Real-time PCR Testing Scheme for Detecting Bacterial Meningitis Pathogens During Routine Surveillance and Outbreak Responses

This testing scheme can be used as a guide to test for bacterial meningitis pathogens during routine surveillance and outbreak testing.

INTRODUCTION
The purpose of this tool is to propose testing schemes for the detection of bacterial meningitis pathogens using the real-time PCR method during routine surveillance and outbreak investigations. The proposed outline of the testing scheme, which may be implemented in the event of an outbreak, to triage a large influx of incoming specimens, to conserve resources (i.e., reagents) and provide faster results for public health response.

TOOL CONTENTS:
- Routine Surveillance Testing scheme that depicts the laboratory tests that should be performed during low levels of activity
- Outbreak Testing Scheme that depicts the laboratory tests that should be performed during high levels of activity

TOOL INSTRUCTIONS:
The first testing scheme (Figure 1) is recommended for routine surveillance use during non-epidemic periods. Laboratories should be routinely confirming species identification for all three meningitis pathogens. Should either of the three species be detected, additional genogrouping/genotyping should be performed to identify the types of pathogen(s) in circulation. The prioritization of genogroup testing of Neisseria meningitidis strains will vary based on the epidemiology of circulating strains within each country. Depending on the country’s epidemiology, a more targeted genogrouping testing algorithm could be used for genogrouping of N. meningitidis strains.

An example of a testing scheme (Figure 2) is provided in the event of an outbreak, where there is a large influx of specimens sent to the laboratory for confirmatory testing. In this event, the testing demand exceeds the current laboratory capacity. Therefore, this scheme details a more targeted testing approach to improve throughput, allowing for rapid reporting and conservation of reagents. In this example, N. meningitidis serogroup C has been detected and confirmed as the major pathogen in circulation during the season. As clinical specimens continue to be positive for serogroup C, the testing scheme could be re-prioritized to detect the suspected species and genogroup initially.

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For information about using the toolkit: MenAfriNet.org
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Scheme 1: Testing Scheme for Routine Surveillance
Early in the season, testing should be considered for all three meningitis pathogens.

- Perform species identification on CSF, T-I, and/or isolates (*sodC*, *hpd*, and *lytA*)

**If** *hpd* (+), **perform and specify genotyping for H. influenzae**
- If (+), report as *H. influenzae* serotype b
- If (+), report as *S. pneumoniae*, specify serotype

**If** *lytA* (+), **perform capsular genotyping for S. pneumoniae**
- If (+), report as *S. pneumoniae* NT
- If (-), report as *N. meningitidis* NG

**If** sodC (+), **perform genogrouping for NmA, NmC, NmW, and NmX.‡**
- If (+), report as *N. meningitidis* serogroup A, C, W, or X

**If** (-), report as *H. influenzae* non-b
- If (-), report as *S. pneumoniae* NT
- If (-), perform genogrouping for NmB and NmY
- If (-), report as *N. meningitidis* NG

**If** (-), report as No *N. meningitidis*, *H. influenzae*, and *S. pneumoniae* DNA detected.

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* *sodC* gene detects *N. meningitidis* species; *hpd* gene detects *H. influenzae*; *lytA* gene detects *S. pneumoniae*

‡ The prioritization of *N. meningitidis* genogroup testing will depend on the epidemiology of circulating strains in the region or country.

**Capsular genotyping for *S. pneumoniae* will be depend on geographical distributions. Refer to [https://www.cdc.gov/streplab/pneumococcus/resources.html](https://www.cdc.gov/streplab/pneumococcus/resources.html).
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Scheme 2: Testing Scheme for Outbreaks

This testing scheme will depend on the major circulating pathogen detected from the early season testing. In this example, Nm serogroup C has been the major circulating pathogen. In this situation, the PCR cycling parameters can be reduced to 40 cycles for rapid results.

- **Perform species identification on CSF, T-I, and/or isolates (sodC, hpd, and lytA)**
  - If sodC and NmC (+), report as *N. meningitidis* serogroup C
  - If sodC (+) and NmC (-), perform genogrouping for NmA, NmW, and NmX
    - If (+), report as *N. meningitidis* serogroup A, W, or X
    - If (-), perform genogrouping for NmB and NmY
    - If (+), report as *N. meningitidis* serogroup B or Y
    - If (-), report as *N. meningitidis* NG
  - If sodC (-) and NmC (-), perform species identification for *H. influenzae* and *S. pneumoniae* (hpd and lytA)
    - If (+), report as *H. influenzae* serotype b
    - If (-), perform genogrouping for *N. meningitidis* NG
    - If (+), report as *H. influenzae* non-b
    - If (-), report as *S. pneumoniae*, specify serotype
    - If (+), report as *S. pneumoniae* NT
    - If (-), report as No *N. meningitidis*, *H. influenzae*, and *S. pneumoniae* DNA detected

* sodC gene detects *N. meningitidis* species; hpd gene detects *H. influenzae*; lytA gene detects *S. pneumoniae*
† The prioritization of *N. meningitidis* genogroup testing will depend on the epidemiology of circulating strains in the region or country
**Capsular genotyping for *S. pneumoniae* will depend on geographical distributions. Refer to [https://www.cdc.gov/streptococci/pneumococcus/resources.html](https://www.cdc.gov/streptococci/pneumococcus/resources.html)