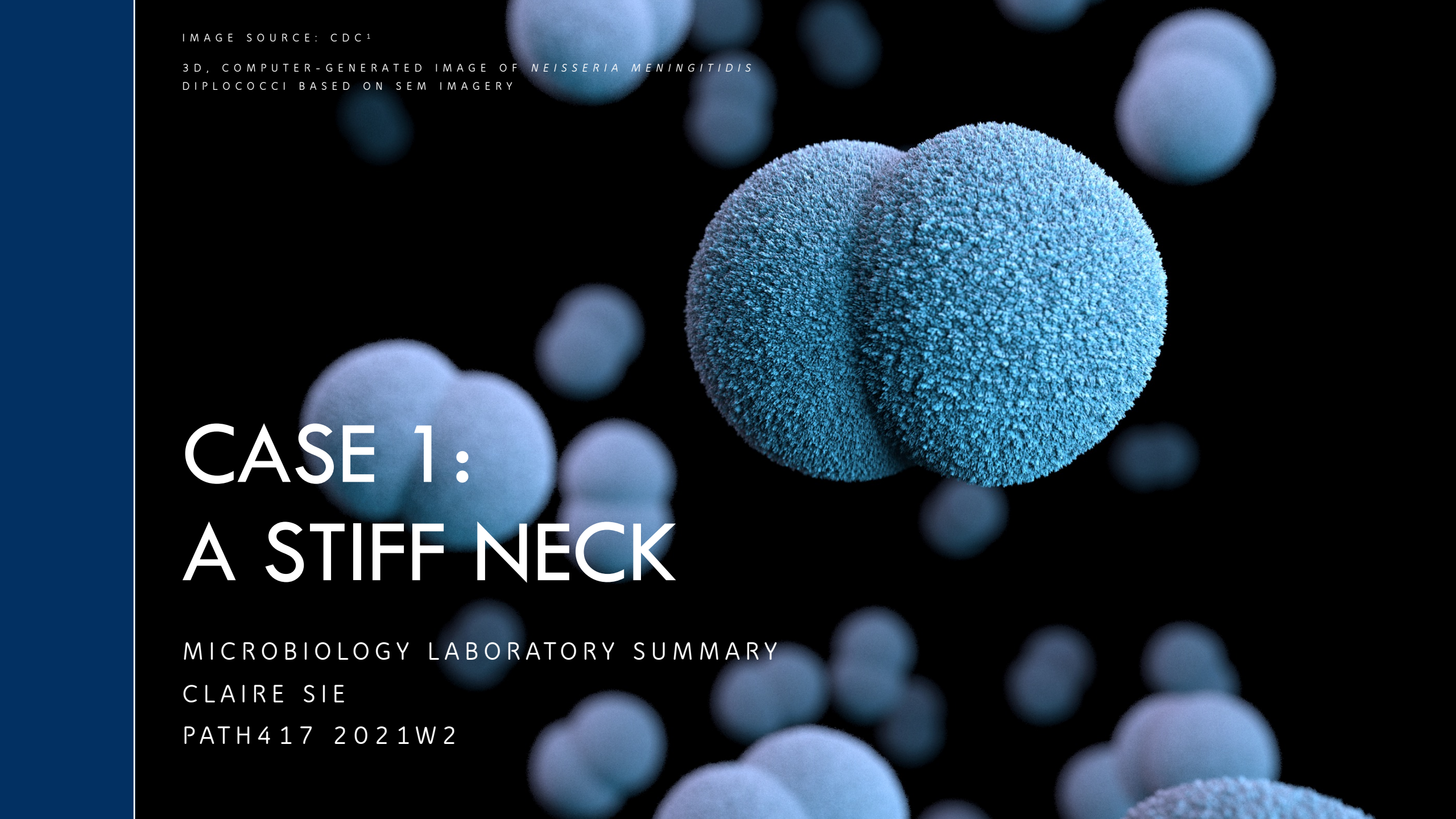


IMAGE SOURCE: CDC[1]

3D, COMPUTER-GENERATED IMAGE OF *NEISSERIA MENINGITIDIS* DIPLOCOCCI BASED ON SEM IMAGERY

CASE 1: A STIFF NECK

MICROBIOLOGY LABORATORY SUMMARY

CLAIRE SIE

PATH417 2021W2

CASE 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 39.2C and low blood pressure. He sends blood and cerebral spinal fluid to the Microbiology Laboratory. She is started immediately on intravenous antibiotics. Mary's blood and cerebral spinal fluid grow *Neisseria meningitidis* and she is diagnosed with meningococcal meningitis."

WHAT IS MENINGITIS?

INFLAMMATION OF THE MENINGES

Meninges – protective membrane surrounding brain and spinal cord

CAN CAUSE DAMAGE TO THE CNS

Increased lymphocyte invasion, neuron damage, and intracranial swelling

VARIETY OF PATHOGENIC CAUSES

Bacterial, fungal, viral – treatments vary

Symptoms:

FEVER AND CHILLS

Elevated body temperature

PAIN

Headache and stiff neck

PHOTOPHOBIA

Eyes become sensitive to light

MAIN CAUSATIVE AGENT: *NEISSERIA MENINGITIDIS*

- Gram-negative, encapsulated, human pathogen
- Leading cause of bacterial meningitis worldwide[1]
- Commensal organism in ~10% of population[2]
- 13 serotypes based on variation in porin protein (6 disease-causing)[3]
- Invades and replicates in cerebral spinal fluid (CSF)
- Capsule-based, conjugate vaccines available and effective

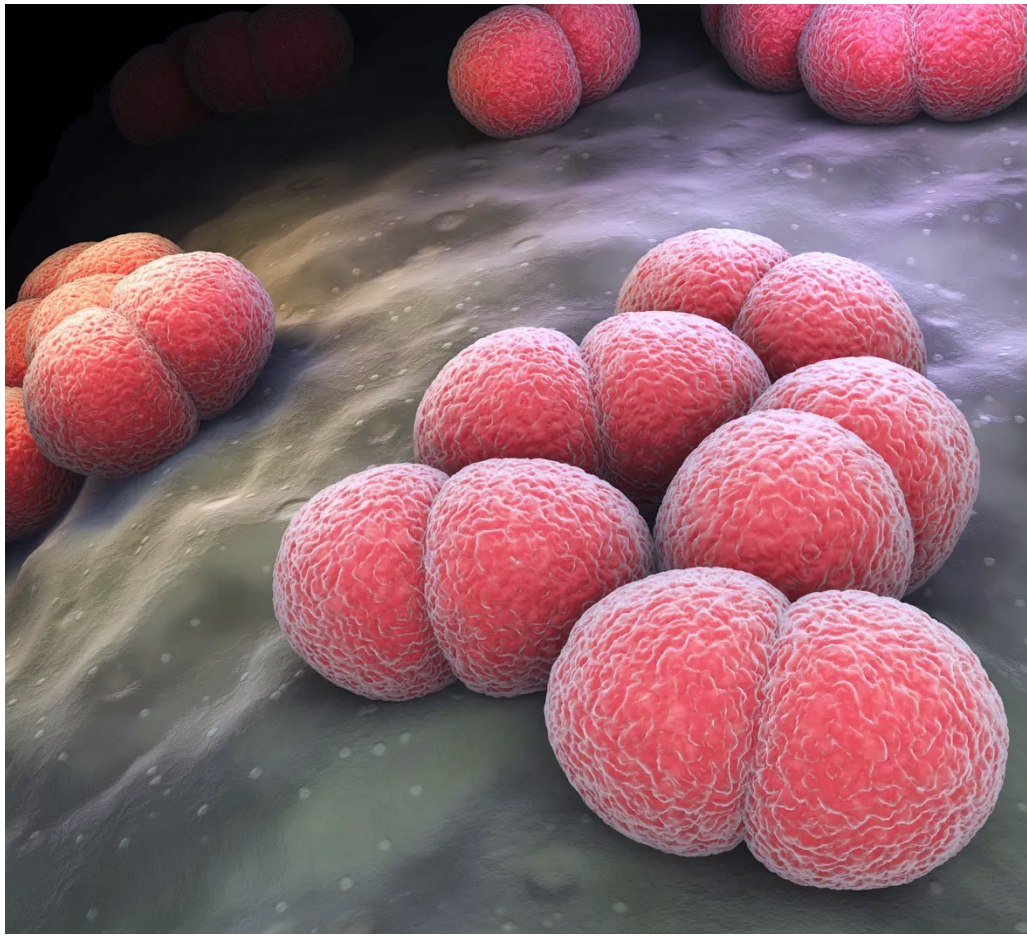
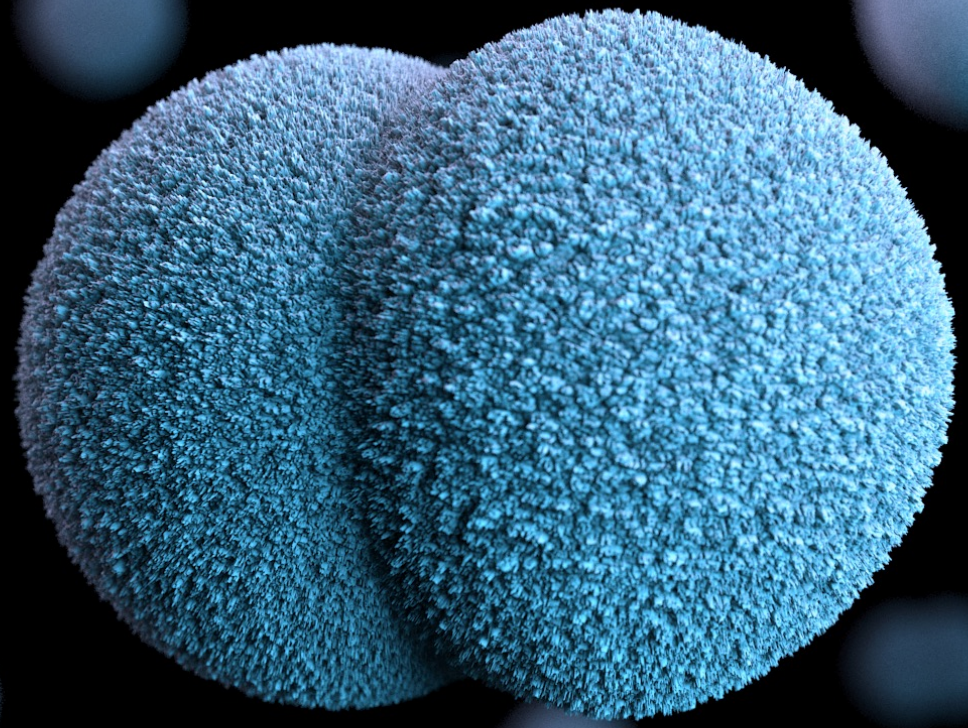


IMAGE SOURCE: *NEISSERIA MENINGITIDIS* BACTERIA, THE CAUSE OF MENINGOCOCCAL MENINGITIS. REPRINTED FROM ENCYCLOPEDIA BRITANNICA[3]. COPYRIGHT ENCYCLOPEDIA BRITANNICA 2021

IMAGE SOURCE: CDC[1]

3D, COMPUTER-GENERATED IMAGE OF *NEISSERIA MENINGITIDIS* DIPLOCOCCI BASED ON SEM IMAGERY



COMMON PATHOGENS

Other than the stated bacterial cause, what are the most common bacterial pathogens associated with this type of infectious scenario?

POTENTIAL CAUSATIVE AGENTS

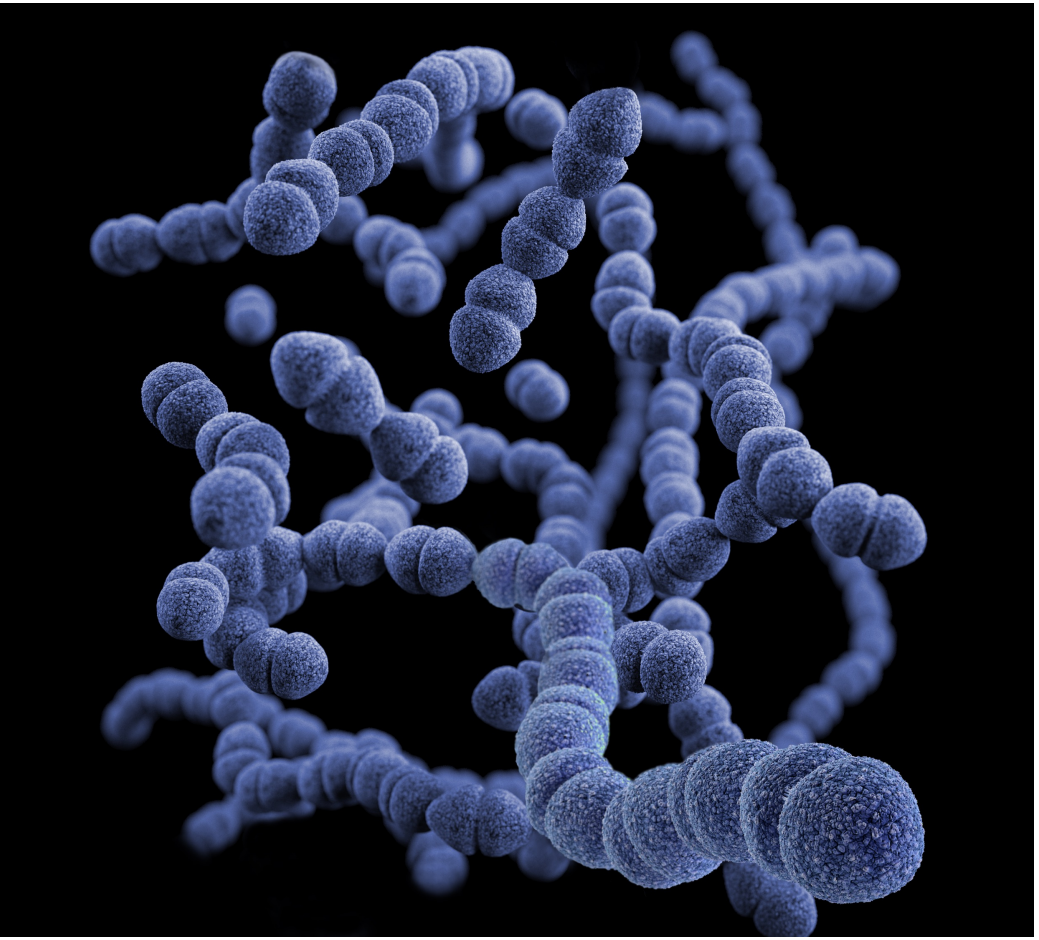
1. *Neisseria meningitidis*
2. *Streptococcus pneumoniae*
3. *Haemophilus influenzae*

Responsible for majority of meningitis cases in the US (all age groups)[3]

4. Group B streptococcus
5. *Escherichia coli*
6. *Listeria monocytogenes*

More common causative agent of bacterial meningitis in infants younger than 30 days[4]

STREPTOCOCCUS PNEUMONIAE



- Gram-positive, lancet-shaped pathogen
- Causative agent in 58% of meningitis cases in US[5]
- Can cross blood-brain barrier to infect central nervous system (CNS)[6]
- Asymptomatic colonization of upper respiratory tract in 5-10% of adults without children[7]
- Transmission through respiratory droplets
- Increasing rates of penicillin resistance[8]
- Preventative vaccine available[9]

IMAGE SOURCE: 3D, COMPUTER-GENERATED IMAGE OF *STREPTOCOCCUS PNEUMONIAE* BASED ON SEM IMAGERY ADAPTED FROM CDC/ANTIBIOTIC RESISTANCE COORDINATION AND STRATEGY UNIT[4]. COPYRIGHT CDC 2013.

HAEMOPHILUS INFLUENZAE

- Gram-negative, encapsulated pathogen
- Subgroup division based on capsule type, *H. influenza* Type B (Hib) dominant[10]
- Particular risk of infection in patients <5 and >65, immunocompromised individuals[10]
- Increasing rates of ampicillin resistance[10]
- Can asymptomatically colonize nasopharynx[11]
- Transmission through respiratory droplets

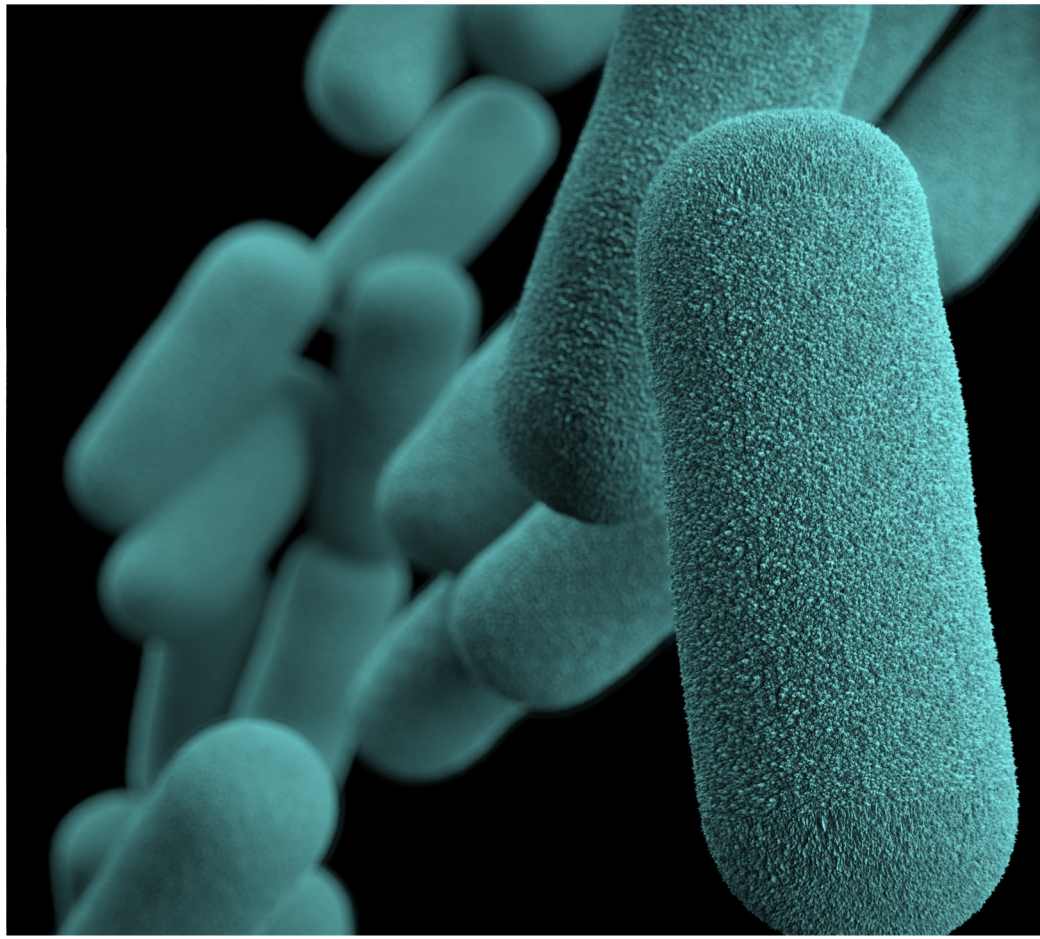
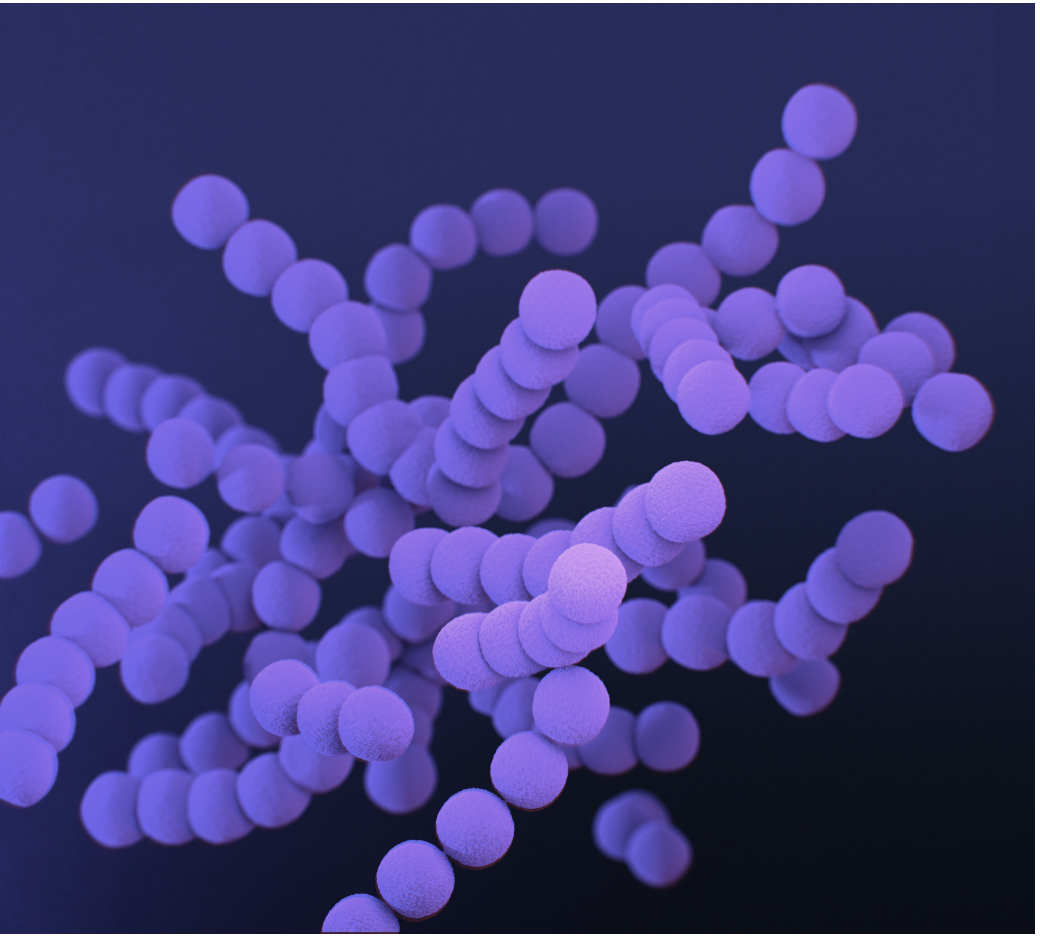


IMAGE SOURCE: 3D, COMPUTER-GENERATED IMAGE OF *HAEMOPHILUS INFLUENZAE* BASED ON SEM IMAGERY ADAPTED FROM CDC/SARAH BAILEY CUTCHIN[5].

GROUP B STREPTOCOCCUS



- Gram-positive cocci, forms pairs or chains
- Commensal organism in reproductive and GI tract in 1/3 of women[12]
- Transmission to newborns during childbirth[13]
- Contact-based transmission is unlikely[13] – therefore Mary is unlikely to be infected with it

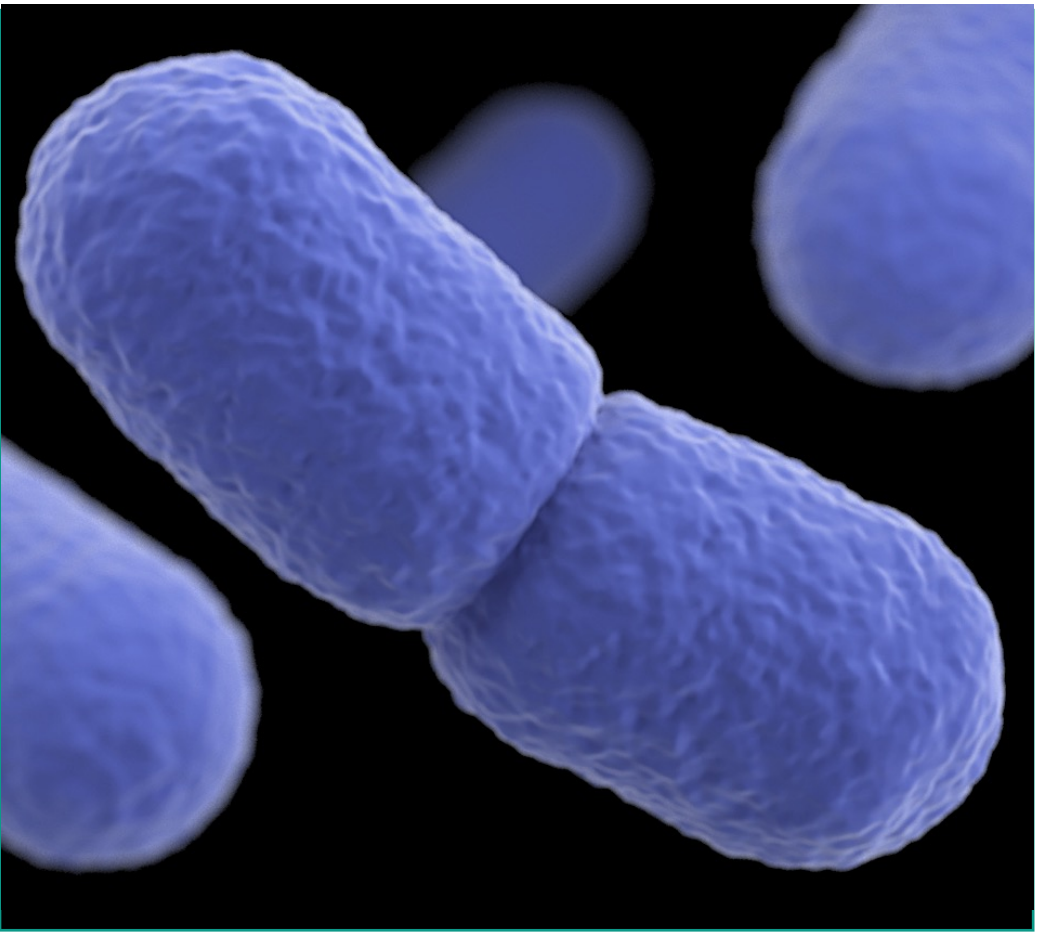
IMAGE SOURCE: 3D, COMPUTER-GENERATED IMAGE OF GROUP B *STREPTOCOCCUS* BASED ON SEM IMAGERY ADAPTED FROM CDC/ANTIBIOTIC RESISTANCE COORDINATION AND STRATEGY UNIT[6].

ESCHERICHIA COLI

- Gram-negative organism
- Establishes infection in infants due to immature immune system
- Meningitis-causing strains possess K1 polysialic capsule – essential for crossing BBB[14]
- Typically harmless and commensal in intestines[15]
- Transmission to newborn occurs during vaginal delivery[16]
- Unlikely to be causative agent in Mary's case (*E. coli* meningitis rare in adults[17])

IMAGE SOURCE: 3D, COMPUTER-GENERATED IMAGE OF *ESCHERICHIA COLI* BASED ON SEM IMAGERY ADAPTED FROM CDC/ANTIBIOTIC RESISTANCE COORDINATION AND STRATEGY UNIT[7]. COPYRIGHT CDC 2016

LISTERIA MONOCYTOGENES



- Gram-positive, intracellular pathogen
- Transmission by ingestion of contaminated food, or during vaginal delivery[18]
- Environmental factors – common cause of bacterial meningitis in sub-Saharan Africa[19]
- Listeriosis – additional symptoms include convulsions and loss of balance[17]
- Unlikely to be causative agent in Mary's case due to her lack of additional symptoms

IMAGE SOURCE: 3D, COMPUTER-GENERATED IMAGE OF *LISTERIA MONOCYTOGENES* BASED ON SEM IMAGERY ADAPTED FROM CDC/JAMES ARCHER[8]. COPYRIGHT CDC 2016.

IMAGE SOURCE: PEXELS[9] 2021. IMAGE FREE FOR USE.

BLOOD SAMPLES IN COLLECTION TUBES

SAMPLING

What samples are taken for laboratory testing and how important is the Microbiology Laboratory in the diagnosis of this particular infectious disease?

IMPORTANCE OF THE MICROBIOLOGY LABORATORY

Clinicians will collect samples for a **three-pronged approach** to microbiological testing:

- Blood culture
- Cerebrospinal fluid (CSF) Gram stain
- CSF culture

Effectively and correctly diagnoses 90% of cases with Mary's symptoms (fever, headache, stiff neck)[20]

Important to identify the causative agent of Mary's symptoms as different pathogens are sensitive/resistant to different antibiotics

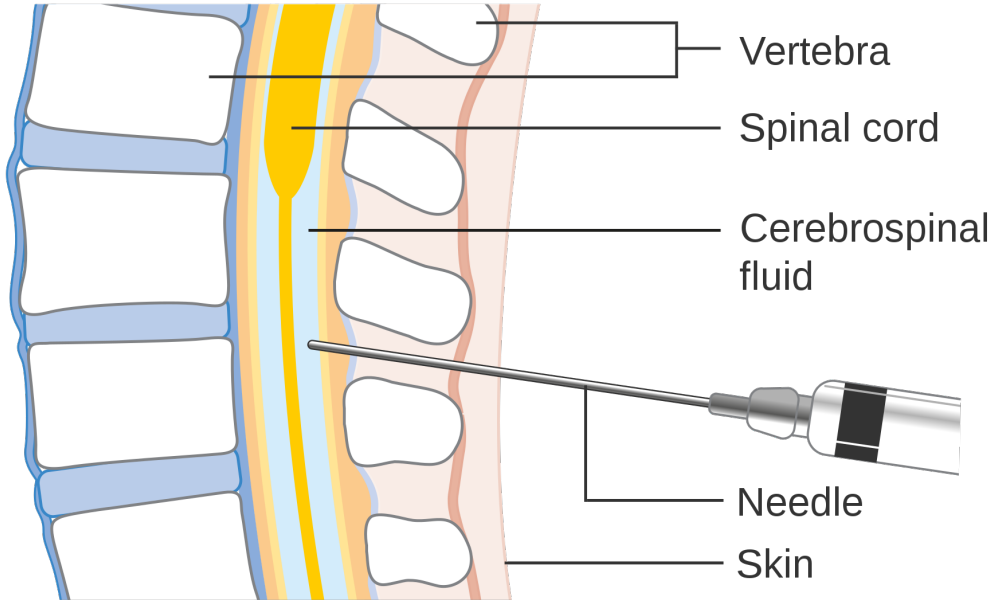
Treatment with broad-spectrum antibiotics can lead to failure to clear pathogen and development of antibiotic resistance!

CEREBROSPINAL FLUID (CSF)

A CLEAR AND COLOURLESS FLUID WHICH CUSHIONS AND PROVIDES NUTRIENTS TO THE CENTRAL NERVOUS SYSTEM (CNS)

Collection Process:

- ❑ Sample collected before or shortly after administration of antibiotics
- ❑ Obtained via lumbar puncture
- ❑ Needle inserted between lumbar vertebrae (image shown) while patient is sitting or on their side; sterility important - contamination can interfere with testing results
- ❑ Sample taken to Microbiology Laboratory for analysis within an hour of collection[21]
  - ❑ If not possible, inoculate into Trans-Isolate (T-I) medium and incubation at 5% CO2, 37C until ready for transport[21]



Examine CSF for:

- ❑ Cell count
- ❑ Glucose level
- ❑ Gram stain
- ❑ Cultures
- ❑ Polymerase Chain Reaction (PCR)

IMAGE SOURCE: DIAGRAM SHOWING HOW TO PERFORM A LUMBAR PUNCTURE FOR THE COLLECTION OF CEREBROSPINAL FLUID ADAPTED FROM CANCER RESEARCH UK/WIKIMEDIA COMMONS[10]. COPYRIGHT WIKIMEDIA COMMONS 2014.

BLOOD

Collection Process:

- ❑ 1-3 ml collected before or shortly after administration of antibiotics[22]
- ❑ Sample should be inoculated within one minute of collection and taken to Microbiology Laboratory immediately[21]

Used for:

- ❑ Complete blood count (CBC)
- ❑ Coagulation studies
- ❑ Electrolyte levels
- ❑ Probe for inflammatory markers

IMAGE SOURCE: 2 VIALS OF BLOOD ON A RACK.
REPRINTED FROM PEXELS[11]. COPYRIGHT PEXELS 2021. IMAGE FREE FOR COMMERCIAL USE. NO ATTRIBUTION REQUIRED.

NOTES ON SAMPLE COLLECTION

- If samples were collected after antibiotic administration, or if no bacteria observed via Gram stain:
  - Perform Polymerase Chain Reaction test (PCR) -
  - PCR can confirm:
    - Meningitis
    - Causative agent – e.g. *N. meningitidis*, *S. pneumoniae*, *H. influenzae*[23]

- Important! Follow biosafety guidelines to reduce risk of being infected with unknown pathogen while collecting CSF and blood samples[21]
  - Wash hands, wear gloves, sterile technique, safe sample transport, safe sharps disposal, immediate and complete reporting of any injuries or contamination

IMAGE SOURCE: PIXABAY[12] 2017. IMAGE FREE FOR COMMERCIAL USE. NO ATTRIBUTION REQUIRED.

A CLOSE-UP IMAGE OF A MICROSCOPE

LABORATORY TESTING

Explain the tests that will be performed on the samples in order to detect any of the potential bacterial pathogens causing this disease.

TESTS PERFORMED ON SAMPLES

CULTURE

Growth on Blood or Chocolate agar plates, macroscopic and microscopic morphology

CSF CYTOLOGICAL ANALYSIS

Glucose level, CSF protein concentration, lymphocyte infiltration

GRAM STAIN

Identify pathogen as Gram-positive or Gram-negative

BIOCHEMICAL TESTING

Catalase, Oxidase, Carbohydrate utilization, Growth requirements

AGGLUTINATION

Identify pathogen using pathogen-specific antisera or antibody-coated latex particles

POLYMERASE CHAIN REACTION

Identify pathogen based on presence of organism-specific DNA sequences

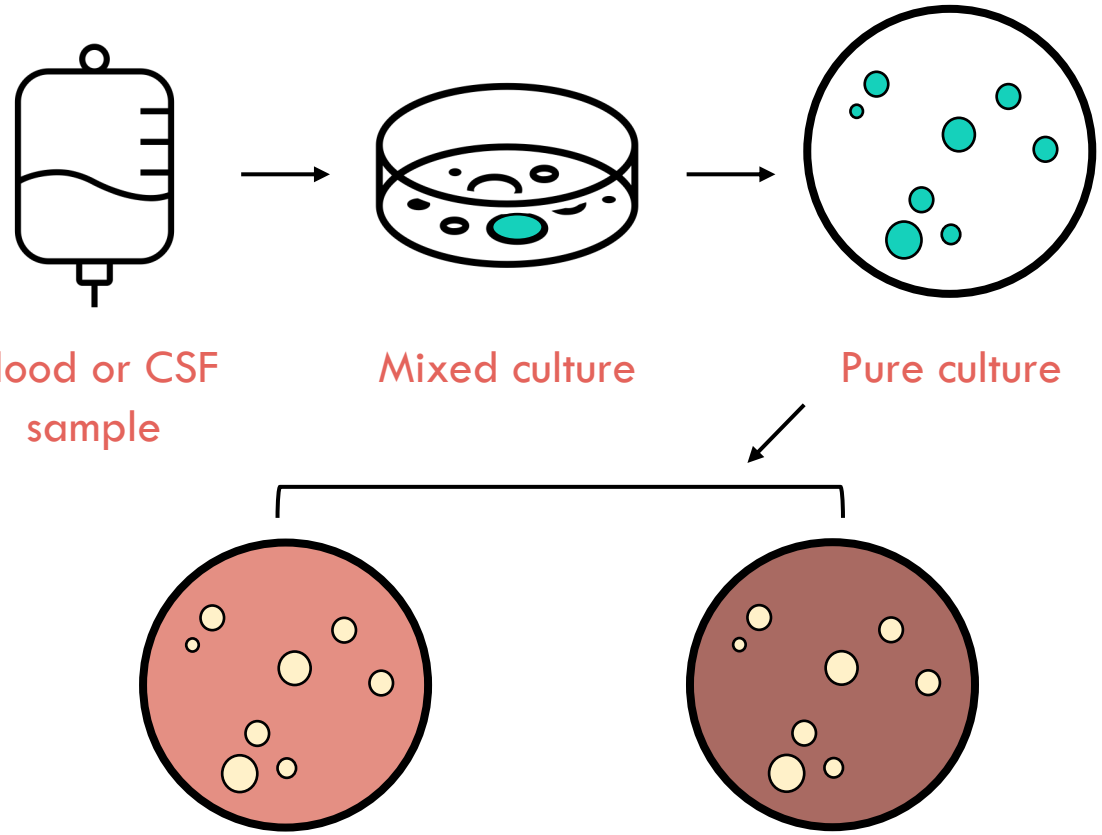
CULTURE

- Isolate a pure culture[24] from sample by:
  - Streak from sample to obtain single colonies
  - Inoculate blood agar plate or chocolate agar plate from single colony[25]
  - For use in further testing
- Observe macroscopic morphology
- Observe growth on selective/differential media

Positive blood culture can confirm[24]:

- 40% of meningococcal meningitis cases
- 50-90% of *H. influenzae* cases
- 75% of pneumococcal meningitis cases

CULTURE


Blood or CSF sample
Mixed culture
Pure culture
Blood agar plate
Differentiates between three types of hemolysis and prevents growth of certain sp.
Chocolate agar plate
A variant of blood agar, nonselective and good for growing fastidious organisms

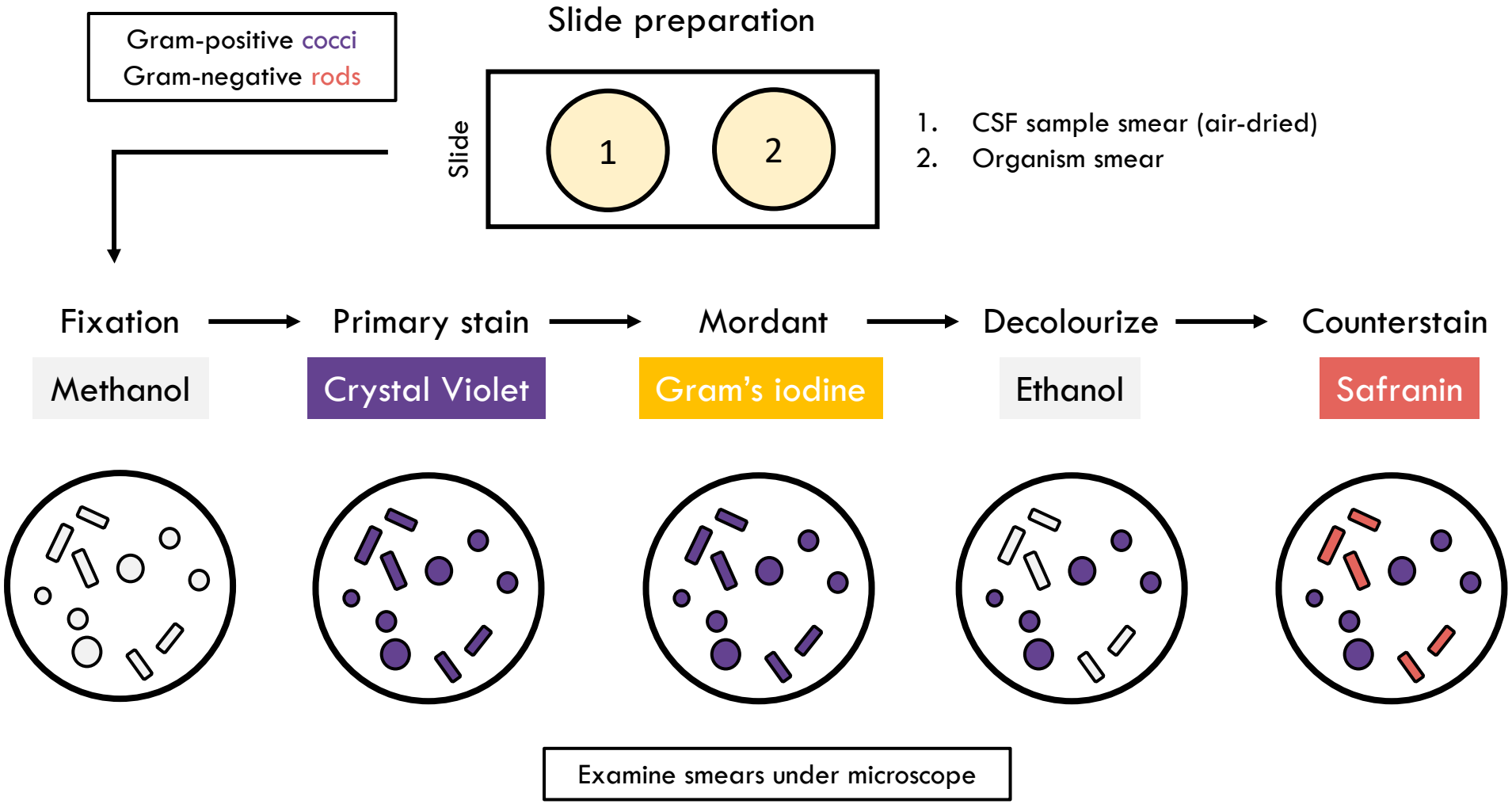
CSF CYTOLOGICAL ANALYSIS

Cerebrospinal fluid property	Healthy child or adult	Bacterial meningitis	Notes
Colour/Turbidity	Clear	Unclear	Presence of WBCs or RBCs[27]
Total leukocytes (cells/mm^3)	<6	>1000	Seen in 87% of bacterial meningitis cases[20,27]
Neutrophils (%)	0	>85-90%	Indicates inflammatory response
Protein (mg/dL)	20-40	>100-150	Sensitive indicator of CNS disease[27]
Glucose level (mg/dL)	40-80	0-<40	0.4 mg/dL 80% indicative of bacterial meningitis[20]
Select biomarkers (C-reactive protein, procalcitonin)		Elevated	Elevated levels point to bacterial meningitis[20,28]

Perform blood cultures in conjunction with CSF cytological analysis and CSF culture

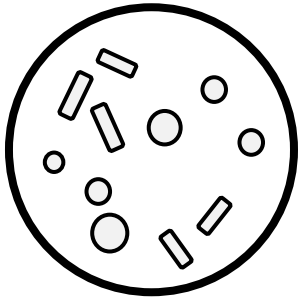
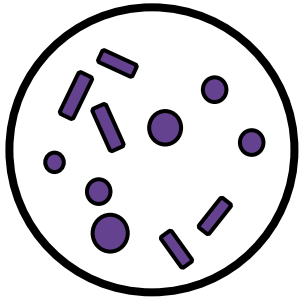
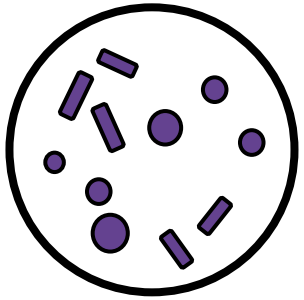
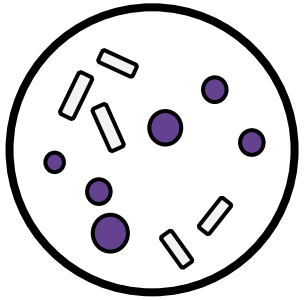
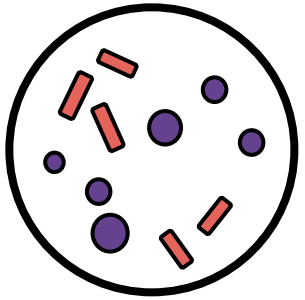
GRAM STAIN PROCEDURE

DIFFERENTIATE BETWEEN ORGANISMS BASED ON BACTERIAL CELL WALL PROPERTIES[29]



GRAM STAIN

DIFFERENTIATE BETWEEN ORGANISMS BASED ON BACTERIAL CELL WALL PROPERTIES

Fixation	Primary stain	Mordant	Decolourize	Counterstain
Methanol	Crystal Violet	Gram's iodine	Ethanol	Safranin
				
Prevent degradation of cells and cell structure	Thick peptidoglycan layer in Gram-positives traps CV[30]	Binds CV to cell wall (CV-iodine complexes)[31]	Disrupts lipids of outer membrane (Gram-negatives), CV washed away	All cells stained pink, but CV in Gram-positives is too dark for pink to be seen[31]

MICROSCOPIC MORPHOLOGY

CAN BE DONE CONCURRENTLY WHILE OBSERVING RESULTS OF GRAM STAIN

- Bacterial organisms exhibit characteristic morphological differences which allow them to be identified
  - Cell shape (shown right)
  - Cell size
  - Cell clustering arrangements
  - Additional structures (endospores, capsules)

- E.g. *N. meningitidis* appears most often as coffee-bean-shaped diplococci, whereas *S. pneumoniae* are typically lancet-shaped cocci which have an increased tendency to form long chains

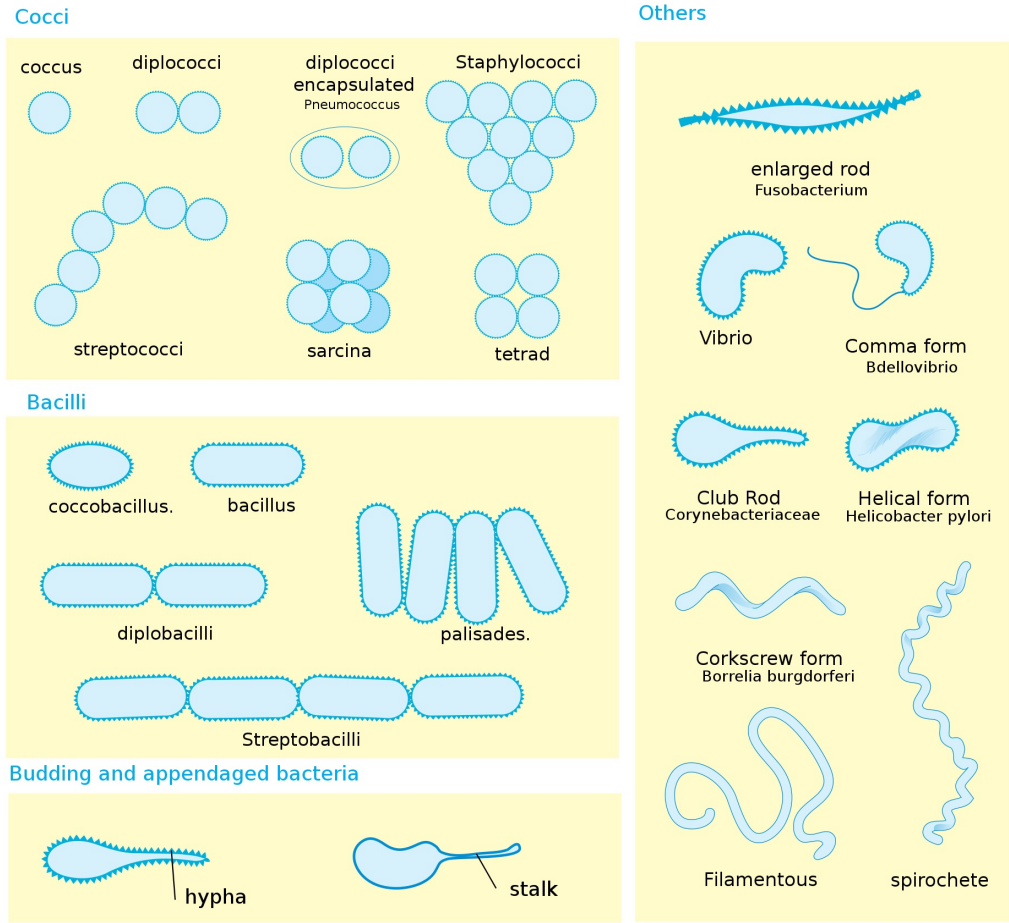


IMAGE SOURCE: BACTERIAL MORPHOLOGY DIAGRAM. REPRINTED FROM WIKIMEDIA COMMONS[13]. COPYRIGHT WIKIMEDIA COMMONS 2006.

BIOCHEMICAL TESTING

Catalase Test

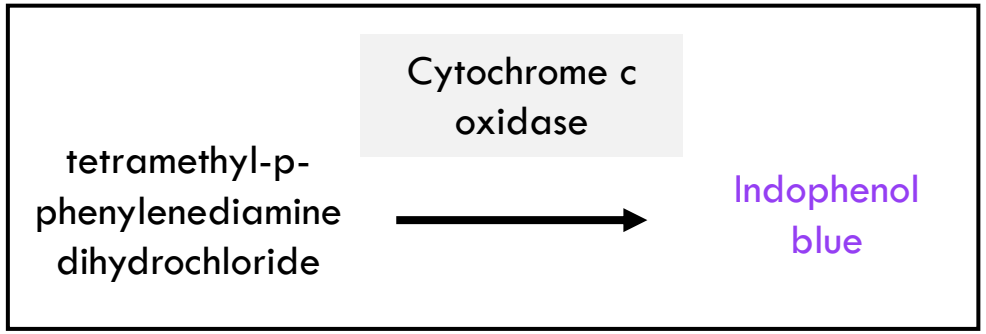
DETECT *S. PNEUMONIAE*

- Catalase decomposes hydrogen peroxide (H_2O_2) to H_2O + O_2 = results in bubbling[32]
- Discriminate between Gram-positive cocci[33]
  - *S. pneumoniae* and *Enterococcus* = negative
  - Staphylococcus = positive
- Procedure[31]:
  - Grow up bacteria on BAP for 24 hours
  - Using loop, transfer a single colony to glass side
  - Add hydrogen peroxide and observe for bubbling

Kovac's Oxidase Test

DETECT *N. MENINGITIDIS* AND *H. INFLUENZAE*

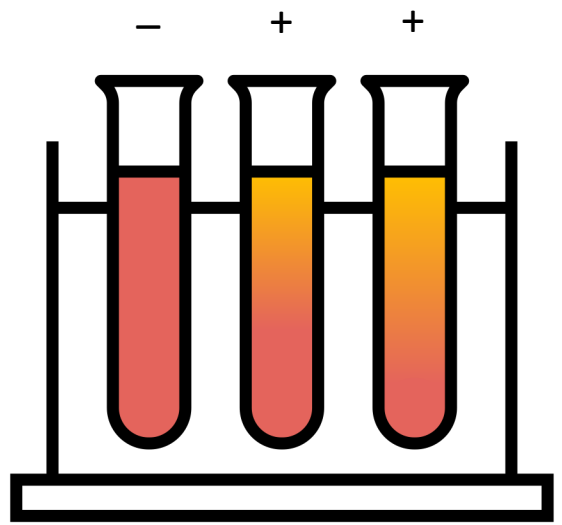
- Determine presence of cytochrome c oxidase in respiratory chain[31]
- Procedure[31]:
  - Grow up bacteria on BAP for 24 hours
  - Kovac's oxidase reagent blotted on filter paper, dried
  - Using loop, transfer a single colony to paper, observe for colour change



CARBOHYDRATE UTILIZATION

DETECT *N. MENINGITIDIS*

- Determine organism's ability to ferment carbohydrates
- *N. meningitidis* only oxidizes glucose and maltose[30]
- Add carbohydrates to tubes containing cystine trypticase agar (CTA) and phenol red (acid indicator)[31]
- Carbohydrate metabolism/oxidization produces acid and sometimes gas
- Procedure[30]:
  - Grow up bacteria on BAP for 24 hours
  - Inoculate CTA-carbohydrate media with a single colony
  - Incubate at least 72 hours
  - Observe colour change (red → yellow = positive)



OPTOCHIN TEST

DETECT *S. PNEUMONIAE*

- Optochin = ethylhydrocupreine hydrochloride
- *S. pneumoniae* strains are sensitive to optochin[32]
- Procedure[32]:
  - Grow up bacteria on BAP for 24 hours
  - Using a loop, streak out a single colony onto half of a new BAP
  - Place an optochin disk within the streak
  - Incubate overnight at 35-37C
  - Measure zone of inhibition the following day
    - >14 mm = organism is sensitive (*S. pneumoniae*)

HEMIN AND NAD GROWTH FACTOR TEST

DETECT *H. INFLUENZAE*

- *H. influenzae* requires hemin and NAD for growth
- Procedure[34]:
  - Grow up bacteria on BAP for 24 hours
  - Create a turbid cell suspension (>1.0 McFarland standard) and vortex
  - Inoculate a trypticase soy agar plate with the cell suspension and allow to dry
  - Place paper strips/disks containing:
    - Hemin
    - NAD
    - Hemin + NAD
  - Observe for growth

- Note: *H. haemolyticus* also requires hemin and NAD for growth[34] – test for hemolysis using rabbit or horse blood agar!

AGGLUTINATION TESTING

Agglutination Serogrouping

DETECT *N. MENINGITIDIS* AND *H. INFLUENZAE*

- A.k.a. Slide agglutination serogrouping (SASG)
- Use serogroup-specific antisera
- Binding of antisera to bacteria causes clumps to form[30]
- Observe for clumping within 1-2 minutes
- Ratings:
  - Negative: 0, 1+, 2+
  - Positive: 3+, 4+

Latex Agglutination

DETECT *S. PNEUMONIAE*

- Quick detection of bacterial antigens in CSF
- Variable sensitivity
- Bacteria expressing specific antigens are mixed with antibody-coated latex particles
- Procedure[31]:
  - Centrifuge CSF to remove sediments
  - Heat CSF supernatant to 100C
  - Add 30-50 uL heated CSF to latex reagent
  - Observe for precipitation and clumping within 5-10 seconds (positive for agglutination)

BILE SOLUBILITY TEST

DETECT *S. PNEUMONIAE*

- Discriminate *S. pneumoniae* from other streptococci sp.[32]
  - *S. pneumoniae* soluble in bile
  - Other streptococci insoluble in bile
- Procedure[32]:
  - Prepare bile solution by dissolving sodium deoxycholate in sterile water
  - Grow up bacteria on BAP for 24 hours
  - Transfer an isolated colony into a tube, dilute to 0.5-1.0 McFarland standard turbidity in saline
  - Add bile solution to cell suspension
  - Incubate tubes in CO_2
  - Vortex and observe for turbidity
    - Positive (bile-soluble): clear

POLYMERASE CHAIN REACTION (PCR) TEST

- Targets a pathogen-specific DNA sequence
- Several technologies exist:
  - Traditional PCR
    - Design primers to specifically bind to and amplify unique DNA sequences[35]
    - Visualize amplicons using agarose gel electrophoresis (some concerns with contamination)
  - Real-time PCR
    - Using fluorescently-conjugated oligonucleotide probe which binds unique DNA sequence[35]
    - If pathogen is present --> amplification --> fluorophore hydrolysis --> fluorescent signal
    - Increasingly common due to reduction of potential contamination
- High specificity and sensitivity
- Can be done with small sample volumes of CSF[27]

IMAGE SOURCE: PEXELS[14] 2020. IMAGE FREE FOR USE

HAND HOLDING PETRI DISH

RESULTS

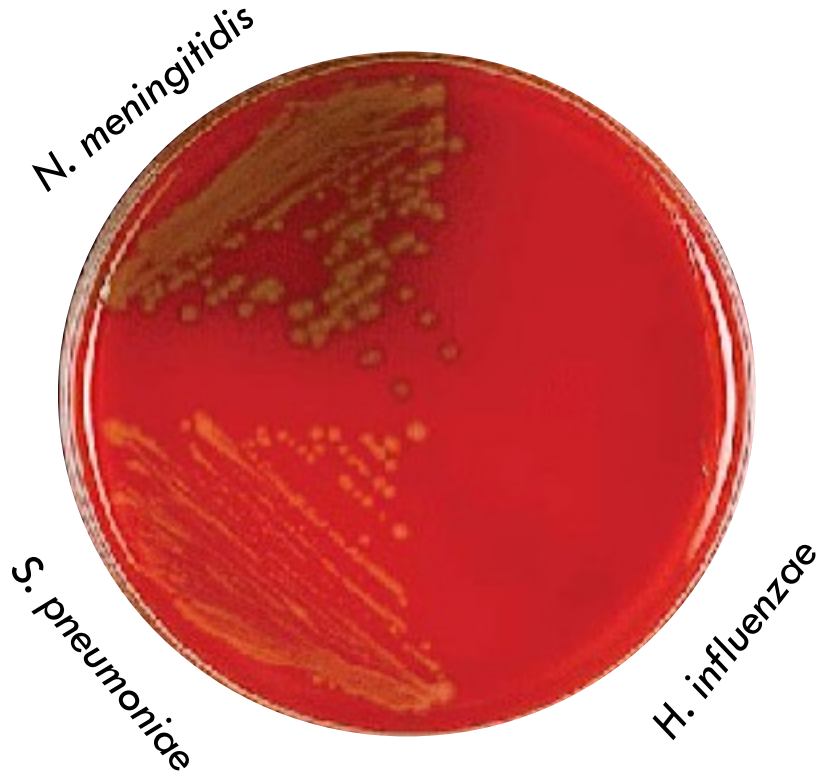
What are the results expected from these tests allowing for the identification of the bacteria named in this case?

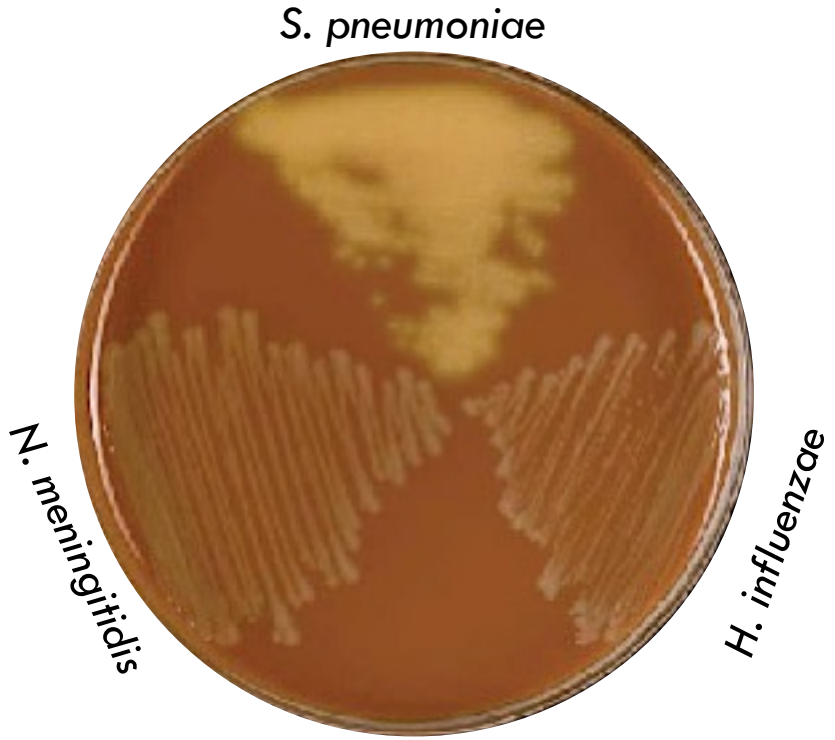
CULTURE

Organism	Blood Agar Culture		Chocolate Agar Culture	
	Colony morphology	Hemolysis	Colony morphology	Hemolysis
N. meningitidis[30,33]	Round, smooth, moist, glistening, convex, grey	No hemolysis	Large, colourless or grey, opaque	No hemolysis
H. influenzae[33,36]	No growth without supplementation	No hemolysis	Large, colourless or grey, opaque	No hemolysis
S. pneumoniae[32,33]	Small, grey, moist, mucoidal	Alpha-hemolysis (green)	Small, grey, moist, mucoidal	Alpha-hemolysis (green)

Note: *N. meningitidis* and *H. influenzae* appear similar on chocolate agar plates. Perform NAD growth factor test to confirm *H. influenzae*

CULTURE





Note: *N. meningitidis* and *H. influenzae* appear similar on chocolate agar plates. Perform a NAD growth factor test to confirm *H. influenzae*.

IMAGE SOURCE: (L) BLOOD AGAR PLATE STREAKED WITH *N. MENINGITIDIS*, *S. PNEUMONIAE*, AND *H. INFLUENZAE*. ADAPTED FROM CDC[15]. COPYRIGHT CDC 2016. (R) CHOCOLATE AGAR PLATE STREAKED WITH ABOVE ORGANISMS. ADAPTED FROM CDC[16]. COPYRIGHT CDC 2016.

GRAM STAIN

- *N. meningitidis* is Gram-negative, should appear pink/red when Gram stained[30]
  - Due to presence of outer membrane composed of lipooligosaccharides and lack of thick peptidoglycan layer[31]
- *H. influenzae* is also Gram-negative[36]
- *S. pneumoniae* is Gram-positive[32], should appear purple
  - Due to presence of thick peptidoglycan layer[31]

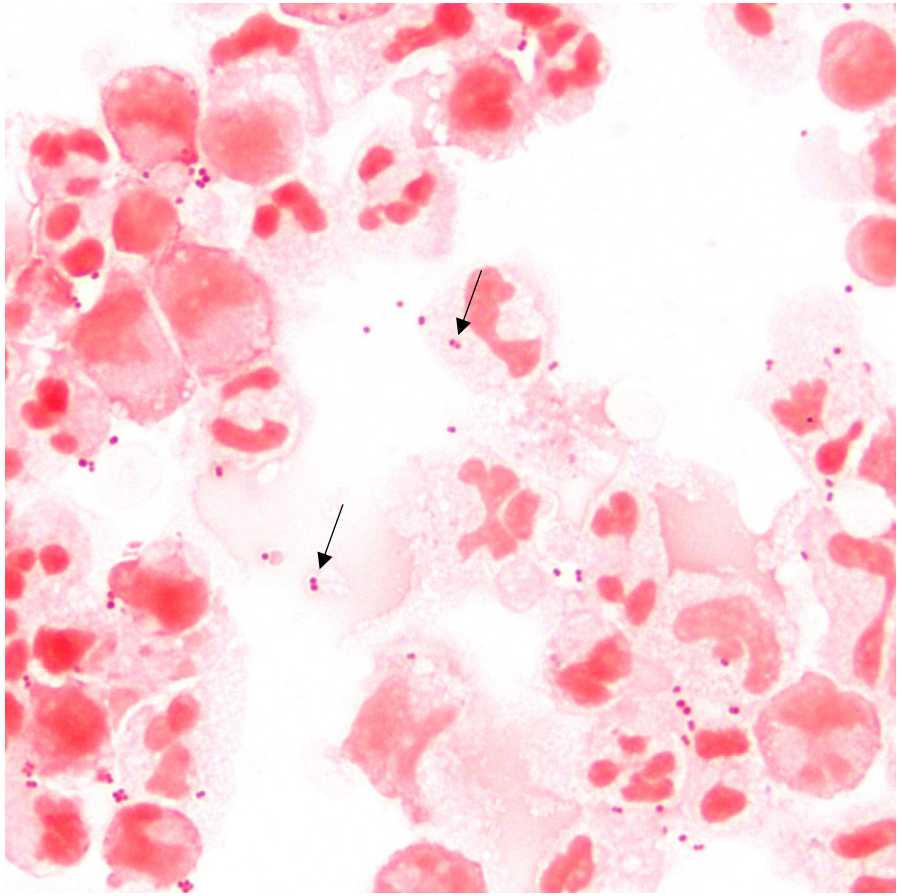
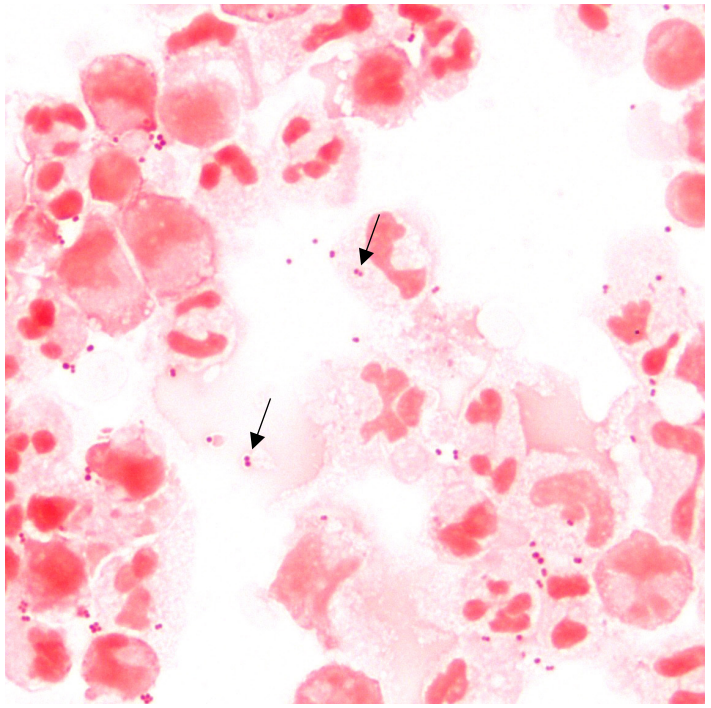


IMAGE SOURCE: (TOP) *NEISSERIA MENINGITIDIS* IN CSF SEEN BY GRAM STAIN AT 1000X MAGNIFICATION. ADAPTED FROM WIKIMEDIA COMMONS[17]. COPYRIGHT WIKIMEDIA COMMONS 2019.

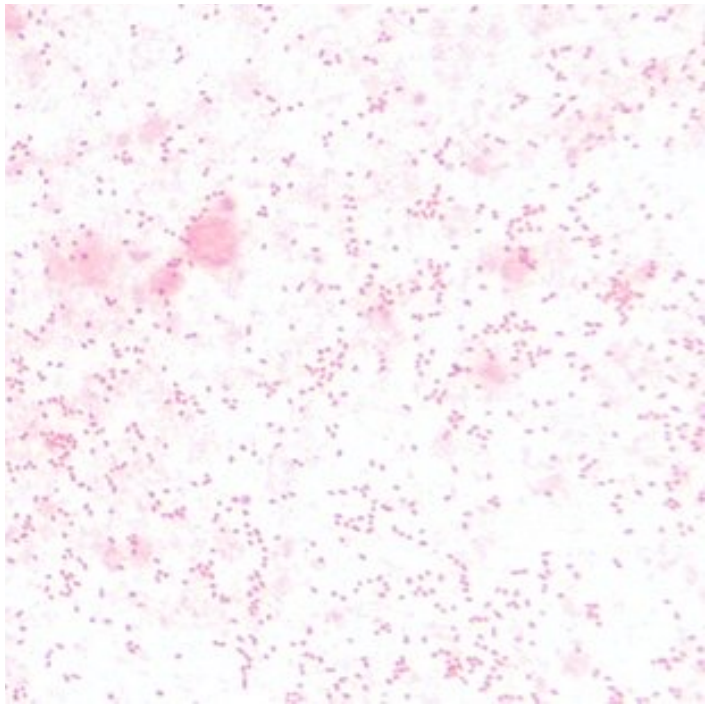
GRAM STAIN

Neisseria meningitidis



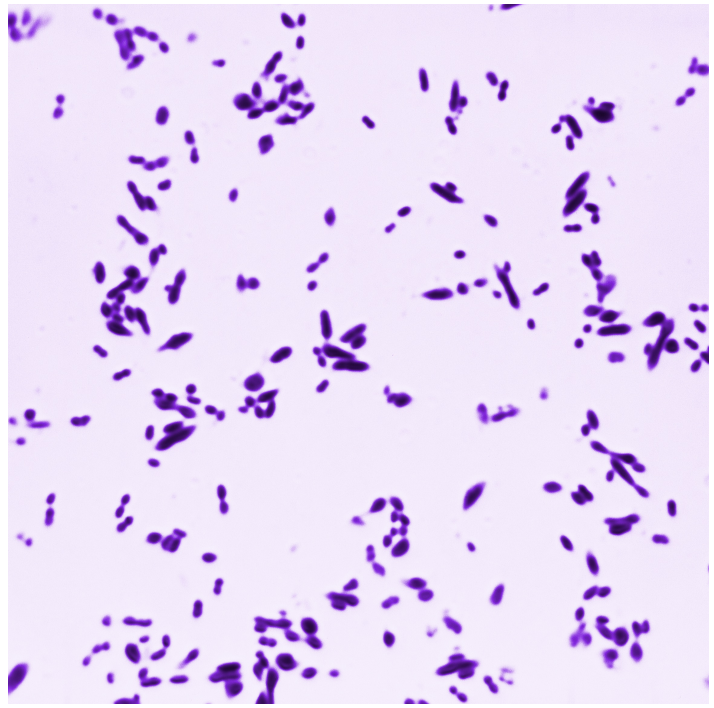
Gram-negative diplococci
Pair clustering[30]

Haemophilus influenzae



Gram-negative, rod-shaped[36]
Rounded ends (coccobacilli)

Streptococcus pneumoniae



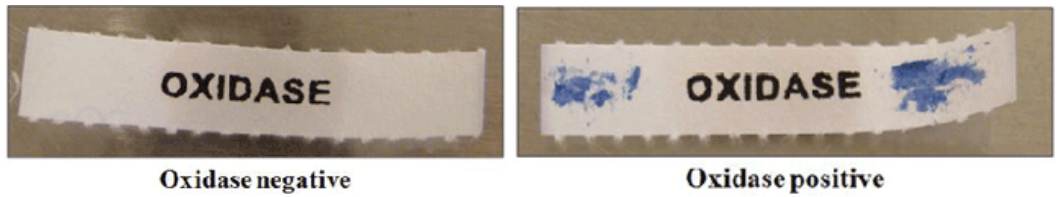
Gram-positive, lancet-shaped cocci[33]
Pair clustering

IMAGE SOURCE: (LEFT) *NEISSERIA MENINGITIDIS* IN CSF SEEN BY GRAM STAIN AT 1000X MAGNIFICATION. ADAPTED FROM WIKIMEDIA COMMONS[17]. COPYRIGHT WIKIMEDIA COMMONS 2019.(MIDDLE) *HAEMOPHILUS INFLUENZAE* IN SPUTUM SEEN BY GRAM STAIN AT 1000X MAGNIFICATION. ADAPTED FROM WIKIMEDIA COMMONS[18]. COPYRIGHT WIKIMEDIA COMMONS 2019. (RIGHT) *STREPTOCOCCUS PNEUMONIAE* SEEN BY GRAM STAIN AT 1700X MAGNIFICATION. ADAPTED FROM CDC[19], COPYRIGHT CDC/ARNOLD KAUFMAN, UNDATED.

BIOCHEMICAL TESTING

Kovac's Oxidase Test

- *N. meningitidis* and *H. influenzae* express cytochrome c oxidase --> positive oxidase test[30,36]
  - Filter paper changes to blue
- *S. pneumoniae* does not express oxidase --> negative test[32]
  - Filter paper remains colourless



Oxidase negative | Oxidase positive

Catalase Test

- *N. meningitidis*, *H. influenzae*, and *S. pneumoniae* do not express catalase[32]
  - No bubbling observed when hydrogen peroxide added

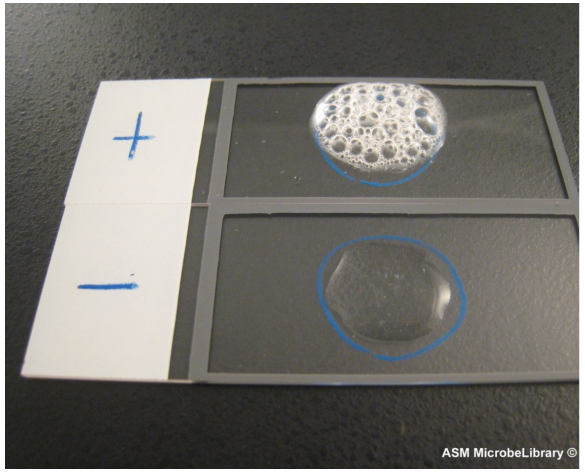


IMAGE SOURCE: (LEFT) KOVAC'S OXIDASE TEST: A NEGATIVE AND POSITIVE REACTION ON FILTER PAPER. ADAPTED FROM CDC[20]. COPYRIGHT CDC/NATIONAL CENTER FOR IMMUNIZATION AND RESPIRATORY DISEASES 2016. (RIGHT) POSITIVE AND NEGATIVE CATALASE TEST RESULTS. REPRINTED FROM AMERICAN SOCIETY FOR MICROBIOLOGY[21]. COPYRIGHT AMERICAN SOCIETY FOR MICROBIOLOGY 2016.

CARBOHYDRATE UTILIZATION

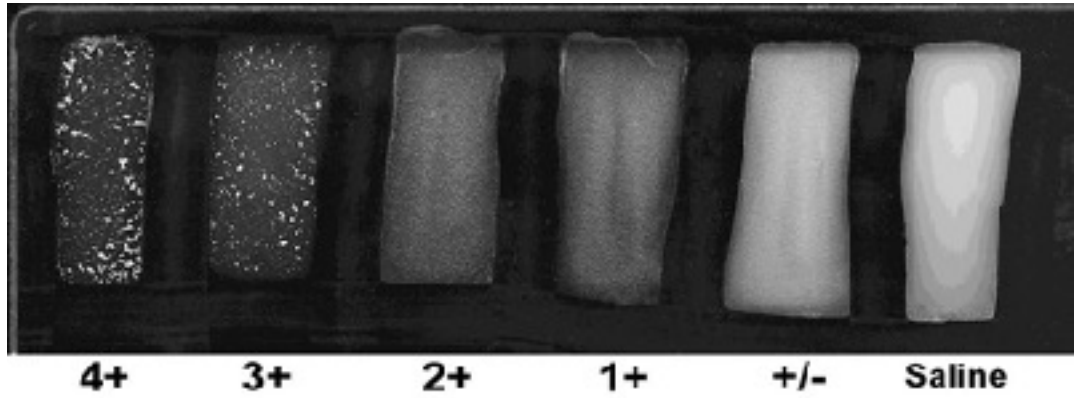
- *N. meningitidis* ferments glucose and maltose (colour change red --> yellow)[30]
  - Does not ferment lactose or sucrose
- *H. influenzae* ferments glucose and maltose[36]
  - Does not ferment lactose or sucrose
- *S. pneumoniae* ferments glucose, lactose, maltose, and sucrose[32]

IMAGE SOURCE: CTA SUGAR REACTIONS FOR *N. MENINGITIDIS*. REPRINTED FROM CDC[22]. COPYRIGHT CDC/ NATIONAL CENTER FOR IMMUNIZATION AND RESPIRATORY DISEASES 2016.

AGGLUTINATION TESTING

Agglutination Serogrouping

- Used to identify serotype of *N. meningitidis*[30]
- A rating of 3+ or 4+ indicates the presence of the unique serotype of *N. meningitidis*



Latex Agglutination

- Positive test indicated by clumping seen within 5-10 seconds
- Typically used as a secondary method of testing if[37]:
  - Gram stain is inconclusive
  - Or meningococcal disease suspected
- Prone to false negatives[37]
  - Positive in 60% of *S. pneumoniae* cases
  - Positive in 93% of *H. influenzae* cases
  - Positive in 39% of *N. meningitidis* cases

IMAGE SOURCE: DIFFERENT INTENSITY RATINGS OF AGGLUTINATION REACTION. REPRINTED FROM CDC[23]. COPYRIGHT CDC/ NATIONAL CENTER FOR IMMUNIZATION AND RESPIRATORY DISEASES 2016.

HEMIN AND NAD GROWTH FACTOR TEST

- *N. meningitidis* and *S. pneumoniae* do not require hemin or NAD to grow
- *H. influenzae* requires hemin and nicotinamide-adenin-dinucleotide (NAD) to grow[39]
  - No growth seen around disk containing only hemin
  - No growth around disk containing only NAD
  - Growth seen around disk containing hemin + NAD

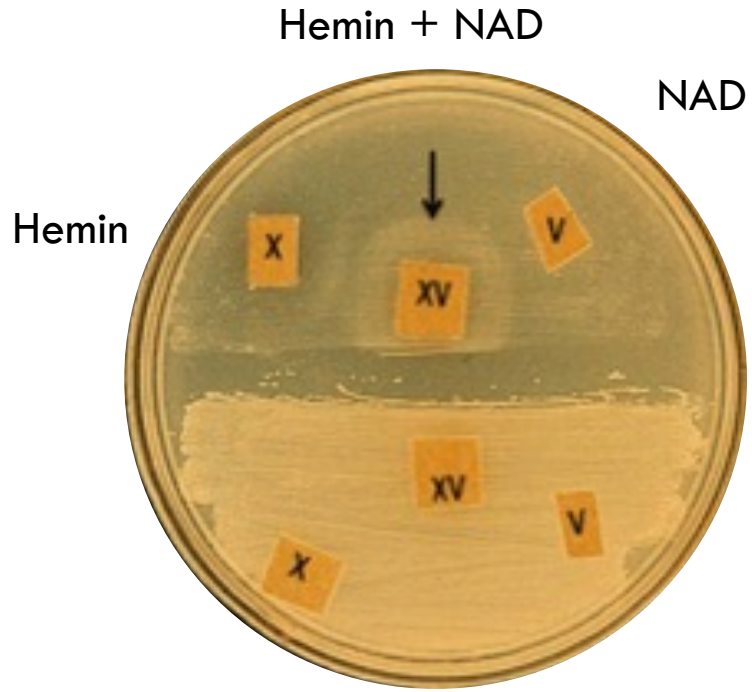


IMAGE SOURCE: IDENTIFICATION OF HEMIN (X) AND NAD (V) AS GROWTH REQUIREMENTS USING PAPER DISKS. GROWTH IS ONLY SEEN AROUND THE DISK CONTAINING BOTH HEMIN AND NAD (ARROW). THE ORGANISM IS PRESUMABLY *H. INFLUENZAE*. ADAPTED FROM CDC[24]. COPYRIGHT CDC/ NATIONAL CENTER FOR IMMUNIZATION AND RESPIRATORY DISEASES 2016.

TESTS TO CONFIRM *S. PNEUMONIAE*

Optochin test

- *N. meningitidis* and *H. influenzae* do not have their growth inhibited in the presence of optochin
- *S. pneumoniae* is optochin-sensitive[32]
  - If zone of inhibition >14 mm, organism is considered sensitive
  - Pathogen is likely to be *S. pneumoniae*

Bile solubility test

- *N. meningitidis* and *H. influenzae* are not bile soluble
  - Negative test = solution remains turbid after 10 minutes, and even after 2 hours
- *S. pneumoniae* is bile soluble, unlike other alpha-hemolytic streptococci[32]
  - Positive test = solution becomes clear/less turbid after 10 minutes

POLYMERASE CHAIN REACTION TEST

- Can be used to pinpoint presence of *N. meningitidis, H. influenzae,* and *S. pneumoniae* with high specificity and sensitivity[35]
- Can also detect serotype

- ***N. meningitidis*** – expresses gene encoding the copper, zinc superoxide dismutase, *sodC*[35]

- ***H. influenzae*** – expresses gene encoding the surface-exposed lipoprotein, protein D, *hpd*[35]
  - Can be found in all *H. influenzae* serotypes

- ***S. pneumoniae*** – targets a specific segment of gene encoding autolysin, *lytA*[35]

Target	Primer or probe name	5' to 3' nucleotide sequence
N. meningitidis		
sodC	F351	GCACACTTAGGTGATT TACCTGCAT
	R478	CCACCCGTGTGGATCATAATAGA
	Pb387	CATGATGGCACAGCAACAAA TCCTGTTT
H. influenzae		
hpd	hpdF822	GGTTAAATATGCCGATGGTGTTG
	hpdR952	TGCATCTTTACGCACGGTGTA
	Pb896i	TTGTGTACACTCCGT"T"GGT AAAAGAACTTGCAC
S. pneumoniae		
lytA	F373	ACGCAATCTAGCAGATGAAGCA
	R424	TCGTGCGTTTTAATTCCAGCT
	Pb400i	TGCCGAAAACGC"T"TGATAC

TABLE ADAPTED FROM CDC/NATIONAL CENTER FOR IMMUNIZATION AND RESPIRATORY DISEASES[35].

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