N. meningitidis - Microbiology Laboratory

A summary of key concepts

Presentation Outline

Associated Bacterial Pathogens

Role of the Microbiology Laboratory

Tests Performed on Collected Samples

Microbiology Test Results

Potential Bacterial Causes

Other than N. meningitidis, potential bacterial pathogens associated with these signs and symptoms include:

- *Escherichia coli*
- *Group B streptococcus*
- *Listeria monocytogenes*
- *Streptococcus pneumoniae*
- *Haemophilus influenzae*

N. meningitidis is present in the nose or throat of 1 in 10 individuals, and is the main cause of bacterial meningitis and sepsis

Escherichia coli

Escherichia coli is a Gram-negative bacteria that is a common cause of meningitis in infants

Most E. coli are harmless, and are commensal in the intestinal tract. E. coli meningitis is very rare in adults, thus Mary is unlikely to have E. coli meningitis.

Strains of E. coli that are associated with meningitis have the K1 polysialic capsule, which is also found in N. meningitidis

K1 polysialic capsule is vital in the bacteria’s ability to survive after crossing the blood-brain barrier

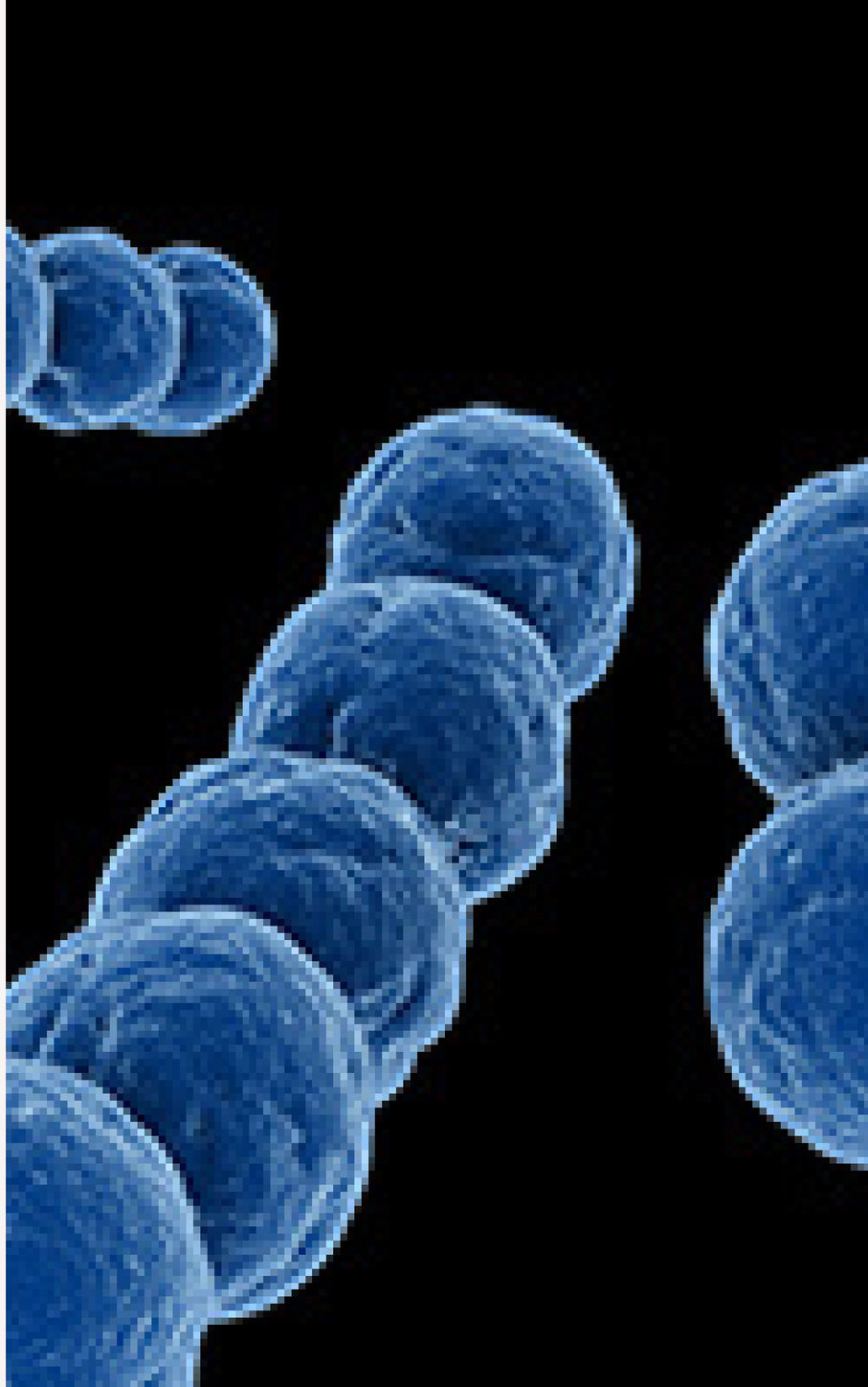
Group B Streptococcus

Group B streptococcus (GBS) is a Gram-positive bacteria present in the reproductive tract of 35% of healthy women

This strain can be transmitted to a newborn during birth, which is why meningitis caused by GBS is so common in infants

GBS, however, is not transmissable disease, it cannot be spread by incidental contact, therefore, its not possible that Mary's meningitis is caused by GBS

GBS can travel in the blood to the brain by crossing the blood-brain barrier into the cerebral spinal fluid (CSF)



Listeria monocytogenes

L. monocytogenes is a Gram-positive bacteria that accounts for 20% of meningitis in newborns and the elderly

Mary does not experience symptoms consistent with Listeriosis, nor does she fit the normal patient profile, therefore, its unlikely that her meningitis is caused by this bacteria

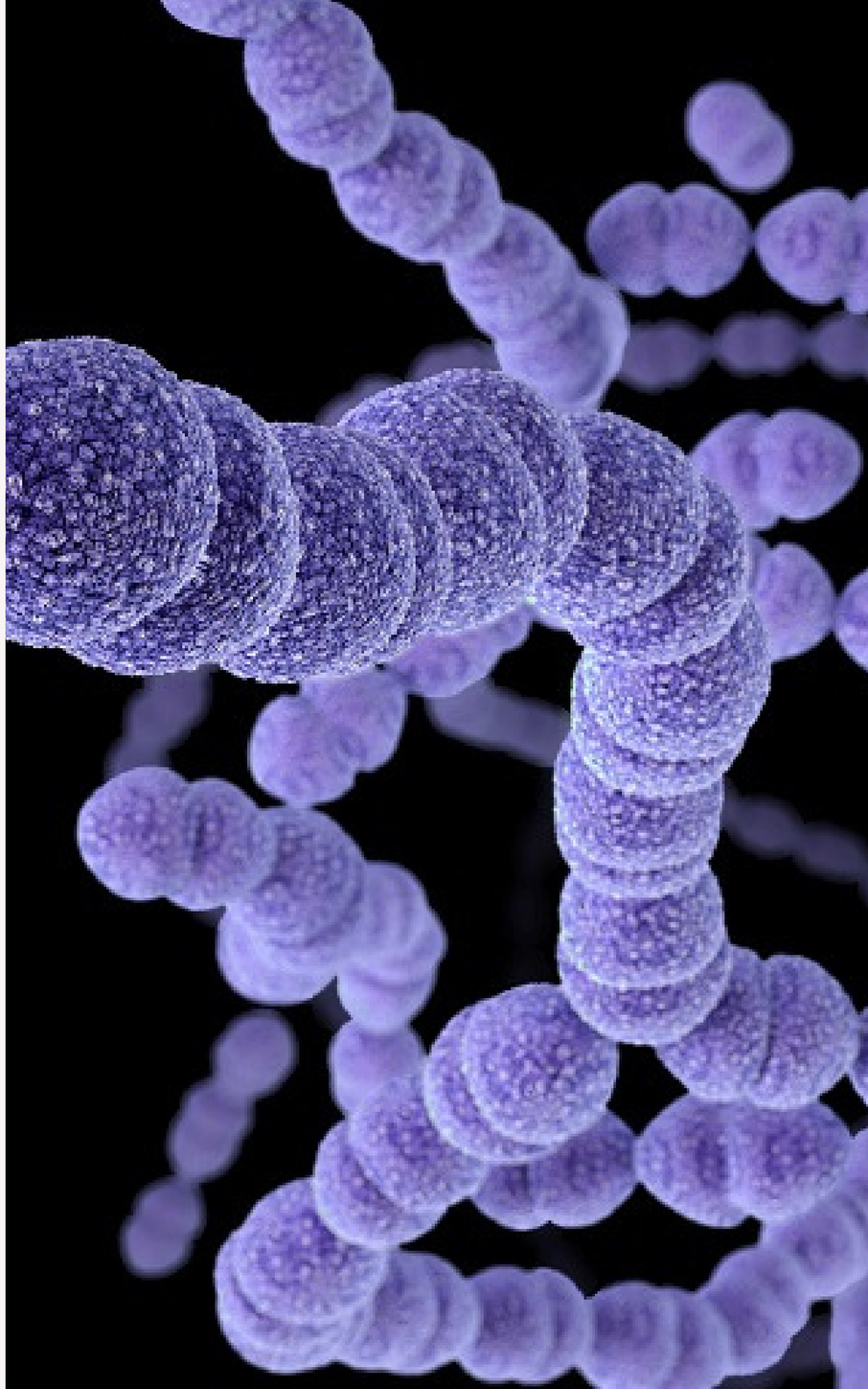
L. monocytogenes is commonly transmitted by ingesting contaminated food, such as meats and cheeses, as well as by vertical transmission from mother to child

Streptococcus pneumoniae

S. pneumoniae is an anaerobic, Gram-positive bacteria that causes 58% of meningitis cases in the United States

S. pneumoniae is transmitted between hosts through sneezing and coughing, where it then colonizes the nasopharynx region of the new host

The incidence of S. pneumoniae causing meningitis decreased by 50% from 1997 to 2009, partly due to the introduction of the pneumococcal conjugate vaccine



Haemophilus influenzae

H. influenzae is an anaerobic, Gram-negative bacteria that can cause meningitis in patients under 5yrs and over 65yrs

H. influenzae is divided into subgroups based on capsule type, with H. influenza type b (Hib) being the predominant group that causes the infection

The Hib conjugate vaccine is largely protective against infections, most current cases primarily involve non-immunized children

Samples Taken - What and Why

CSF and Blood samples are taken to the Microbiology Laboratory to confirm that Mary's symptoms and signs are caused by meningococcal meningitis

Blood culture, CSF gram stain, CSF culture and Blood/CSF metabolic factors must be sent to and examined by the Microbiology laboratory to confirm a meningococcal infection

While IV antibiotic treatment begins immediately following the suspicion of meningococcal infection, confirmation of the infection by the microbiology laboratory informs subsequent treatment

Samples Taken - CSF

CSF samples are collected by a physician via a lumbar puncture and sent to the laboratory

CSF samples are examined for its cell count, glucose level, Gram staining, cultures, and polymerase chain reaction (PCR)

Ideally, CSF samples are collected prior to, or immediately after, starting antibiotic treatment, as antibiotics can alter the cellular, metabolic, and biochemical character of the CSF

Samples Taken - Blood

Analysis of blood samples by the microbiology laboratory is important for diagnosing meningococcal meningitis and necessary for developing a complete clinical picture

Blood, like CSF, can be used to provide a definitive diagnosis of meningococcal meningitis

Blood measurements not exclusive to meningococcal meningitis, such as complete blood count (CBC), inflammatory markers (C-reactive protein), electrolyte levels, and total protein count are also measured by the microbiology laboratory to inform future treatment decisions

Microbiology Lab Tests

Cytological processes are used to examine the CSF and blood samples for pressure, colour, glucose level, protein count, lactate count, turbidity, and pleocytosis

Cytological examination - looking at cells underneath a microscope - can provide information about the quantity and quality of cells in the CSF

Potential bacterial pathogens causing bacterial meningitis can be determined by isolating and culturing the bacteria

Because tests have a margin of error, CSF analysis can produce normal results or negative cultures in a patient with meningitis symptoms; blood cultures should be performed in conjunction with CSF analysis to confirm the cause of infection

Growing a Culture

Growing a bacterial culture is key to several of the tests used to identify and isolate the cause of infection

To prepare the serological sample for culturing, the sample is centrifuged to separate bacteria from other blood/CSF components. Drops from the sediment will be used to 'streak' the primary culture media, and bacterial growth rates can be manipulated and measured from there

The culture can be grown on either a blood agar plate (BAP) or a chocolate agar plate (CAP). To favor the growth of *N. meningitidis*, a small container of water is added to the incubator to create sufficiently humid conditions

Lab Tests Performed

Kovac's Oxidase Test

Kovac’s oxidase test is to determine the presence of cytochrome c oxidase. A colony from the culture will be transferred to filter paper treated with a reagent. positive result would be shown by a purple colour change. Used to detect *N. meningitidis* or *H. influenzae*

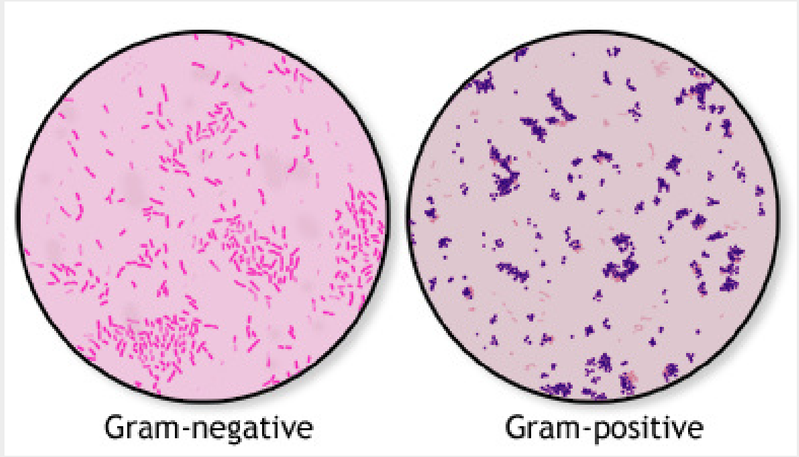
Carbohydrate utilization

A carbohydrate utilization test examines a pathogens’ ability to ferment carbohydrates, determined by the acid indicator in the medium. This test is used specifically for the identification of N. meningitidis as it can only oxidize glucose and maltose

Slide agglutination serogrouping (SASG)

If the antisera binds to the bacterial cells then a clump will form and an intensity rating can be determined. The ratings 3+ and 4+ within one to two minutes corresponds to a positive result whereas a rating of 0, 1+ or 2+ corresponds to a negative result. Used to detect any bacteria of choice, including *N. meningitidis*

Lab Tests Performed



Latex agglutination

Latex agglutination reaction is carried out when a sample with a specific antigen is mixed with latex particles that have antibodies coating its surface. If aggnulation occurs, it indicates that bacteria is present in the sample which binds to the antibody. A positive test result is indicated by cells clumping together within 5-10 seconds

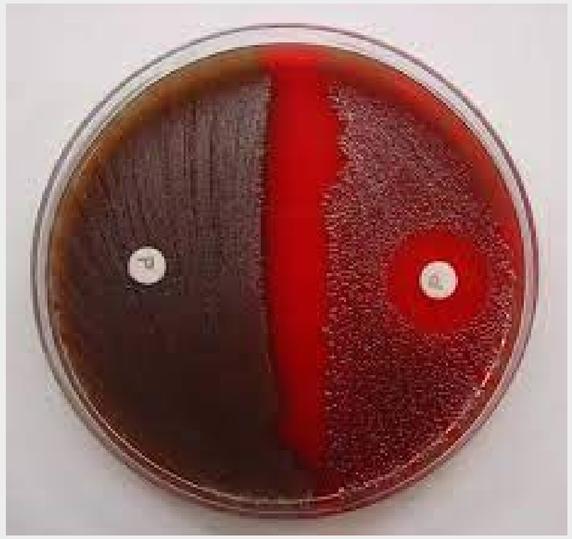
Gram Stain

A Gram stain is used to differentiate between the two main categories of bacteria: Gram-positive and Gram-negative. If the bacteria is gram-positive, then its thick peptidoglycan layer will trap the dye and produce a purple hue. In a gram-negative bacteria, since the violet dye is not trapped, a pink/red hue is observed. *N. meningitidis* is a gram-negative bacteria

Catalase Test

A catalase test uses the enzyme catalase, which decomposes hydrogen peroxide into water and oxygen gas, to discriminate between Gram-positive cocci. A positive result indicates a *S. pneumonia* infection

Lab Tests Performed



Optochin test

Optochin tests utilize ethylhydrocupreine hydrochloride (optochin) to determine if the bacterial pathogen causing bacterial meningitis is *S. pneumoniae*. *S. pneumoniae* strains are sensitive to this chemical, hence inhibition of growth in the culture in response to Optochin indicates an *S. pneumoniae* infection

Hemin and (NAD) growth factor requirement test

H. influenzae can be identified based on its requirement for Hemin and NAD to reproduce. *H. influenzae* can only grow around a disk/strip with both hemin and NAD

Polymerase chain reaction (PCR) test

PCR is a method used to rapidly make millions of copies of a specific DNA sample. A complementary fluorescent oligonucleotide probe is used to target an identifying DNA sequence. If the specific bacteria is present in culture, this will be indicated by a corresponding fluorescent signal. PCR has high sensitivity and specificity for many bacteria

Blood & CSF Tests - Results

Culture Test

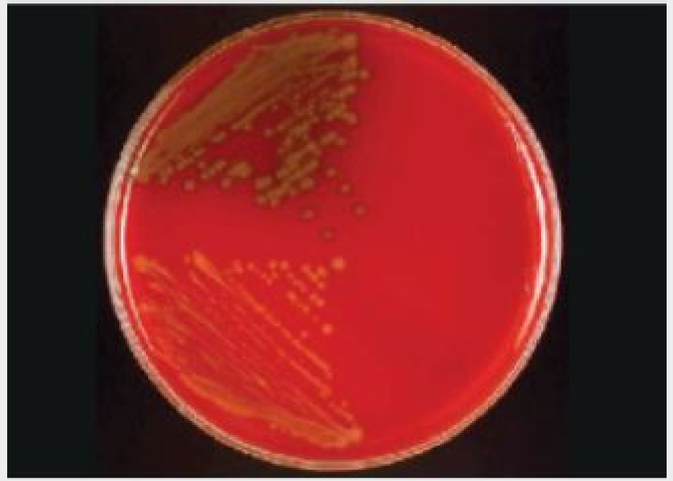
Used to visually confirm bacterial infection. Growth of *N. meningitidis* can be observed as round, smooth, glistening, moist, convex edged colonies on BAP with no hemolysis. On CAP, there would be large, colorless, opaque cultures also without hemolysis or discoloration

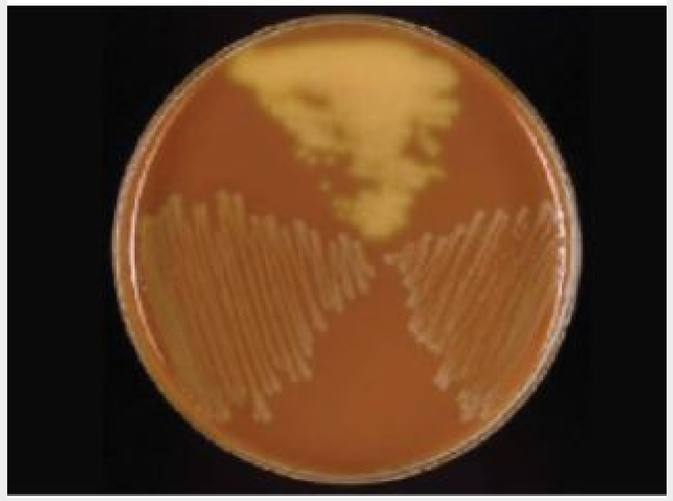
Gram Stain

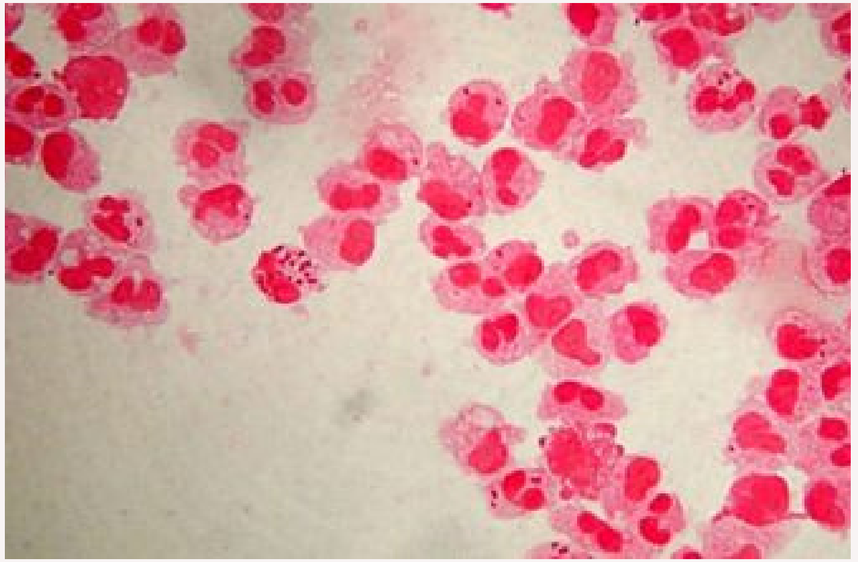
Since N. meningitis is a gram-negative bacteria, it would be expected for the sample to turn a pink or red colour with the addition of crystal violet dye and iodine. The pink/red colour occurs due to the lack of a thick peptidoglycan layer in gram-negative bacteria.

Blood & CSF Workup

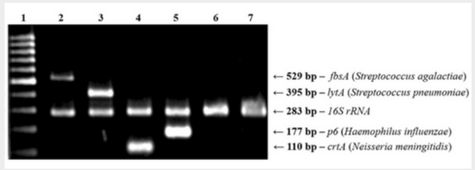
Upregulated immune cell levels, downregulated glucose levels, and upregulated protein levels in CSF/Blood are characterisitcs of bacterial meningitidis, through these results cannot be used to discriminate between potential causative agents





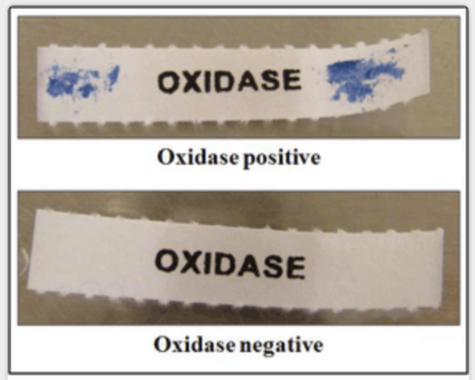


CSF Sample Tests - Results



PCR Test

If N. meningitidis is in the sample, we would expect that Superoxide dismutase genes, sodC, or Capsule Transport to Cell Surface genes, crtA, to be detected



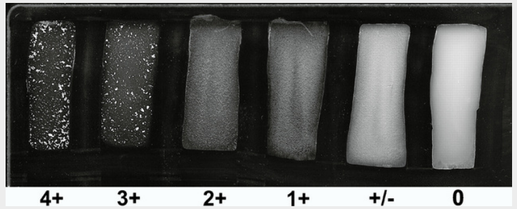
Kovac’s oxidase test

When either N. meningitis or H. influenzae are present in the tested culture, a positive reaction on the filter paper would be expected. A positive result is indicated by a blue colour change on the filter paper

Carbohydrate utilization

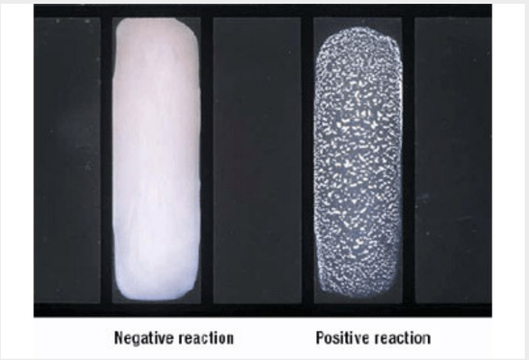
A positive test for N. meningitis, would be indicated by a colour change to yellow in samples with glucose and maltose, and no colour change in lactose and sucrose samples.

CSF Sample Tests - Results



Slide agglutination serogrouping (SASG)

It would be expected that a rating of 3+ or 4+ is present in samples positive for a target strain of *N. meningitidis*. This indicates aggnulation of the bacteria with the antisera used



Latex agglutination

Functioning in a similar manner as antigen rapid tests, a positive result can be visually observed by the clumping of cells within 5-10 seconds of starting the test. In a negative result, no clumping will be observed. Latex agglutination test was only positive in 39% of N. meningitidis infections, hence it should be used as a secondary measure

Summary - Microbiology Laboratory

Associated Bacterial Pathogens

Outside of *N. meningitidis*, Escherichia coli, Group B streptococcus, Listeria monocytogenes, Streptococcus pneumoniae, Haemophilus influenzae

Role of the Lab & Samples Taken

Blood and CSF samples are taken, lab tests necessary to confirm *N. meningitidis* diagnosis

Microbiology Lab Tests Performed

Bacterial Culture, PCR tests, Blood workup, and various metabolic and serological tests on CSF

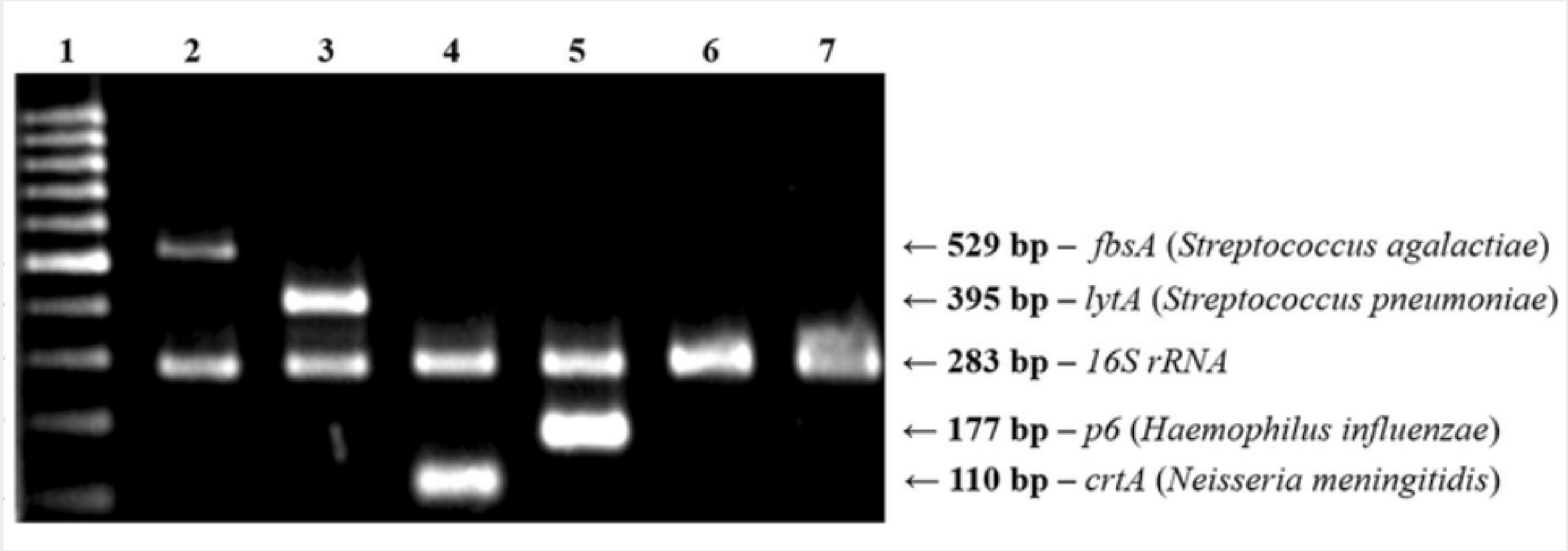
Microbiology Lab Test Results

Varies by experiment

Citations

Please see references used in the PATH 417 UBC Wiki - Case 1 2021 - Question 2 (i, ii, iii and iv)

https://wiki.ubc.ca/Course:PATH417:2021W2/Case1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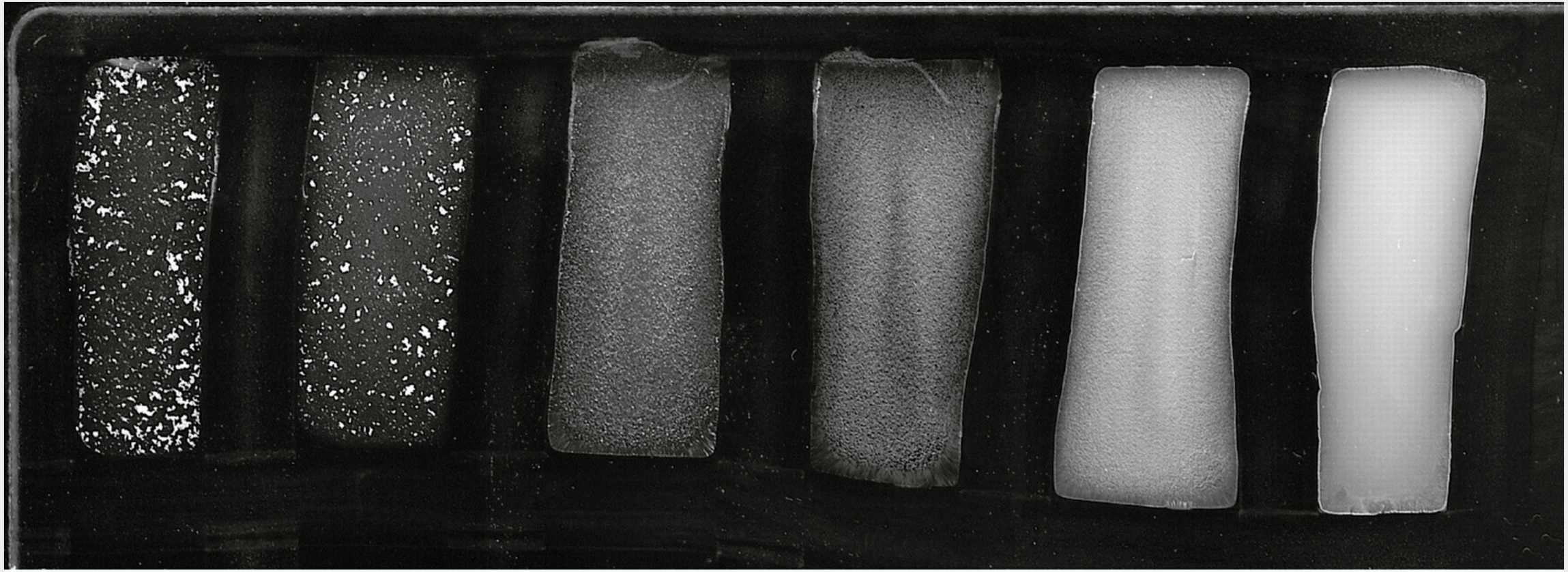
← 529 bp – fbsA (Streptococcus agalactiae)
← 395 bp – lytA (Streptococcus pneumoniae)
← 283 bp – 16S rRNA
← 177 bp – p6 (Haemophilus influenzae)
← 110 bp – crtA (Neisseria meningitidis)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