the same patient identified $\geq$ 3 months after the initial or previous isolate will be tested; all others will be referred to the initial or previous isolate test result.
- *M. abscessus* isolates will also be tested for the presence of an intact *erm*41 gene, and *erm*(41) sequencing will be performed for *M. abscessus ssp abscessus* and *M. abscessus ssp bolletii* to identify non-functional intact *erm*(41).
- All *M. abscessus* isolates will be tested by sequencing for mutations in the *rrl* locus known to confer resistance to macrolides.
- *M. abscessus ssp abscessus* and *M. abscessus ssp bolletii* isolates where the *erm*(41) has been identified as non-functional and *rrl* is not mutated (predictive of macrolide susceptibility) will be sent to the National Microbiology Laboratory for confirmation by phenotypic drug susceptibility testing (DST), as isolates may still be macrolide resistant due to unknown mechanism.

Reporting:

Where an isolate is identified as *M. abscessus* by either the line-probe assay or 16S rRNA sequencing, the reporting will be as follows:

Test	Result	Note
M. abscessus subspeciation sequencing	*M. abscessus subspecies* [*abscessus, bolletii* or *massiliense* or unable to subspeciate]	
Functional *erm*41 gene sequencing	Detected/not detected	The presence of a functional *erm*(41) gene is associated with macrolide resistance (e.g. clarithromycin, azithromycin) with the potential for treatment failure.
rrl gene sequencing	Mutation detected/not detected	The presence of *rrl* gene mutation is associated with macrolide resistance and potential for treatment failure.

M. abscessus ssp abscessus and *M. abscessus ssp bolletii* isolates where the *erm*(41) has been identified as non-functional and *rrl* is not mutated (predictive of macrolide susceptibility) will be sent to the National Microbiology Laboratory for confirmation by phenotypic drug susceptibility testing (DST), as isolates may still be macrolide resistant due to unknown mechanisms

Turn-around time:

Culture identification results by the line-probe assay (HAIN Lifescience) will be reported in 24 to 36 hours, followed within 7 days by the subspeciation and presence/absence of a functional *erm*(41) gene and *rrl* mutation (acquired resistance). The DST results may not be available for up to four weeks, depending on the current turnaround time at the NML.

References:

1. Griffith DE et al. An official ATS/IDSA statement: Diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med 2007;175:367
2. Leao SC et al. Proposal that *Mycobacterium massiliense* and *Mycobacterium bolletii* be united and reclassified as *Mycobacterium abscessus subsp. Bolletii* comb. Nov., designation of *Mycobacterium abscessus subsp. abscessus subsp.* nov. and emended description of *Mycobacterium abscessus*. Int J Syst Evol Microbiol 2011;61:2311
3. Koh WJ et al. Clinical significance of differentiation of *Mycobacterium massiliense* from *Mycobacterium abscessus*. Am J Respir Crit Care Med 2011;183:405.
4. Shallom SJ et al. New rapid scheme for distinguishing the subspecies of the *Mycobacterium abscessus* group and identifying *Mycobacterium massiliense* isolates with inducible clarithromycin resistance. J Clin Microbiol 2013;51(9):2943
5. Bastian S et al. Assessment of clarithromycin susceptibility in strains belonging to the *Mycobacterium abscessus* group by *erm*(41) and *rrl* sequencing. Antimicrob Agents Chemother 2013;55(2):775

For further information:

- Contact the PHOL Customer Service Centre at (416)235-6556 or1-877-604-4567 (toll-free) or by email at CustomerServiceCentre@oahpp.ca
- For the PHOL specimen collection information and previous Labstracts, refer to www.publichealthontario.ca/labstracts
- The current version of the PHO laboratory requisition form is available at www.publichealthontario.ca/requisitions
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