



Clinical Practice Change: Clinical Microbiology

Date: March 5, 2014

To: Physicians in the Section of Infectious Diseases

From: Dr. James Karlowsky, Medical Director, and Shirley Hoban, Technical Director, Clinical Microbiology Discipline, Diagnostic Services Manitoba.

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Re: 16S rRNA Sequencing for Bacterial Pathogens from Sterile Fluids is now available

Take Home Messages:

1. Starting March 17, 2014, 16S rRNA sequencing for bacterial pathogens from sterile body fluids will be available on-site by DSM laboratories; the test will be performed in the Health Sciences Centre (HSC) Clinical Microbiology Laboratory.
2. The test may only be requested through consultation with a site Microbiologist and will only be performed if certain criteria (outlined below) are met.
3. Tissue cannot be tested at the current time and will continue to be forwarded to the National Microbiology Laboratory (NML) for testing.

The definitive diagnosis of bacterial infections from infected sites requires the culture of organisms from an appropriately collected sample. However, in some instances, viable organisms cannot be recovered from an infected site due to either pre-administration of antimicrobials or fastidious growth requirements. In these instances the PCR amplification and sequencing of a bacterium-specific gene, known as the 16S ribosomal RNA gene (16S rRNA gene), may provide a bacterial etiology for the infection. In the past, this assay was available through the NML. Starting March 17, 2014, 16S rRNA gene sequencing from **sterile fluids** will be performed by DSM laboratories in the HSC Clinical Microbiology Laboratory. Tissue specimens will not be tested at this time and will continue to be forwarded to the NML; DSM continues to work on the in-house validation of 16S rRNA sequencing for tissues samples and anticipates offering this test when the validation study is completed.

Criteria for testing sterile fluids by 16S rRNA sequencing will be as follows:

1. Requests for 16S rRNA sequencing must be directed to a site Microbiologist for approval.
2. Only sterile site fluid specimens (collected with a needle and syringe) will be accepted; the test excludes whole blood or blood components. A minimum of 200 µL (preferably 500 µL) is required for testing. **Swabs or swabs of fluid will not be accepted (no exceptions)** due to the risk of contamination of the fibers of these products with RNA from environmental organisms, which are rendered non-viable by the sterilization process but remain detectable by PCR.
3. Aerobic and anaerobic bacterial cultures must be negative after 48 hours and there should be indication of one or more of the following:
 - a. Organism visible in Gram-stain. Stains that show more than one type of organism will not be tested.
 - b. Recent or current antibiotic use, or
 - c. High suspicion of a non-culturable or difficult to recover pathogen.

Assay performance:

In-house verification of the assay demonstrated that the limit of detection of the assay in a sterile biological fluid is 1,000,000 bacteria per mL. This corresponds roughly to the concentration required for an organism



to be visible in a Gram-stain preparation. Correspondingly, the sensitivity of the assay was 94% when organisms were visible on Gram-stain and only 50% when infection was present but no organisms were visible on Gram-stain. Specificity was 96.6%.

Culture remains the gold standard for detection of bacterial infections and 16S rRNA gene sequencing should not be used to rule out infection when organisms are not visible on Gram stain due to its very poor sensitivity in this setting. The negative predictive value of the assay in a population where 80% of individuals have a bacterial infection despite a negative Gram stain is only 32%.

Scheduling:

Tests for direct 16S rRNA sequencing from sterile site fluid specimens will be performed Monday and Wednesday mornings. To be included in a run, the specimen must be received by the HSC Clinical Microbiology Laboratory no later than 8:00 am on the day of the run.

Feel free to direct any questions to Dr. Philippe Lagacé-Wiens (Section Lead, Molecular Diagnostics, Clinical Microbiology) at 204-237-2483 or to Dr. James Karlowsky (Medical Director, Clinical Microbiology Discipline) at 204-237-2105.