MICROBIOLOGY LABORATORY IN DIAGNOSING BACTERIAL MENINGITIS

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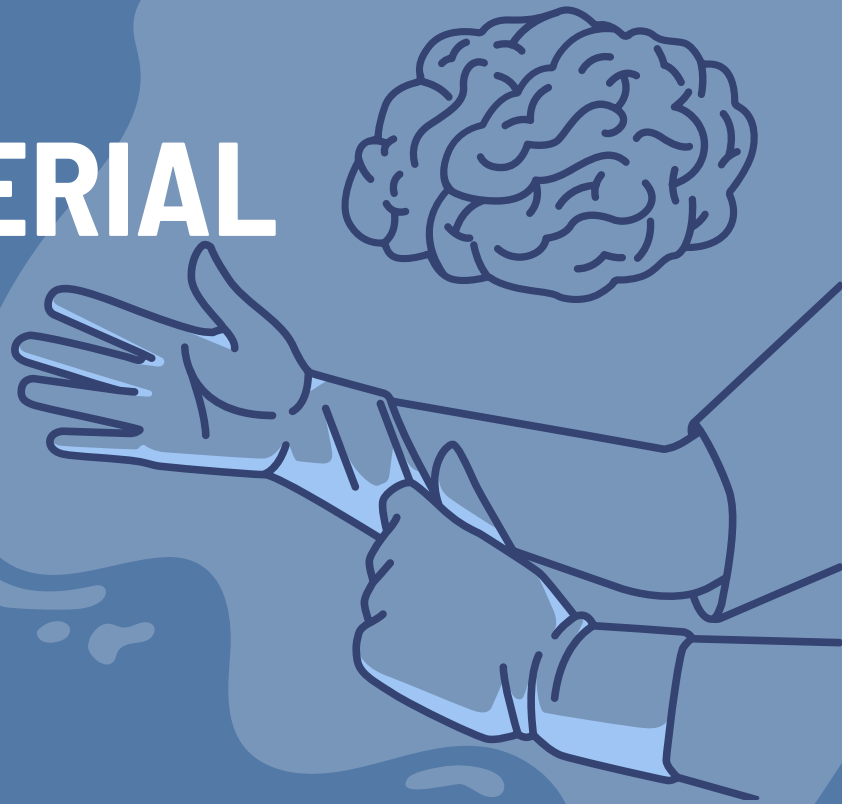
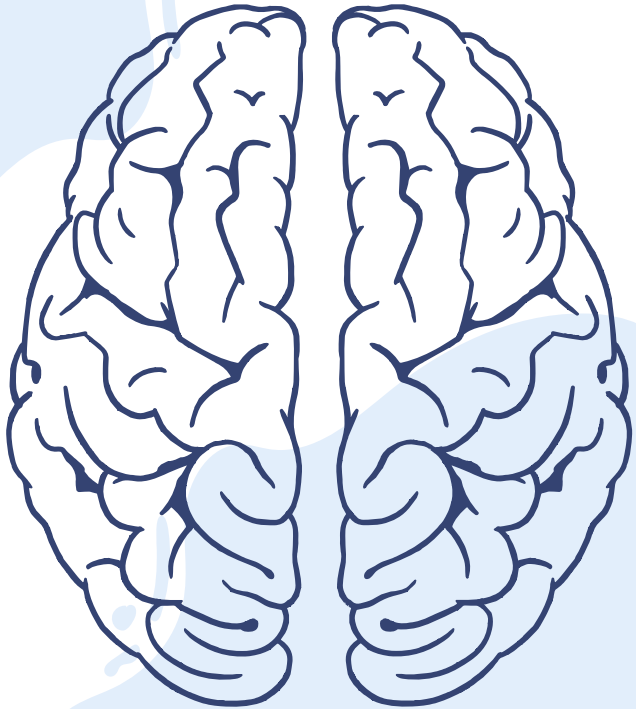




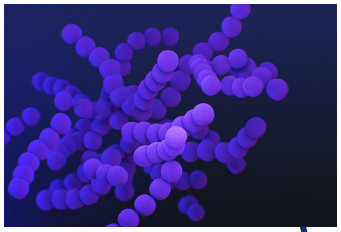
PATIENT OVERVIEW

18-year-old Mary has just moved into the dormitory at her university. One day, her roommate finds her lying in bed under her sheets. She is complaining of fever, chills, bad headache and a stiff neck. She is staying under the covers because the light is hurting her eyes. Her roommate calls 911 and an ambulance takes Mary to the local hospital.

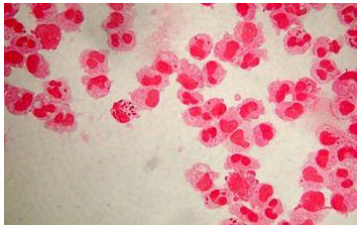
The emergency room physician asks Mary about her recent vaccinations and she reports that she has not had any since she was in elementary school. The physician documents a fever of 39.2°C and low blood pressure. He sends blood and cerebrospinal fluid to the Microbiology Laboratory. She is started immediately on intravenous antibiotics. Mary's blood and cerebrospinal fluid grow Neisseria meningitidis and she is diagnosed with meningococcal meningitis.

Common Bacterial Pathogens Causing Meningitis

Group B streptococcus (8)

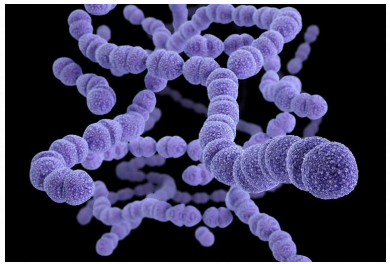


Neisseria Meningitidis (7)

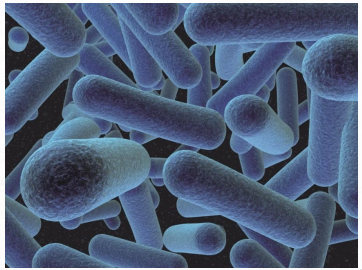


Escherichia coli (9)

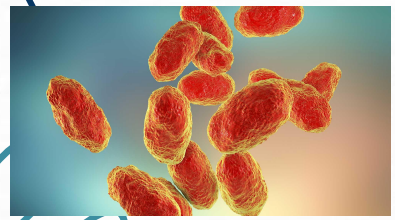
Streptococcus Pneumonia (12)



Listeria monocytogenes (10)



Haemophilus influenzae (11)



Neisseria Meningitidis	Streptococcus Pneumoniae	Haemophilus influenzae
• Gram-negative bacteria • 13 capsular serogroups (serogroups A, B, C, W-135 and Y are most commonly found in meningococcal infection in humans) • leading cause of bacterial meningitis as well as sepsis • only form of bacterial meningitis known to occur epidemically, mainly in Africa and Asia • spread through saliva and respiratory secretions during coughing, sneezing, kissing, chewing on toys and even through sharing a source of freshwater • a single dose of intramuscular antibiotic is often given at the earliest possible opportunity	• lancet-shaped, facultatively anaerobic, Gram-positive bacteria • causative agent of 58% of the meningitis cases in the U.S. • Transmitted through sneezing and coughing; normally resides in the upper respiratory tract • Infect hosts that do not have prior immunity, or have altered immune systems • can cross the blood-brain barrier and infect the central nervous system, leading to inflammation of the CNS • Symptoms include hearing loss and cognitive impairment • Can be prevented with the pneumococcal conjugate vaccine, PCV 7	• Gram-negative, facultatively anaerobic bacteria • divided into subgroups based on capsule type, with H. influenzae type b (Hib) being the predominant group • Patients under 5 years old, over 65 years old and immunocompromised individuals are at risk • Transmitted through respiratory droplet inhalation and can cross the blood-brain barrier • Symptoms include fever, headache, stiff neck, nausea, photophobia and altered mental status • Can be prevented with Hib conjugate vaccine

Group B streptococcus	Escherichia coli	Listeria monocytogenes
• Gram-positive bacteria present in the reproductive and gastrointestinal tract of 35% of healthy women • resides harmlessly in the bowels, vagina, and sometimes the back of the nose and throat • could be transmitted to the newborn during birth • not spread through food and water, and not spread through contact • can cross the blood-brain barrier into the cerebral spinal fluid (CSF) • Treatment includes antibiotic administration and CSF culture monitoring • 20-30% of infants infected with GBS have severe neurological defects	• Gram-negative bacteria that is the common cause of meningitis in infants • Can cross the blood-brain barrier • E. coli infection establishes due to an immature immune system in the newborn • Strains causing meningitis have the K1 polysialic capsule • Most E. coli are harmless, and are commensal in the intestinal tract • some strains can cause diarrhea or other illness • second most common cause of neonatal meningitis • very rare in adults	• Gram-positive bacteria that accounts for 20% of meningitis in newborns and the elderly • transmitted by ingesting contaminated food and vertical transmission • listeriosis of the CNS can cause meningitis • symptoms include headache, stiff neck, confusion, loss of balance, and convulsions • individuals living in the region of sub-Saharan Africa are at risk due to malnutrition and weakened immune system • Current treatment includes antibiotic administration such as penicillin and ampicillin

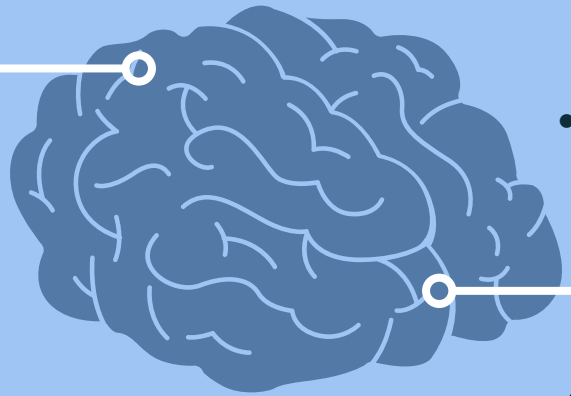
IMPORTANCE OF MICROBIOLOGY LABORATORY

- Able to correctly diagnose 90% of cases
- To identify the causative microorganism in order to render proper treatment
- To monitor efficacy of treatment since lab testing can quantify bacteria and white blood cells

PATIENT SAMPLES TAKEN FOR LABORATORY TESTING

CEREBRAL SPINAL FLUID

- Obtained through lumbar puncture
- The CSF should be clear and colorless, and fluid will drip out into a vial for collection
- Should not be contaminated with blood if multiple samples are collected
- Within an hour of collection, the sample should be taken, at 20-35 degrees Celsius, into the Microbiology Laboratory for analysis
- If analysis is not possible within the hour, then inoculation into Trans-Isolate (T-I) medium should be done with around 5% CO2, at 35-37 degree Celsius
- Neutrophil count >1180/mm3 or leukocyte count >2000/mm3 rules out viral cause
- Gram stain and culture most important tests
- PCR and agglutination reserved as secondary tests for cases where Gram stain results are negative



BLOOD

- About 1-3 ml can be collected and diluted to obtain a blood culture with adequate amount of bacterial growth
- Within 1 min of collection, the blood sample should be inoculated into culture so that the syringe is not clogged with blood
- incubated with around 5% CO2, at 35-37 degree Celsius
- used for blood work such as complete blood count (CBC), coagulation studies, and electrolyte levels
- Inflammatory markers in the blood, such as C-reactive protein and procalcitonin may also be used to determine if it is aseptic or bacterial meningitis

TESTS PERFORMED TO IDENTIFY PATHOGENS

CULTURE

- Culture of CSF can identify the causative bacteria, which can be grown on either a blood agar plate (BAP) or a chocolate agar plate (CAP)
- Increased presence of WBCs and RBCs can cause CSF to appear unclear, indicating the presence of infections (99% of patients have WBC count > 100/mm3 and 87% of patients have WBC count > 1000/mm3)
- Low WBC cell count may indicate viral meningitis
- Increased levels of CSF proteins indicate the presence of infections
- CSF glucose level of 0.4mg/dL is 80% indicative of bacterial meningitis

KOVAC'S OXIDASE TEST

- to detect *N. meningitidis* and *H influenzae*
- Can determine the presence of cytochrome c oxidase
- Reagent: tetramethyl-p-phenylenediamine dihydrochloride (turns purple by compounds that have cytochrome c in their respiratory chain)
- Bacteria grown on a blood agar plate (BAP) for 24 hours prior to the test
- filter paper strip submerged in a few drops of Kovac's oxidase reagent, and an inoculating loop will transfer a colony from the plate and rubbed onto the treated filter paper

CARBOHYDRATE UTILIZATION

- to detect *N. meningitidis*
- examines a pathogens' ability to ferment carbohydrates, determined by the acid indicator in the medium
- glucose, maltose, lactose, and sucrose are added into four different tubes with cystine trypticase agar (CTA) and phenol red indicator
- Colonies from the plate removed via a disposable loop, then stabbed in the upper portion of the CTA sugar medium
- incubation for at least 72 hours

SLIDE AGGLUTINATION SEROGROUPING

- to detect *N. meningitidis* and *H. influenzae*
- uses a serogroup-specific antisera
- a clump will form if the antisera binds to the bacterial cells. An intensity rating can then be determined

LATEX AGGLUTINATION

- to detect *S. pneumonia*
- quick detection of bacterial antigens in CSF
- a sample with a specific antigen is mixed with latex particles that have antibodies coating the surface
- visible precipitate can be observed if agglutination occurs
- uses the supernatant aspect (heated to 100 degrees Celsius) of the previous centrifuged sample, then add 30-50 ul of the liquid to disposable latex reagent

GRAM STAIN

- to differentiate between Gram-positive and Gram-negative bacteria
- divide a glass slide in two, smearing one side with the sample and smearing the other side with the organism in question
- Smear fixed by flooding with methanol, washed with water → flooded with crystal violet ammonium oxalate to stain → flooding with Gram's iodine
- slide rinsed with ethanol to decolourise, counterstained with safranin or carbol-fuchsin → smear examined under a microscope when washed and dried

CATALASE TEST

- to detect *S. pneumonia*
- uses the enzyme catalase, which decomposes hydrogen peroxide into water and oxygen gas
- the isolate will be grown on a BAP for 24 hours prior → a disposable loop removes a colony from the dish and place it onto a glass slide → Hydrogen peroxide added to the slide and mixed with the bacteria
- positive catalase test: vigorous bubbles in the liquid; negative test: absence of bubbling

OPTOCHIN TEST

- to detect *S. pneumonia*
- Uses the chemical ethylhydrocupreine hydrochloride (optochin)
- Strains to be tested are grown on a BAP for 24 hours → isolated colony transferred via a loop and streaked onto half of a new BAP → optochin disk placed within the streaked area incubated overnight at 35-37 degree Celsius

BILE SOLUBILITY TEST

- discriminate *S. pneumoniae* from other streptococci since it is bile soluble whereas the latter are resistant
- bile solution prepared with sodium deoxycholate (bile) dissolved in sterile water → isolate of bacteria grown on a BAP for 24 hours prior to the test → colony transferred to a tube with saline in the 0.5-1.0 McFarland standard of turbidity → adding sodium deoxycholate

POLYMERASE CHAIN REACTION (PCR) TEST

- has high sensitivity and specificity for many bacteria, and can be done with small volumes of CSF
- Target a unique sequence of the bacterial pathogen using a fluorescent oligonucleotide probe
- If the specific bacteria is present in culture, this will be indicated by a corresponding fluorescent signal
- sensitivity of PCR is 95-100% for enterovirus meningitis and 54-100% for tuberculous meningitis

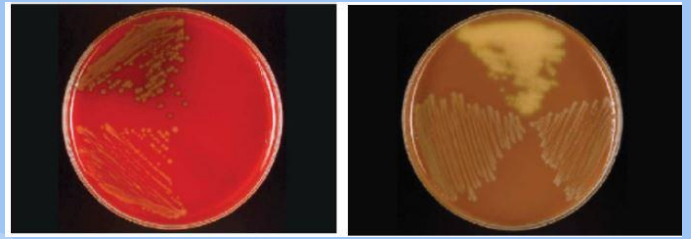
- to detect *H. influenzae*
- *H. influenzae* can be grown on a plate containing chocolate agar for 24 hours → colonies formed are then used to create a heavy suspension of cells (above 1.0 McFarland standard) and vortexed
- Inoculate a heart infusion or trypticase soy agar plate with the cell suspension and place the paper strips containing hemin, NAD, and hemin + NAD
- *H. influenzae* can only grow around the disk with both hemin and NAD
- *H. Haemolyticus* grows using the same factors, hence a hemolysis test must be checked on rabbit or horse blood agar to differentiate between the two

HEMIN AND NICOTINAMIDE-ADENINE-DINUCLEOTIDE (NAD) GROWTH FACTOR REQUIREMENT TEST

RESULTS EXPECTED FROM TESTS

01 CULTURE TESTS

	N. meningitidis	H. influenzae	S. pneumoniae
blood agar plate (BAP)	round, smooth, glistening, moist, convex edged colonies with no hemolysis	Not growing without supplementation	small, grey, moist and mucoidal colonies with alpha hemolysis
chocolate agar plate (CAP)	large, colorless, opaque cultures also without hemolysis or discoloration	large, colorless, grey and opaque cultures	small, grey, and moist culture with alpha hemolysis



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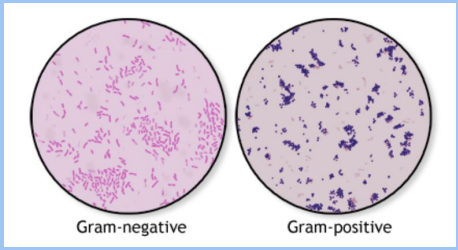
02 GRAM STAIN

	N. meningitidis	H. influenzae	S. pneumoniae
Gram-positive or Gram-negative	**Gram-negative**	**Gram-negative**	**Gram-positive**
Sample color change	turn pink or red with crystal violet dye and iodine followed by safranin	a purple color after safranin is added	a pink or red colour with safranin



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03 PCR TEST

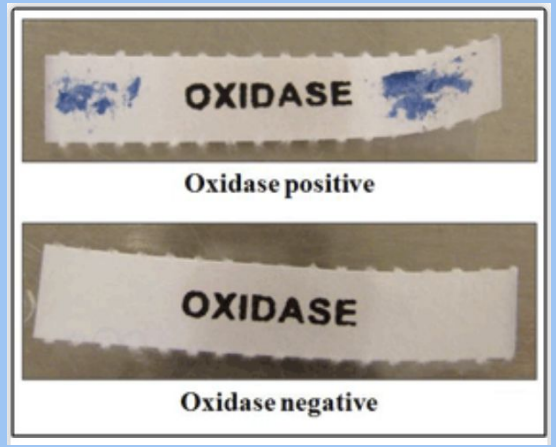
	N. meningitidis	H. influenzae	S. pneumoniae
Expected results	Superoxide dismutase genes, sodC can be detected	presence of bexA should be observed	specific segment of autolysin gene, lytA, should be detected

04 KOVAC'S OXIDASE TEST

	N. meningitidis	H. influenzae	S. pneumoniae
Filter paper method	the filter paper turns medium blue	the filter paper turns medium blue	Not applicable
Plate method	Culturing plates turn purple	Culturing plates turn purple	Not applicable

05 SLIDE AGGLUTINATION SEROGROUPING (SASG)

- **Used to specifically detect N. meningitidis**
- **Positive test results:** a rating of 3+ or 4+
- **Negative test results:** a rating of 0, 1+ or 2+



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06 CARBOHYDRATE UTILIZATION

- Used to specifically detect N. meningitidis
- Positive test result: a color change of yellow
- Negative test result: no color change

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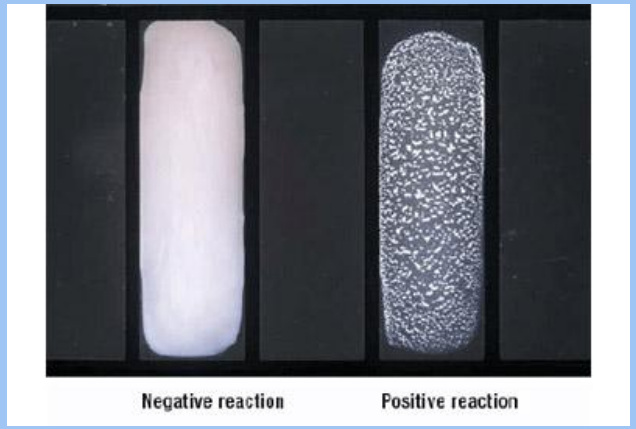
07 LATEX AGGLUTINATION

	N. meningitidis	H. influenzae	S. pneumoniae
Expected test results	clumping of cells within 5-10 seconds	clumping of cells within 5-10 seconds	clumping of cells within 5-10 seconds
Test accuracy	positive in 39% of all cases	positive in 93% of all cases	positive in 60% of all cases



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08 CATALASE TEST

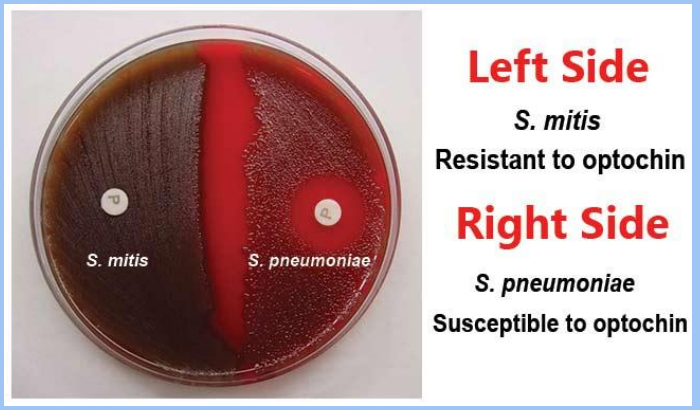
- **Positive test results:** vigorous bubbles in the liquid, indicating that the bacteria is of the genus Staphylococcus
- Negative test results: no bubbling, indicating the bacteria detected in culture is S. pneumoniae

10 OPTOCHIN TEST

- **Used to specifically detect** S. pneumonia
- a zone of inhibition of 14 mm or greater would indicate that the sample bacteria is sensitive, identifying that the bacterial pathogen is indeed S. pneumonia

09 HEMIN AND NAD GROWTH FACTOR REQUIREMENT TEST

- used to determine the presence of H. influenzae
- Positive test results: a pink gram stain should be detected
- to discriminate H. influenzae from H. Haemolyticus, there should be an absence of hemolysis on horse or rabbit blood agar



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CONCLUSION

- While the symptoms Mary is experiencing are very characteristic of meningitis, including fever, headache, and a stiff neck, microbiology laboratory testing is essential to further confirm a meningitis diagnosis
- It is essential to administer intravenous antibiotics immediately after symptom recognition to limit the progression of the infection

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