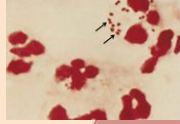


# Microbiology Laboratory in Diagnosing Bacterial Meningitis

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# Common bacterial pathogens causing meningitis



## *Neisseria meningitidis*

1°

- Gram-negative coccus, in pairs
- Polysaccharide capsule
- Resides in human nasopharynx

## *Neisseria gonorrhoeae*

- Intracellular, gram-negative
- Diplococcus
- Severe infections and disseminated infection can lead to meningitis

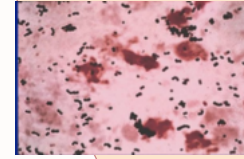
2°



## Group B *Streptococcus*

- Gram-positive coccus
- Can be passed from mother to baby during labour and birth
- More commonly found in newborns

2°



## *Haemophilus influenzae*

- Gram-negative coccobacillus
- Can be encapsulated
- Type B distinguished by capsular polysaccharides

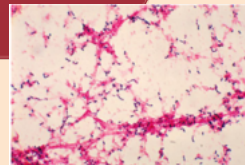
1°



## *Streptococcus pneumoniae*

1°

- Gram-positive lancet-shaped with capsule-forming cocci or diplococci
- Normally resides in human upper respiratory tract



## *Listeria monocytogenes*

- Gram-positive, rod-shaped
- Infection can lead to listeriosis, caused by contaminated food
- Can infect gut as well as spread throughout body

2°



## *Escherichia coli*

- K1 known to cause meningitis in newborns or babies under 3 months
- Meningitis very rare, but those with compromised immune systems, head injuries, recent head surgery have increased risk

2°



## Patient samples taken to laboratory

### Cerebrospinal Fluid

- Obtained through lumbar puncture
- Neutrophil count  $>1180/\text{mm}^3$  or leukocyte count  $>2000/\text{mm}^3$  rules out viral cause
- Gram stain and culture most important tests
- Culture of CSF is gold standard
- PCR and agglutination reserved as secondary tests for cases where Gram stain results are negative

### Blood

- CSF glucose levels interpreted relative to blood glucose – ratio decreases in infection
- Can reveal underlying pathology
- Useful in diagnosis when CSF sample is contradicted, blood cell counts and differential can confirm active infection

# Tests performed to identify pathogen

## Culture

- CSF sample concentrated by centrifugation
- Cultured on solid media, usually chocolate or peptone-blood base agar, *N. meningitis* grows on both
- Cultivated in moist chamber with 5-10% CO<sub>2</sub>, important to warm medium to 37C before inoculation
- Thayer-Martin medium contains antimicrobials that inhibit growth of most organisms other than *N. Meningitis*

## Sensitivity

- Kovac's oxidase test
  - Confirms presence of cytochrome oxidase
- Carbohydrate utilization test
  - Sample enriched with glucose, maltose, lactose, sucrose in 4 separate tubes – production of acid from glucose and maltose confirms *N. meningitis*
- *N. Meningitis* utilizes glucose and maltose

## Gram Staining

- Preliminary step in identifying bacterial organism, yields rapid confirmation, distinguishes between Gram-positive and negative
- After centrifugation, bacterial sediment spread onto slide and stained with crystal violet dye for 30s, then rinsed and counter-stained with safranin for 30s
- Gram-positive retain purple colour of crystal violet dye
- Gram-negative susceptible to decolourization, lose crystal violet dye when rinsed and adapt pink/red of safranin

## PCR

- Used to identify serogroup of pathogen
- Complementary strands of DNA initiated from a pair of oligonucleotide primers, DNA polymerase elongates complementary strand to copy a strand of DNA
- *crgA* gene of *N. meningitis* is amplified, *lytA* gene of *S. pneumoniae* and *bexA* gene of *H. influenzae* often amplified as well to distinguish *N. meningitidis* from these other pathogens
- Serogroups B, C, Y, and W135 of *N. meningitidis* can be identified via amplification of *siaD* gene, serogroup A via *mynB* gene

## Latex Agglutination

- Determines serogroup by initiating an antigen-antibody reaction between potential capsular antigens of infectious pathogen and serogroup-specific artificial antibody
- Results available within 15 min, high sensitivity for bacterial meningitis
- May provide advantage of rapid identification of causative pathogen and the serogroup

# Expected results of laboratory tests in identification of bacteria

## CSF and Blood

- CSF can be examined for altered cell counts, glucose, and protein levels in order to determine cause of symptoms
- Elevated white blood cell numbers and proteins levels characteristic of viral and bacterial meningitis
- Good indication of bacterial infection: 1000-5000 leukocytes/mm<sup>3</sup> with neutrophil dominance of 80-95%
- During bacterial meningitis, CSF to glucose ratio drops dramatically, ratio <0.4 highly indicative of bacterial

## Culture

- Growth on culture plates differentiates between viral, bacterial, and fungal
- If viral, plate remains empty, fungi and bacteria will grow depending on plate used
- Recommended to use blood and cholate agar plates, can differentiate between 3 main causes: *N. meningitidis*, *S. pneumoniae*, *H. influenzae*
- Thayer-Martin agar plates can be used to directly select for Neisseria due to containing antibiotics and fungicides



*N. meningitidis* on blood agar

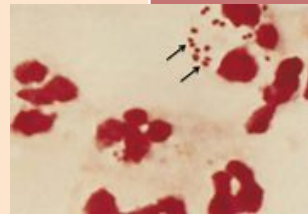


*N. meningitidis* on chocolate agar

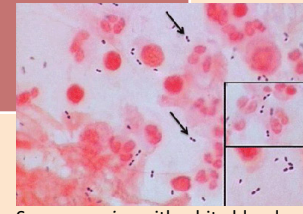
- *N. meningitidis* is haemolytic, able to grow on blood and chocolate agar plates; colonies appear moist, grey/unpigmented, with round, smooth edges
- *H. influenzae* unable to lyse red blood cells so unable to grow on blood agar plates
- Blood agar plates narrow down to *N. meningitidis* and *S. pneumoniae*

## Gram Stain

- Differentiates between *N. meningitidis* and *S. pneumoniae*
- *N. meningitidis* are Gram-negative, will appear pink/red diplococci
- *S. pneumoniae* are Gram-positive and will appear violet



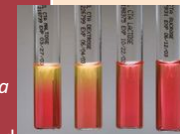
*N. meningitidis* with PMNs



*S. pneumoniae* with white blood cells

## Sensitivity

- Kovac's oxidase test detects whether organism produces cytochrome c oxidases (partakes in aerobic respiration)
- *N. meningitidis* treated as strictly aerobic, turns testing reagent purple, indicating positive result
- Carbohydrate utilization test detects whether organism can ferment specific carbohydrate substrates, can help differentiate between different *Neisseria* species
- *N. meningitidis* able to ferment glucose and maltose, these samples should turn yellow

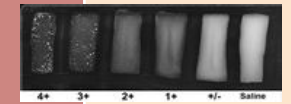


## PCR

- *crgA* gene will be only one amplified for *N. meningitidis*
- Amplification of *SiaD* gene indicates B, C, Y, or W135 serogroup, whereas *mynB* gene indicates A serogroup

## Agglutination

- Used to determine serogroup of *N. meningitidis*
- Positive result is determined when sample reacts with antigen specific antibody to result in an obvious precipitation pattern
- Positive conclusion can only be reached if precipitation pattern is at a +3 or +4 rating and sample does not react with saline



## Rapid Diagnostic Tests

- Used to directly test CSF in a quick, simple to use technique, however not sensitive or specific
- 2 commonly used tests identify 4 of the serogroups – one identifies A and W135/Y and other C and Y
- Positive indicator results in 2 lines appearing on test paper stick
- Negative result only has 1 line
- Where the second line appears determines which serogroup is present

