CNS module practical
Meningitis

Instructor Information

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Learning outcomes

By the end of this lecture the students will be able to:

1. Become acquainted with the procedure of CSF sample collection.
2. Lay out the CSF sample processing.
3. Describe the criteria of CSF in the different kinds of meningitis.
4. Explain the process for detecting meningitis-causing microbes in a laboratory setting.
5. List rapid methods for diagnosing meningitis.
6. Link their understanding to real-world clinical issues.

Practice Content

- **CSF sample collection and processing.**

- **Laboratory diagnosis of bacterial meningitis caused by *Neisseria meningitides* and viral meningitis.**

Case Scenario

- A 54-year-old Caucasian female presents to the emergency department with worsening headache, neck pain, neck stiffness, and back pain for 2 days duration. She also complains of low-grade fevers and chills that developed over the past 24 hours. Her son states that she seems more lethargic and has difficulty maintaining her balance. In addition, she reports 3 to 4 episodes of nausea and vomiting, and not being able to look at any light. A complete blood count showed neutrophilia. A CSF sample was obtained by lumbar puncture. CSF was turbid, there was an increase in neutrophils and proteins with a decrease in glucose. Direct film from CSF demonstrated intracellular Gram-negative diplococci.

Case Scenario

1. **What is the most likely clinical diagnosis?**
2. **What is the causative organism in this case?**
3. **Mention selective media for isolating the causative organism.**
4. **Mention biochemical reactions for identifying the causative organism.**

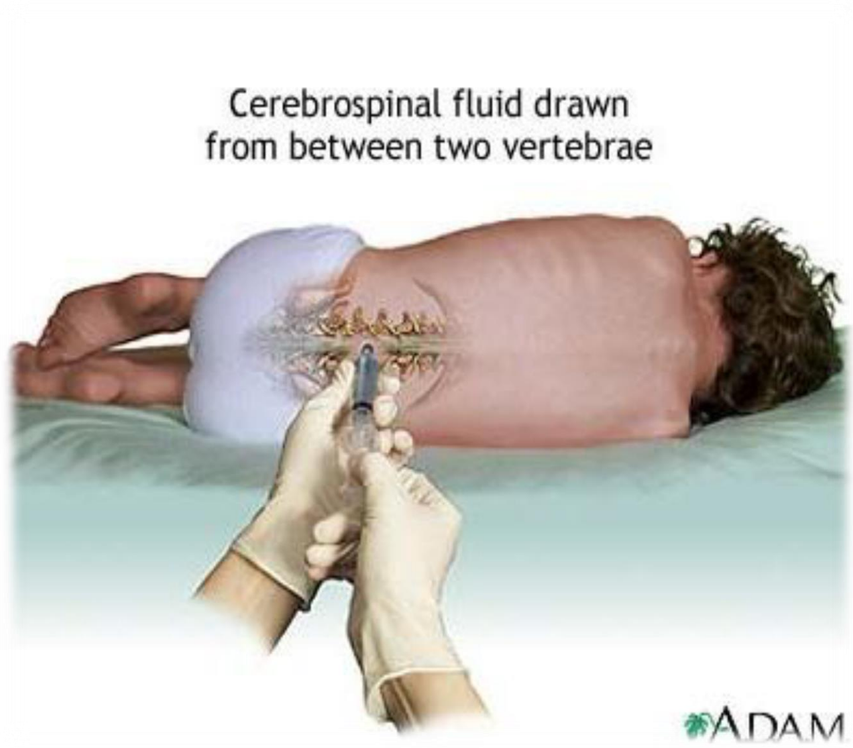
Lab diagnosis of meningitis

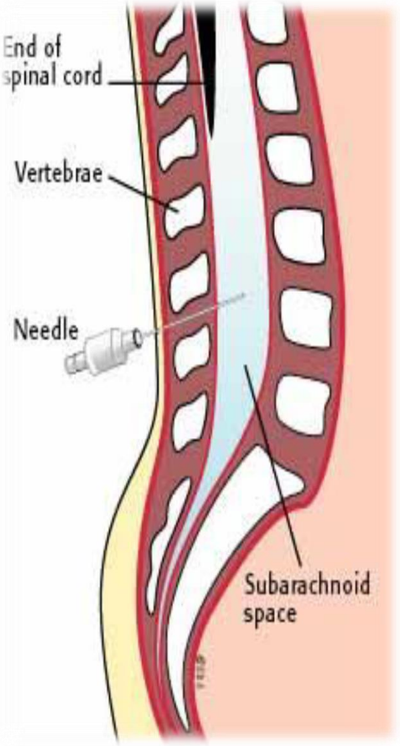
- **Lab diagnosis of meningitis**
- ***Neisseria meningitides***
- **Viral Meningitis**

Lab diagnosis of meningitis

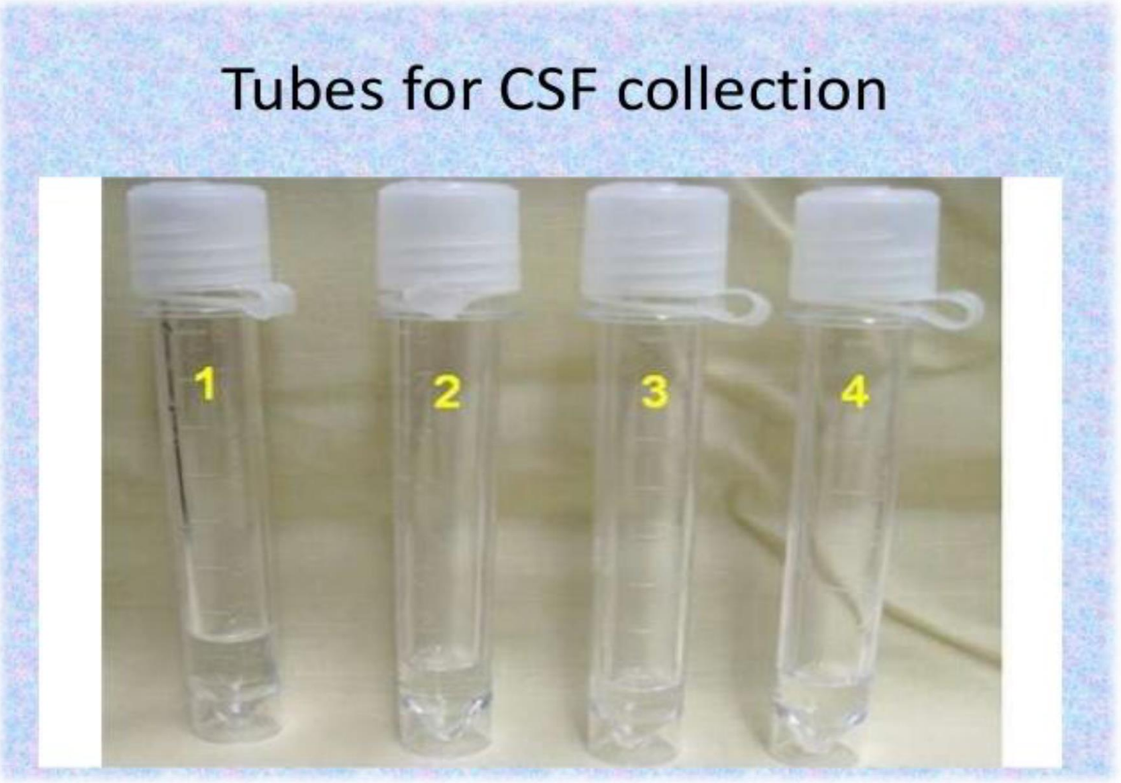
Sample: CSF Collection

- Lumbar puncture.
- Aseptic conditions.
- Between 4th and 5th lumbar vertebrae.
- Screw-capped bottles.
- Sent to the laboratory at once.





Lab diagnosis of meningitis



Lab diagnosis of meningitis

CSF is examined for:

- Physical (pressure , turbidity)
- Chemical (protein, and glucose)
- Haematological (cells)
- Bacteriological characters (microscopy and culture)

In acute bacterial meningitis, CSF is

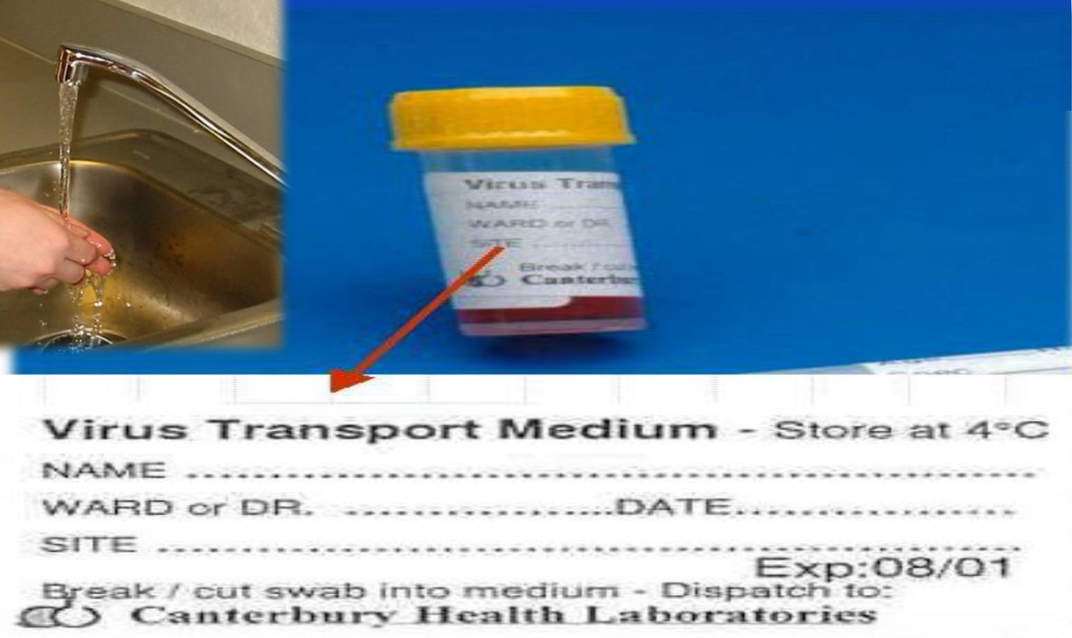
- Turbid, under tension, low glucose value, high protein level, and contains polymorphonuclear cells

Lab diagnosis of meningitis

- CSF is collected and examined as follows:

Type of meningitis	Cells	Glucose	Protein
Bacterial (Septic)	Neutrophils	Low	High
Tuberculous	Lymphocytes	Low	High
Aseptic	Lymphocytes	Normal	Moderately high

General rules for specimen



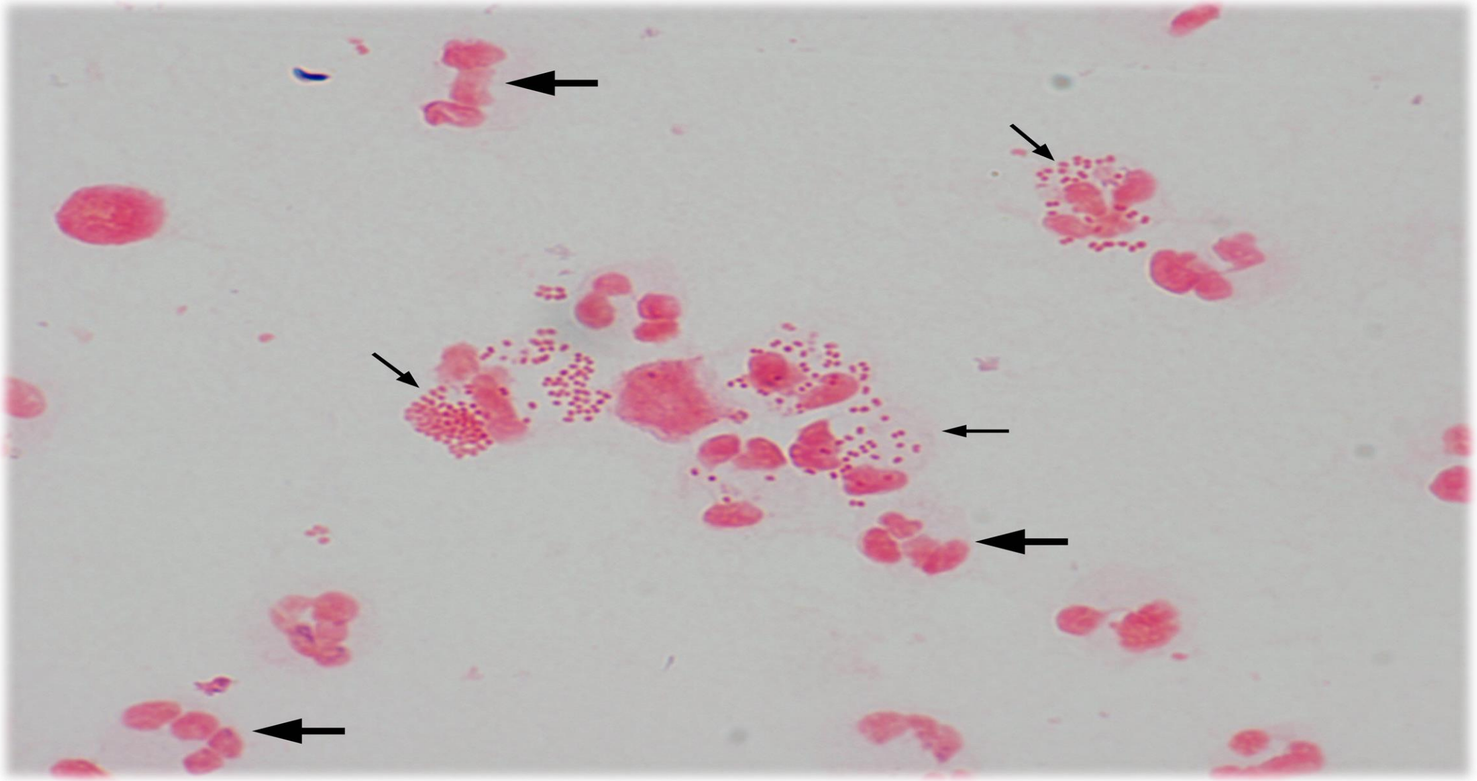
- Wash hands before and after sample collection.
- Samples must be:
    - Before the start of antimicrobial therapy.
- CSF specimens are best taken at the bedside where suitable media are directly inoculated.
- Viral transport media for transporting swabs for viral cultures and if there is a delay should be kept at -70C.

Neisseria meningitides

• Neisseria meningitides cause acute cerebrospinal meningitis (**meningococcal meningitis**).

General Characters:

- Gram-negative, kidney shape, diplococci, piliated & capsulated.
- Intracellular.



Laboratory diagnosis of acute cerebrospinal meningitis

I. Diagnosis of a case:

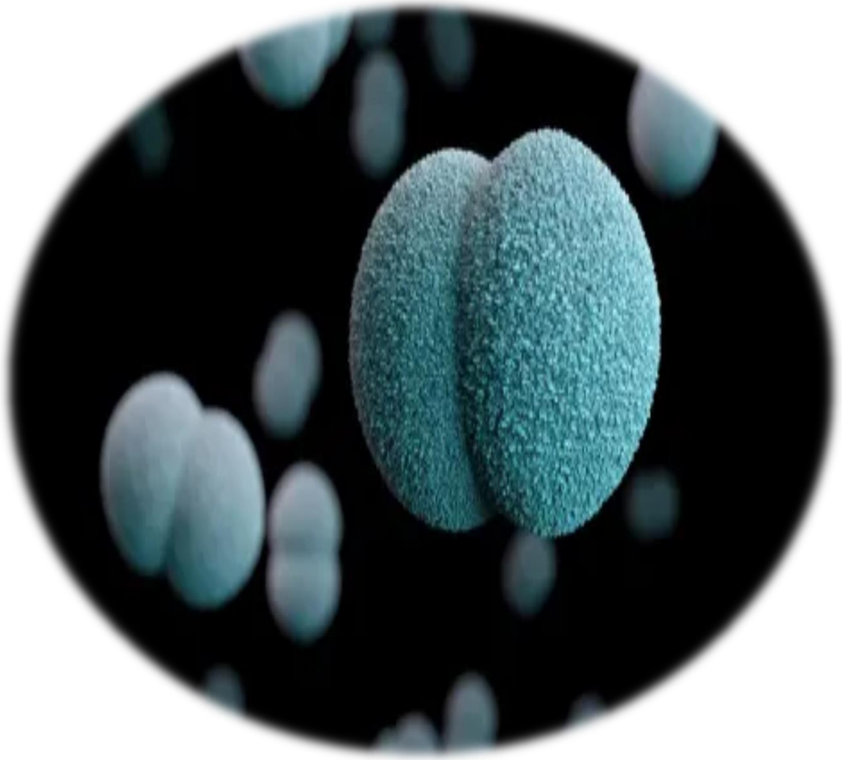
1- Samples:

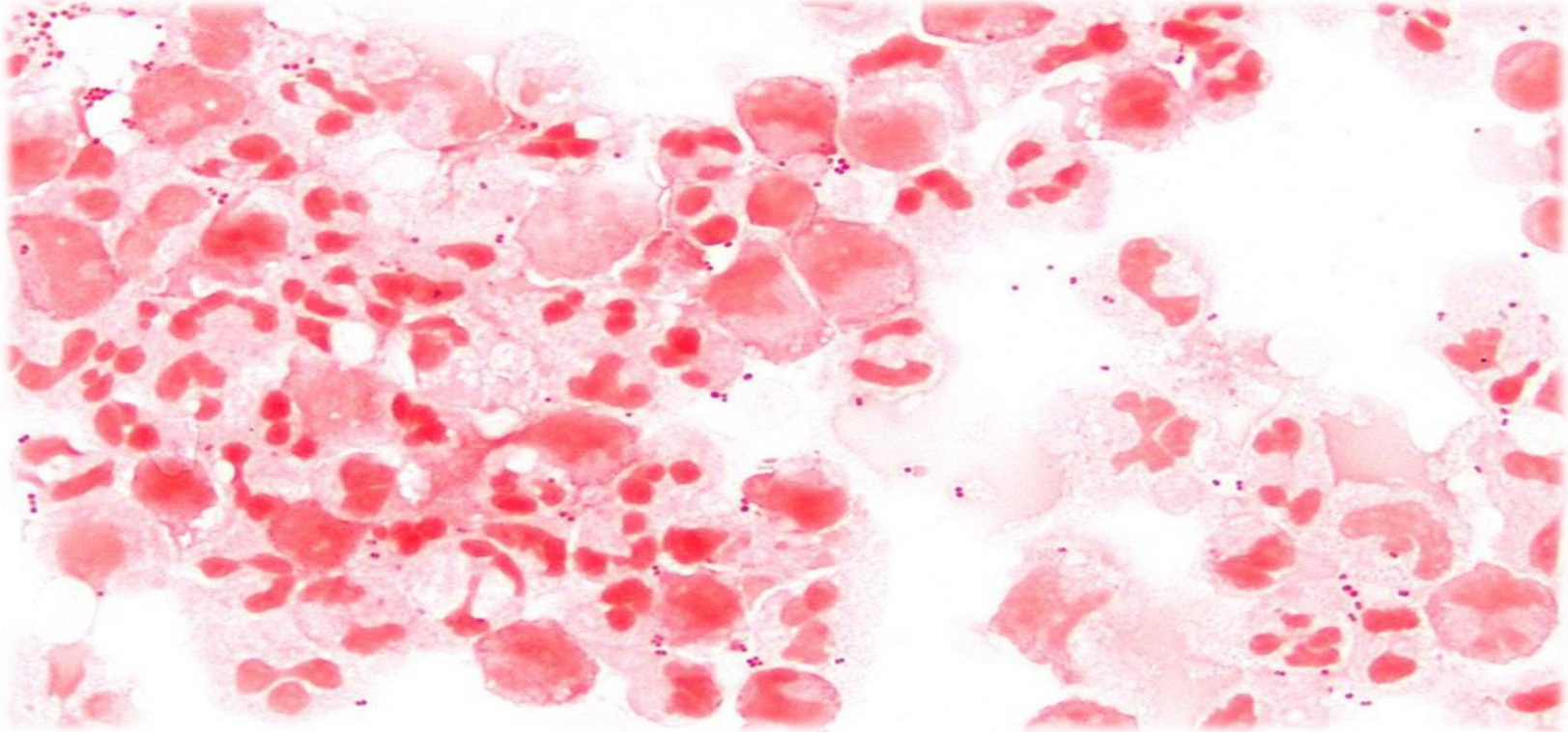
- Meningococci are found in CSF, blood, and the nasopharynx early in the disease.
- C.S.F is obtained by lumbar puncture under complete aseptic precautions.
- C.S.F is turbid and under tension due to the presence of pus cells.

2 -Direct film stained by Gram: (Rapid)

- Detection of intracellular Gram-negative diplococci is diagnostic.

Laboratory diagnosis of acute cerebrospinal meningitis





Laboratory diagnosis of acute cerebrospinal meningitis

3- Culture:

Culture characters:

- Aerobic.
- Optimum temperature: 37 °C, with narrow temperature range (30-38°C),

no growth at 22°C.

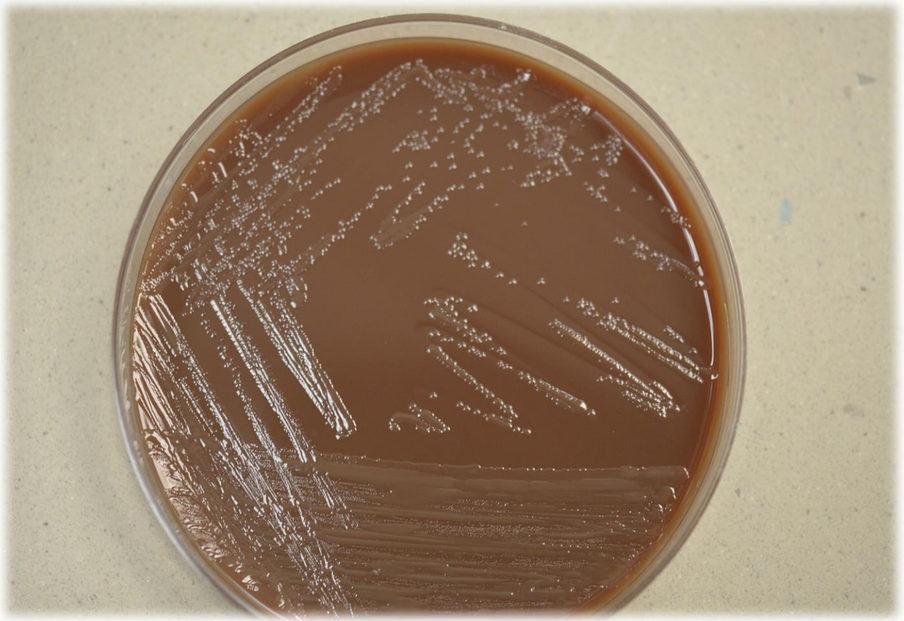
- 10% Co2 and moist atmosphere are required for growth.

Laboratory diagnosis of acute cerebrospinal meningitis

3- Culture:

Culture media:

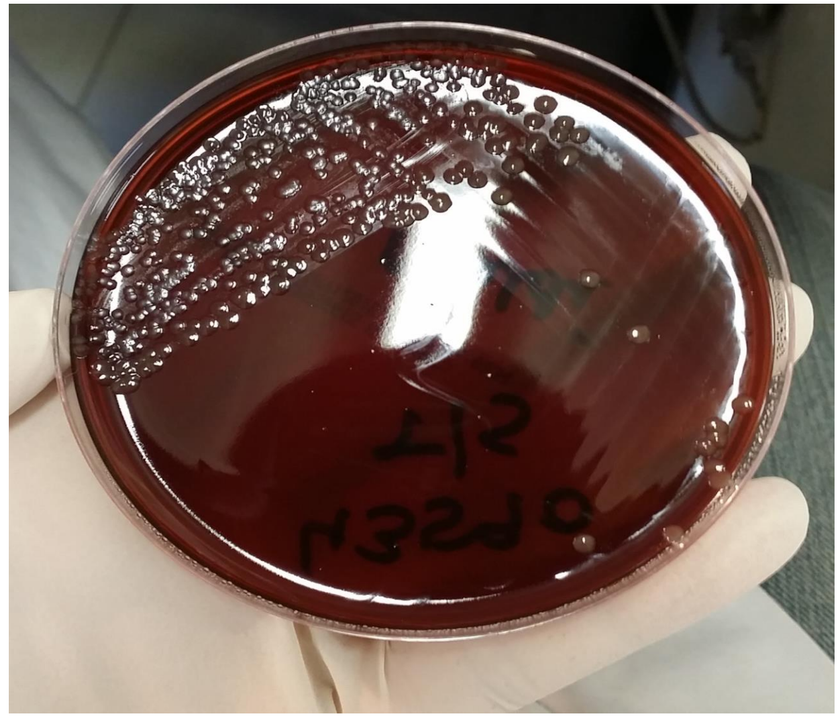
- **Ordinary media**: no growth.
- **Enriched media**: chocolate agar plate.



Laboratory diagnosis of acute cerebrospinal meningitis

- **Selective media**: Thayer-Martin agar. **is selective media for Pathogenic Neisseria, contains antimicrobials that inhibit the growth of organisms other than pathogenic Neisseria.**

  - ✓ Vancomycin kill most gram-positive bacteria
  - ✓ Colistin kill most gram-negative bacteria including the commensal Neisseria spp., except Pathogenic Neisseria
  - ✓ Nystatin kill most fungi.

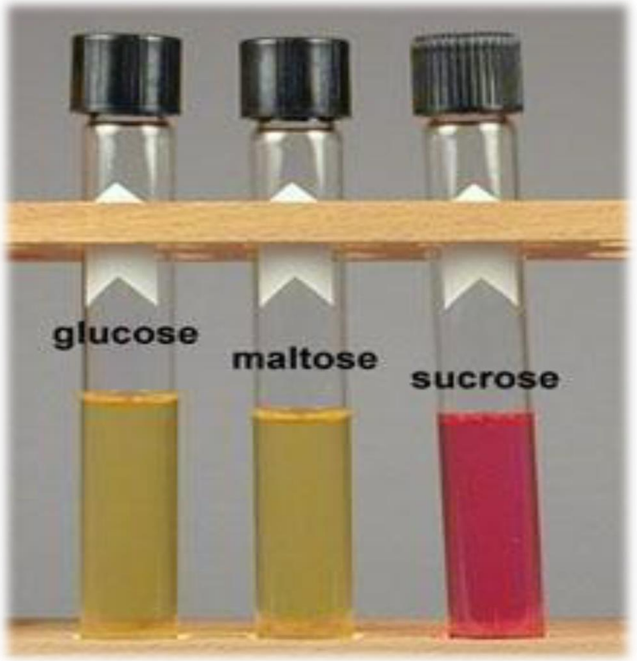


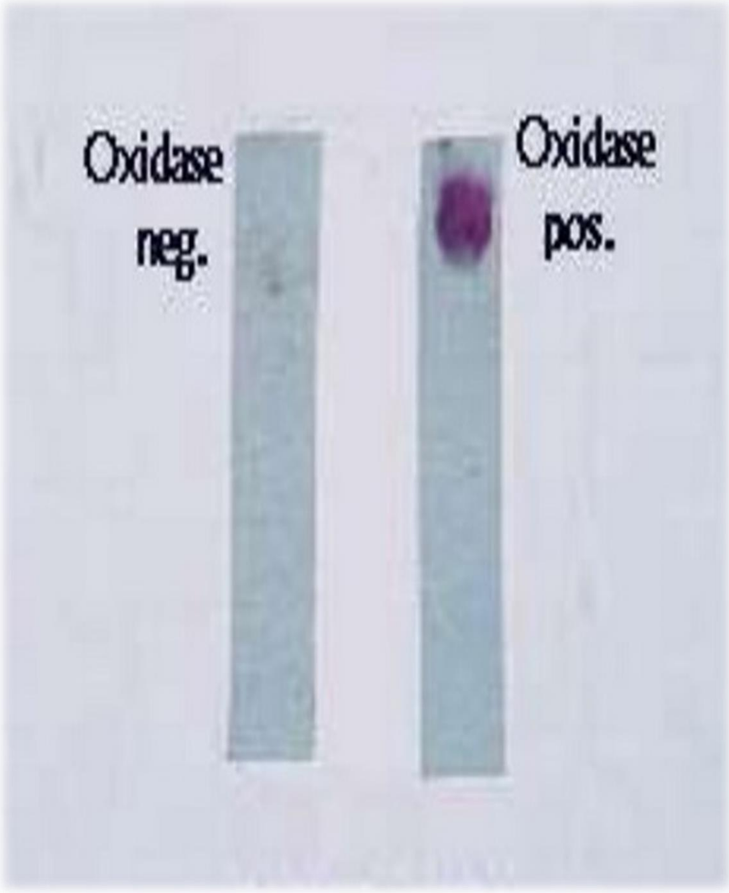
Laboratory diagnosis of acute cerebrospinal meningitis

4- Identification:

- **Gram-stained film from culture**
- **Biochemical reactions**:

✓ Oxidase positive.



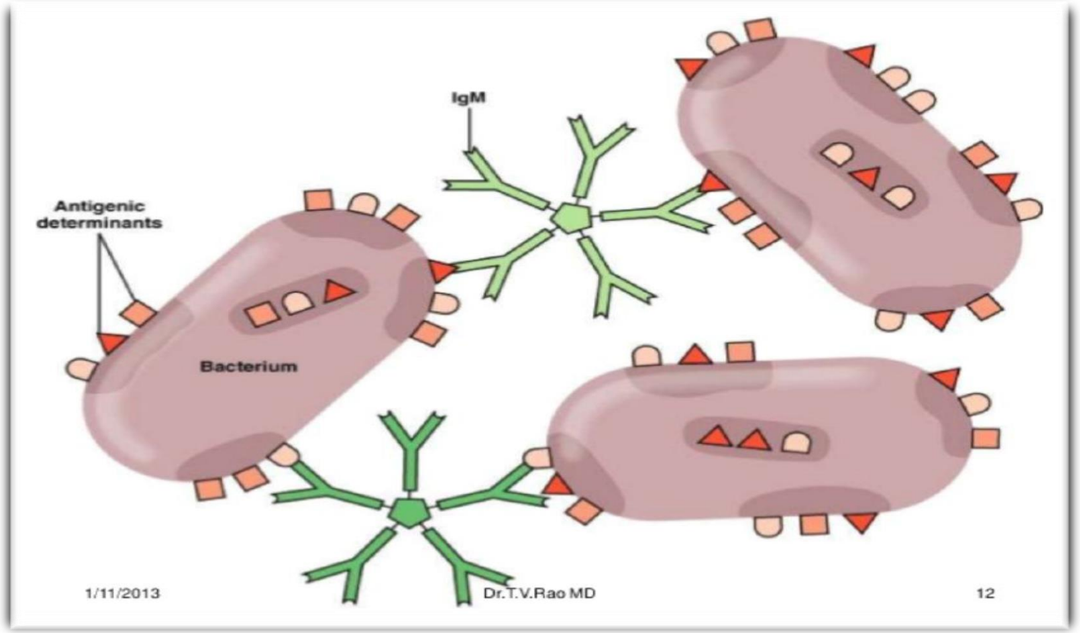


✓ Sugar fermentation: glucose and maltose (acid production only).

Laboratory diagnosis of acute cerebrospinal meningitis

5- Definitive identification: by agglutination with specific meningococcal antisera.

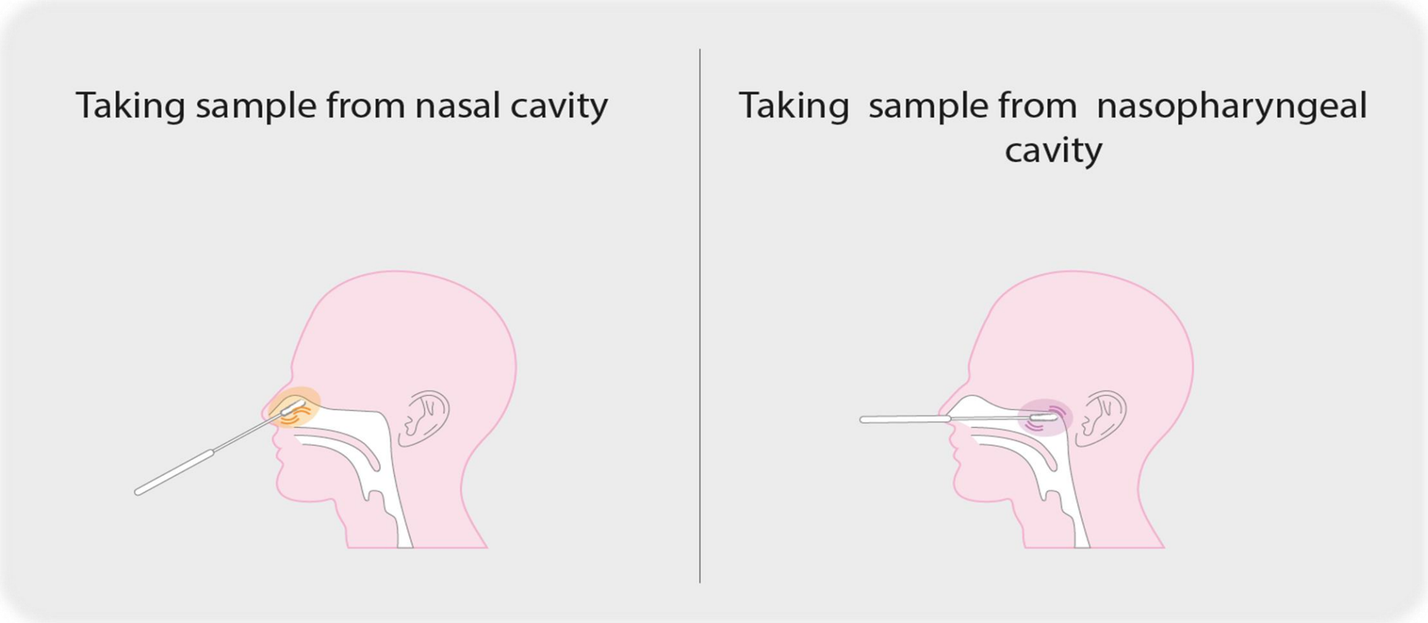
6- Rapid diagnosis: by detection of meningococcal antigen in CSF by latex agglutination available for N. meningitidis groups A,B,C,Y, and W135.

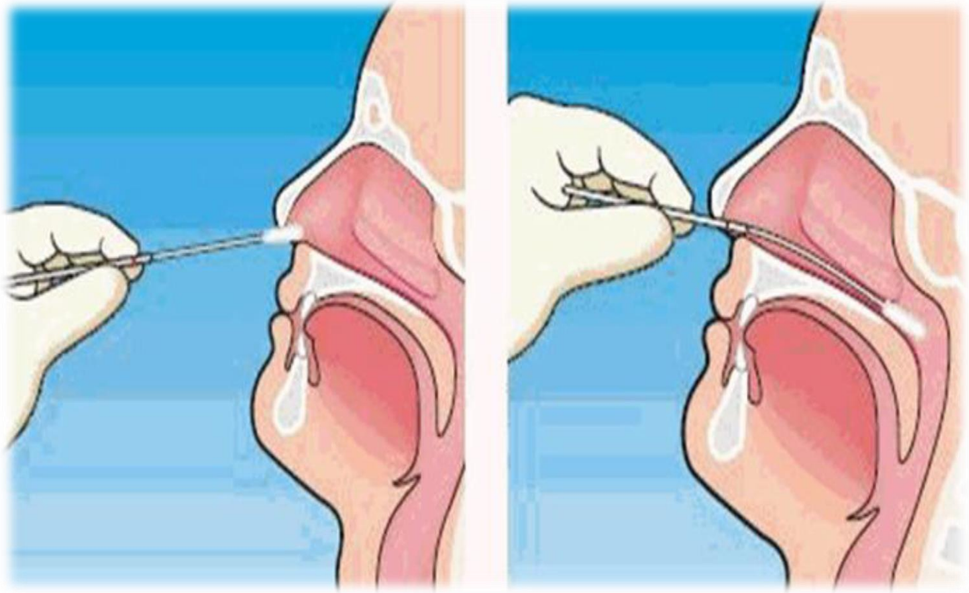


Laboratory diagnosis of acute cerebrospinal meningitis

II. Diagnosis of carrier:

- Nasopharyngeal swab is examined as in the diagnosis of the case.
- The obtained growth should be differentiated from commensal neisseria.





Laboratory diagnosis of acute cerebrospinal meningitis

Neisseria	Pathogenic	Commensal
Temperature	37	Room temp.
Ordinary media	No	Grow
Thayer Martin medium	Grow	No
Colony pigment	No	May produce
Reaction with antimeningococcal sera	Yes	No

* Enriched media used for isolation of *Neisseria meningitidis*…

a) Blood agar plate.

b) Nutrient agar plate.

c) Chocolate blood agar.

d) CLED agar plate.

e) Thayer-martin media.

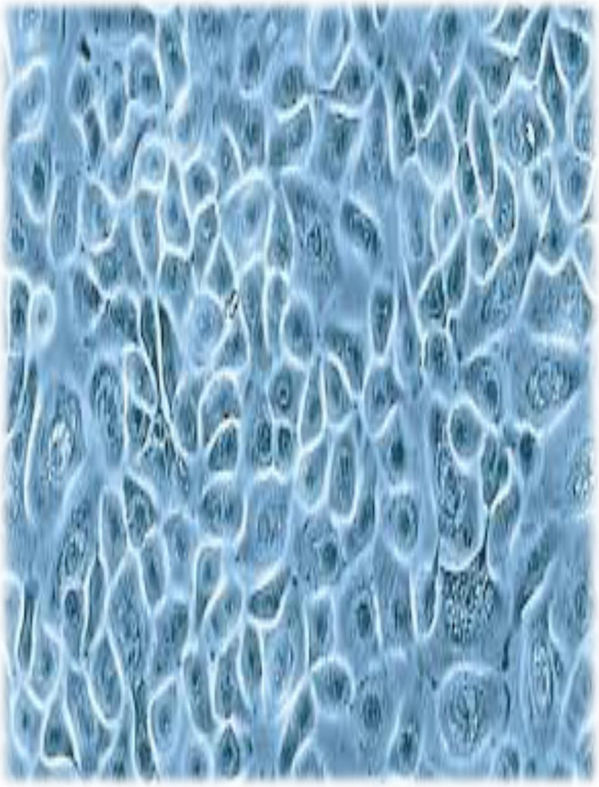
Viral Meningitis

- **Enteroviruses:** Echovirus, Poliovirus, Coxsackie A virus
- **Herpes:** Herpes simplex virus type 1 (HSV-1 / HHV-1) or type 2 (HSV-2 / HHV-2), Varicella zoster (VZV / HHV-3), Epstein–Barr virus (EBV / HHV-4), Cytomegalovirus (CMV / HHV-5).
- **Measles, Mumps, influenza.**
- **Human immunodeficiency virus (HIV).**
- **Lymphocytic choriomeningitis virus (LCMV).**
- **St. Louis encephalitis virus, West Nile virus.**

Laboratory diagnosis of viral infection

1- Direct detection:

- Detection of Virus particles by (electron microscope).
- Detection of viral antigens by (EIA, RIA…) and nucleic acid by (PCR).



2- Virus isolation: Viruses are obligate intracellular parasites and can be cultivated on:

- Tissue cultures: cytopathic effect (CPE).
- Embryonated egg.
- Animal inoculation.

3- Serologic detection of antiviral antibodies: By serological methods (EIA, RIA…).

Laboratory diagnosis of viral infection

References

- Topley and Wilson’s Microbiology and Microbial Infections: 10th edition.

- Brooks, G. F., Jawetz, E., Melnick, J. L., & Adelberg, E. A. (2013). Jawetz, Melnick, & Adelberg's medical microbiology. New York: McGraw Hill Medical.

- Cheesbrough M. (2006): Microbiological tests. Cited by Cheesbrough, M., (ed.) District Laboratory Practice in Tropical Countries, Part 2, Microscopical techniques used in microbiology, Cambridge University Press, UK.


THANK YOU