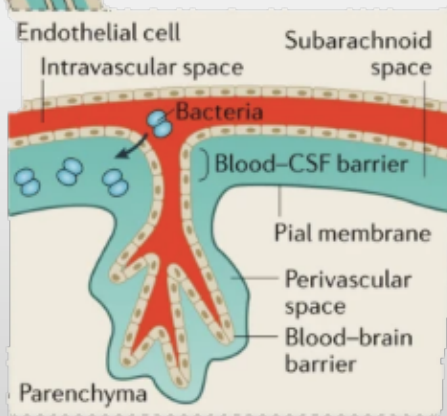
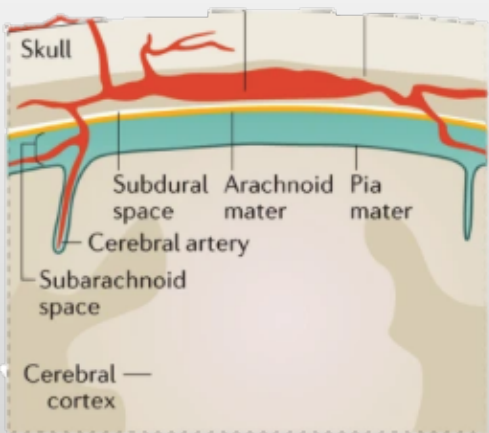


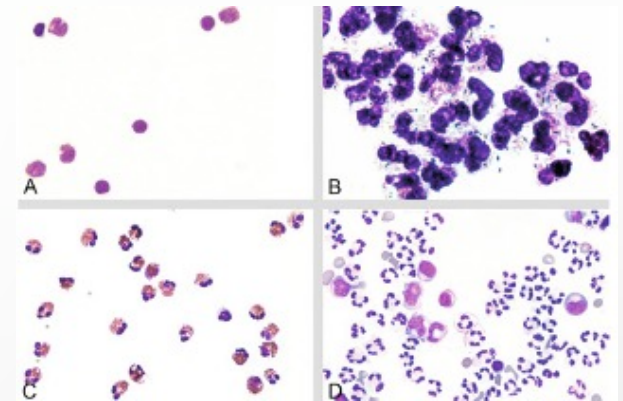
# PATH 417 CASE 1 A STIFF NECK

## MICROBIOLOGY LABORATORY



CATHERINE GAI

# PRESENTATION OVERVIEW



## (1) COMMON BACTERIAL PATHOGENS

- Summary of what can lead to meningitis, in addition to *N. meningitidis*:
  - Other bacterial pathogens
  - Mechanism of transmission / prevention with vaccine
  - Signs & symptoms

## (2) SAMPLES FOR LABORATORY TESTS

- Summary of samples taken for laboratory testing:
  - Method for collection
  - How they contribute to diagnosis

## (3) TESTS & RESULT INTERPRETATION

- Tests for detecting / identifying the pathogens & result interpretation
  - Cell culture & CSF cytological analysis
  - Gram stain
  - Additional tests for CSF samples

# (I) COMMON BACTERIA PATHOGENS

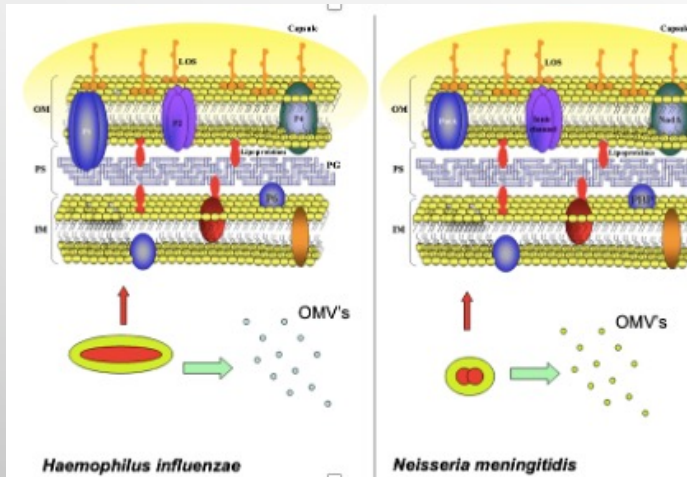
## Gram Positive Bacteria

Bacterial Pathogen	Mechanism of Transmission / Prevention	Signs & Symptoms
Streptococcus pneumoniae	Coughing, sneezing; Prevented with vaccine	Fever, stiff neck, coughing, hearing loss, cognitive impairment
Group B Streptococcus	Vertical transmission	Fever, neurological impairment
Listeria monocytogenes	Ingestion of contaminated food; Vertical transmission	Headache, stiff neck, confusion, loss of balance, convulsion

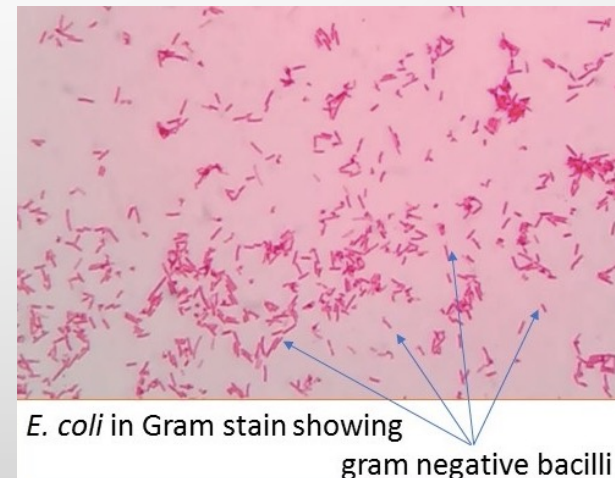
# (I) COMMON BACTERIA PATHOGENS

## Gram Negative Bacteria

Bacterial Pathogen	Mechanism of Transmission / Prevention	Signs & Symptoms
Haemophilus influenzae	Respiratory droplet; Prevented with vaccine	Fever, headache, stiff neck, nausea
Escherichia coli	Ingestion of contaminated food; Vertical transmission	Fever, headache, vomiting, diarrhea



Membrane proteins of *H. influenzae* and *N. Meningitidis*



*E. coli* Gram staining

## (2) SAMPLES FOR LABORATORY TESTS

### Cerebrospinal Fluid

Method to obtain sample:

- lumbar puncture

Normal CSF appearance:

- clear

Components of CSF sample being analyzed:

- cell count (higher PMN level during infection)
- glucose level (lower during infection)
- protein level (higher during infection)

This will be followed by Gram Staining / PCR

Table 23.4

Typical biochemical changes seen in cerebrospinal fluid secondary to bacterial meningitis

Cerebrospinal fluid finding	Healthy newborn	Healthy child or adult	Bacterial meningitis
Total leukocytes (cells/mm <sup>3</sup> )	<30	<6	>1000
Neutrophils (%)	20–60	0	>85–90
Protein (mg/dL)	30–150	20–40	>100–150
Glucose (mg/dL)	30–120	40–80	0–<40
Positive gram stain (%)	0	0	>85
Positive culture (%)	0	0	>95

## (2) SAMPLES FOR LABORATORY TESTS

### Blood Sample

Method to obtain sample:

- Serology test: take 1-3 ml of blood and dilute it to obtain a blood culture before or shortly after antibiotic treatment

Analysis for the following to observe any abnormalities:

- Complete blood cell count (CBC)
- Coagulation study
- Electrolyte level
- Inflammatory marker (eg., C-reactive protein or procalcitonin) which distinguishes aseptic meningitis from bacterial meningitis

# (3) TESTS & RESULTS

## Cell Culture & CSF Cytological Analysis

A new colony should be streaked; the CSF culture is grown on either Blood Agar Plate (BAP) or Chocolate Agar Plate (CAP)

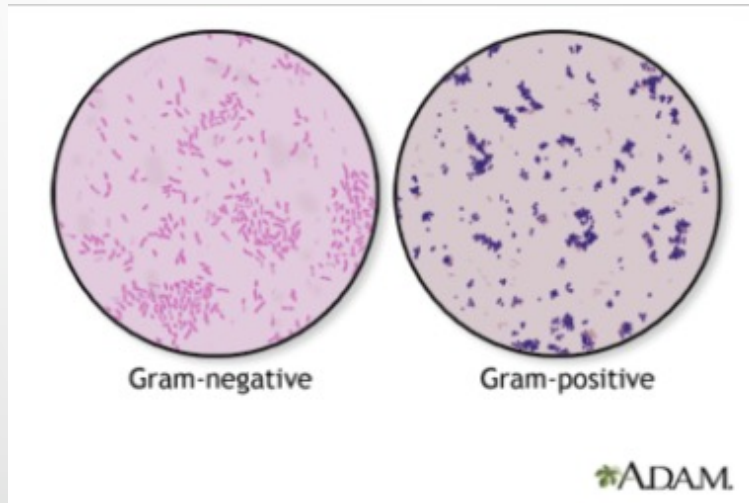
- Bacteria culture in BAP or CAP:
  - *N. meningitidis*:
    - BAP: smooth, glistening, convex edged colonies; no hemolysis
    - CAP: large and opaque cultures; no hemolysis
  - *S. pneumoniae*:
    - BAP: small and mucoidal colonies with hemolysis
    - CAP: small and moist culture with hemolysis

Cytological examination of CSF's composition; the test involves analyzing the following:

- Cell count (elevated PMN level)
- Glucose level (low during infection)
- Protein level (high during infection)
- Other inflammatory markers
- Abnormal electrolyte level

### (3) TESTS & RESULTS

## Gram Stain



Visualization of gram-negative (left) and gram-positive (right) bacteria using gram staining test

Purpose & method:

- To differentiate between gram-positive and gram-negative bacteria, based on their ability to retain crystal violet dye in the cell wall during solvent treatment
- Procedure brief summary:
  - Divide a glass slide into two, where one side is smeared with the sample and the other side with the organism in question
  - Prepare the sample, where the key step is to prepare for staining with crystal violet ammonium oxalate

Result:

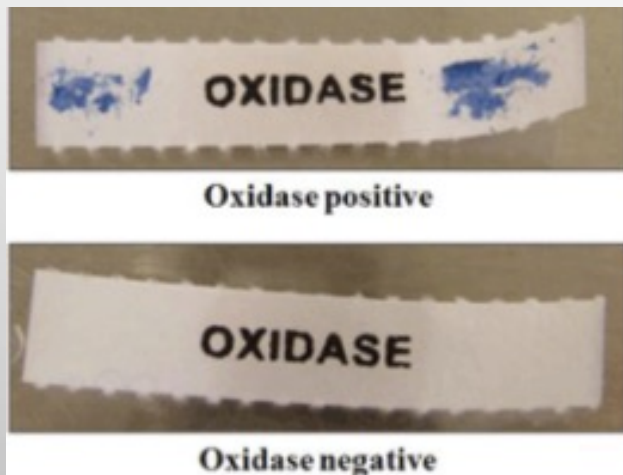
- Gram-positive bacteria: result in purple color
- Gram-negative bacteria: result in pink/red color



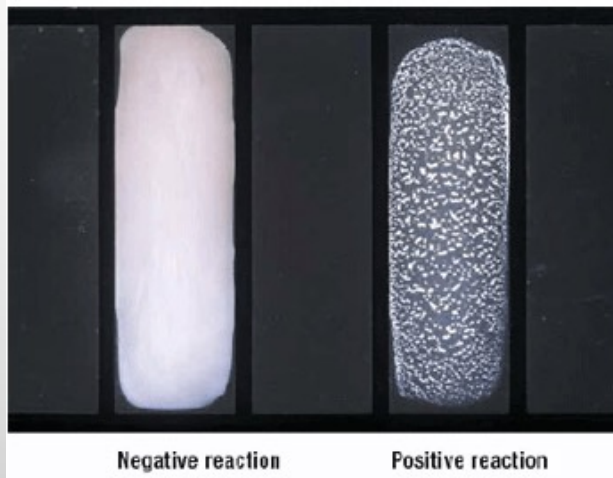
# (3) TESTS & RESULTS

## Additional Tests for CSF Sample Overview

(1) Tests for *M. meningitidis* & *H. influenza* & result interpretation



(2) Tests for detecting the presence of bacterial pathogens that can cause meningitis in general & result interpretation



(3) Other tests & what bacterial pathogen they identify & result interpretation




### (3) TESTS & RESULTS

## Tests for *N. meningitidis* & *H. influenzae*

Test	Bacterial Pathogen	Result
<b>Kovac's Oxidase Test</b> - determines presence of cytochrome c oxidase in bacterium	<i>N. meningitidis</i> & <i>H. influenzae</i>	Positive (filter paper): color change (blue) Positive (plate method): color change (purple)
<b>Carbohydrate Utilization Test</b> - tests pathogen's ability to produce acid from carbohydrate by oxidation of glucose and maltose	<i>N. meningitidis</i>	Positive: glucose & maltose medium turns yellow with turbidity development
<b>Hemin &amp; NAD Requirement Test</b> - tests if the pathogen requires both hemin and NAD for growth	<i>H. influenzae</i>	Positive: <i>H. influenzae</i> only grow around disk with hemin + NAD
<b>Slide Agglutination Sero-grouping Test</b> - tests for agglutination of bacterium and serogroup-specific antisera for serotype identification	<i>N. meningitidis</i> & <i>H. influenzae</i>	Positive: strong agglutination with intensity rating of 3+ within 2 minutes

# (3) TESTS & RESULTS

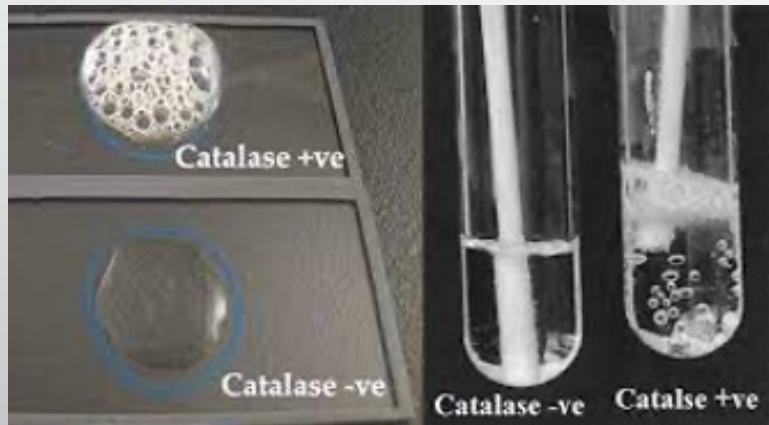
## Testing the Presence of Bacterial Pathogens

Test	Bacterial Pathogen	Result
<b>PCR Test</b> - targets a unique sequence of the pathogen using complementary fluorescent oligonucleotide probe	<i>N. meningitidis</i> ; <i>H. influenzae</i> ; <i>S. pneumoniae</i>	Positive: fluorescent signal observed: <ul style="list-style-type: none"><li>• <i>N. meningitidis</i>: sequence for SodC</li><li>• <i>H. influenzae</i>: sequence for BexA</li><li>• <i>S. pneumoniae</i>: sequence for LytA</li></ul>
<b>Latex Agglutination Test</b> - detects pathogen by mixing a sample with latex particles coated with Ab to observe agglutination	<i>N. meningitidis</i> ; <i>H. influenzae</i> ; <i>S. pneumoniae</i>	Positive: agglutination within 5-10 seconds  Negative reaction (left) and positive reaction (right)

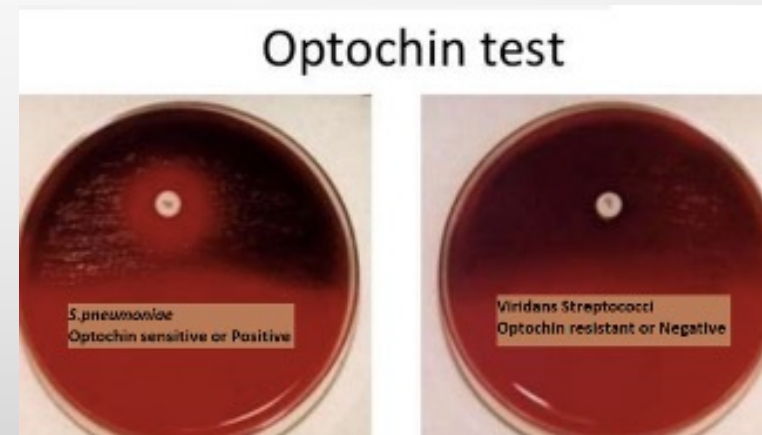
### (3) TESTS & RESULTS

#### Other Tests

Test	Bacterial Pathogen	Result
<b>Catalase Test</b> - differentiates between gram-positive cocci based on if bubbles occur due to catalase enzyme	Staphylococcus vs Streptococcus	Positive: bubbles in the liquid (Staphylococcus)
<b>Optochin Test</b> - tests pathogen's susceptibility to optochin	<i>S. pneumoniae</i>	Positive: inhibition zone > 14 mm around an optochin disk



Catalase test observations



Optochin test observations



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