INTRODUCTION

The typical laboratory network structure is generally divided into levels, such as (1) district/peripheral, (2) regional/intermediate, and (3) national/reference. Laboratories at each level are expected to be able to perform a minimum set of test services, with the national/reference level encompassing the capacity for all types of testing. For example, in addition to PCR, a national/reference laboratory should also have the capacity to perform all tests listed under the regional/intermediate and peripheral level laboratories. These tests, illustrated in the testing algorithm flowchart, provide a framework for the identification and characterization of the three predominant causative agents of bacterial meningitis: *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae*. For more information on how to perform each test and interpret their results, please refer to their respective SOPs and manual from the manufacturer.

TOOL CONTENTS:

- Flowchart that describes the steps necessary for laboratory identification and confirmation of the three predominant causative agents of bacterial meningitis at the district/peripheral, regional/intermediate, and national/reference laboratories

For information about using the toolkit: MenAfriNet.org
TOOL INSTRUCTIONS:

I. District/peripheral level

At the district/peripheral level, specimen(s) collected will need to be sent to the laboratory within two hours for testing. If the specimen cannot be transferred to the laboratory or testing cannot be performed within that time, inoculate a minimum of 0.5 ml into T-I media.

Upon arrival at the laboratory, the specimen should be aliquoted into two tubes: a cryotube for PCR testing and a dry tube. The cryotube should be immediately stored at -70°C until ready for shipment to the national/reference laboratory. If an ultra-low freezer is not available, store the cryotube at 4°C or lower for no more than a week. Tests such as macroscopic appearance, gram stain from the clinical specimen, cell count, and/or rapid diagnostic tests (RDTs) should be immediately performed on the dry tube to provide immediate results back to clinicians for patient management.

Transport the T-I, unvented, or dry tube at room temperature to the regional laboratory for culture. The cryotube should be transported at 4°C or on ice packs to the national/reference laboratory for PCR testing.

II. Regional/intermediate level

Regional/intermediate level laboratories should have the capacity to perform culture. Culture can be performed using the sediment portion of the centrifuged specimen or from T-I media within two hours of specimen collection. Common media types for culture include blood and chocolate agar plates (BAP and CAP). While *N. meningitidis* and *S. pneumoniae* grow on both BAP and CAP, *H. influenzae* grows only on CAP. Additionally, *S. pneumoniae* has α-hemolytic properties, which distinguishes it from *N. meningitidis*.

Biochemical testing should be performed on pure cultures of isolates to identify the bacterial pathogen. Once the species has been confirmed, slide agglutination and antimicrobial susceptibility testing could be performed to further characterize the pathogen. If there is a lack of antisera to perform slide agglutination, the regional laboratory should refer the isolate to the national level for characterization by real-time PCR testing. All results should be shared with the national reference laboratory and the clinician and/or referring laboratory.

III. National/reference level

The national or reference laboratories should have the capacity to perform all types of testing, including real-time PCR. All clinical specimens should be sent to the national or reference laboratory for confirmatory testing by PCR, as this method is more sensitive and specific than conventional tests. The national or reference laboratories should be responsible for conducting quality assurance programs to assess results of the peripheral level laboratories. The laboratory should consider performing routine quality control testing on a subset of isolates from the lower level laboratories to ensure the quality of the laboratory results being reported from those laboratories.

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Testing Algorithm for the Identification of Bacterial Meningitis

SPECIMEN

Aliquot 1: 250 μL (Cryotube)
Aliquot 2: ≥ 1 mL (Dry tube)
Aliquot 3: 0.5-1 mL (T-I media)†

Freeze at -70°C

Cytology/Cell count
Centrifugation
Supernatant

Rapid Diagnostic Testing; E.g.,
- CERMES dipstick
- Directogen
- Pastorex
- Wellcogen

Sediment
Gram stain

Plating: CAP/BAP†
Gram stain

If gram result: (+) cocci or diplococci
Catalase test: (-)
Optochin test: Susceptible
Bile Solubility Test: Soluble
Confirmed: S. pneumoniae

If gram result: (-) diplococci
Oxidase test: (+)
Optochin test: Resistant
Sugar Utilization Test (glucose & maltose)
Confirmed: N. meningitidis

If gram result: (-) coccobacilli
Oxidase test: (+)
Growth factor test: Requires X & V
Optional: Rapid biochemical test (e.g. NH Strip)
Confirmed: H. influenzae

Slide agglutination

PCR

† If specimen cannot reach the laboratory within 1 hour, inoculate into T-I media
† H. Influenzae grows only on CAP. N. meningitidis and S. pneumoniae grows on both BAP and CAP.

Store isolates at -70°C in 5% sheep blood or 10% skim milk w/glycerol solution
Perform additional tests as needed (e.g. antimicrobial susceptibility testing)