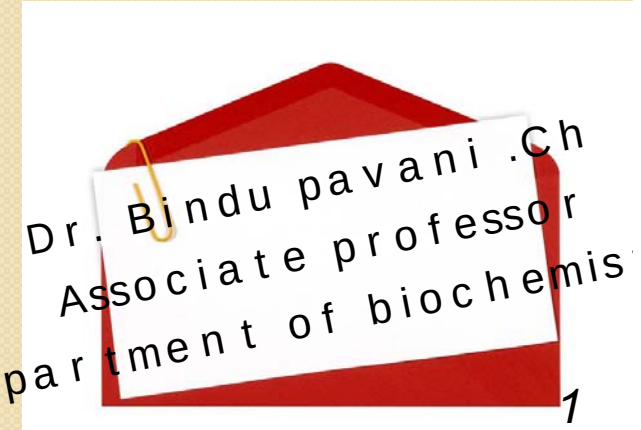
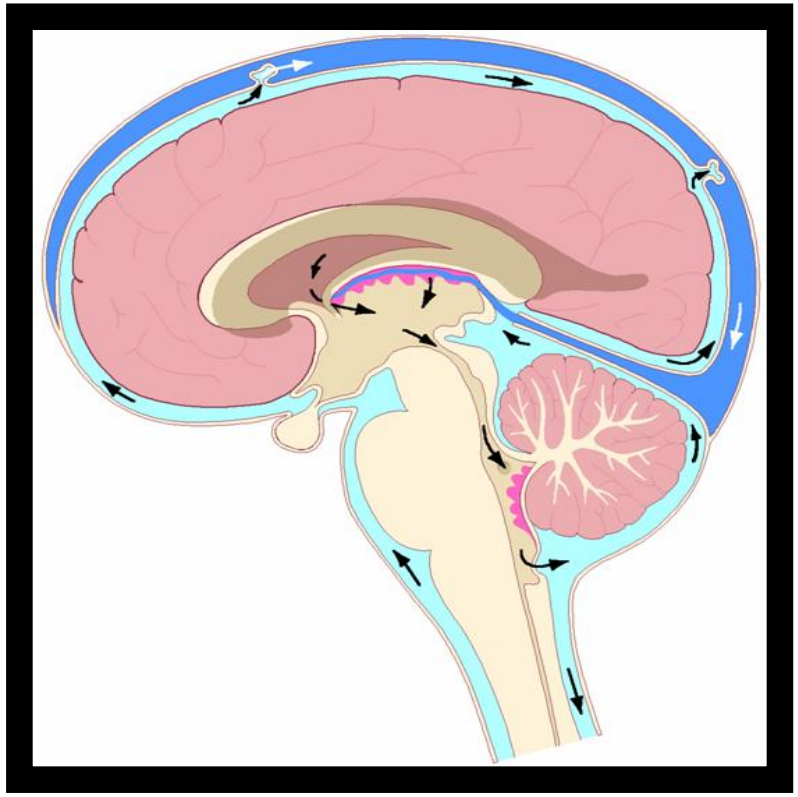
Laboratory diagnosis of meningitis



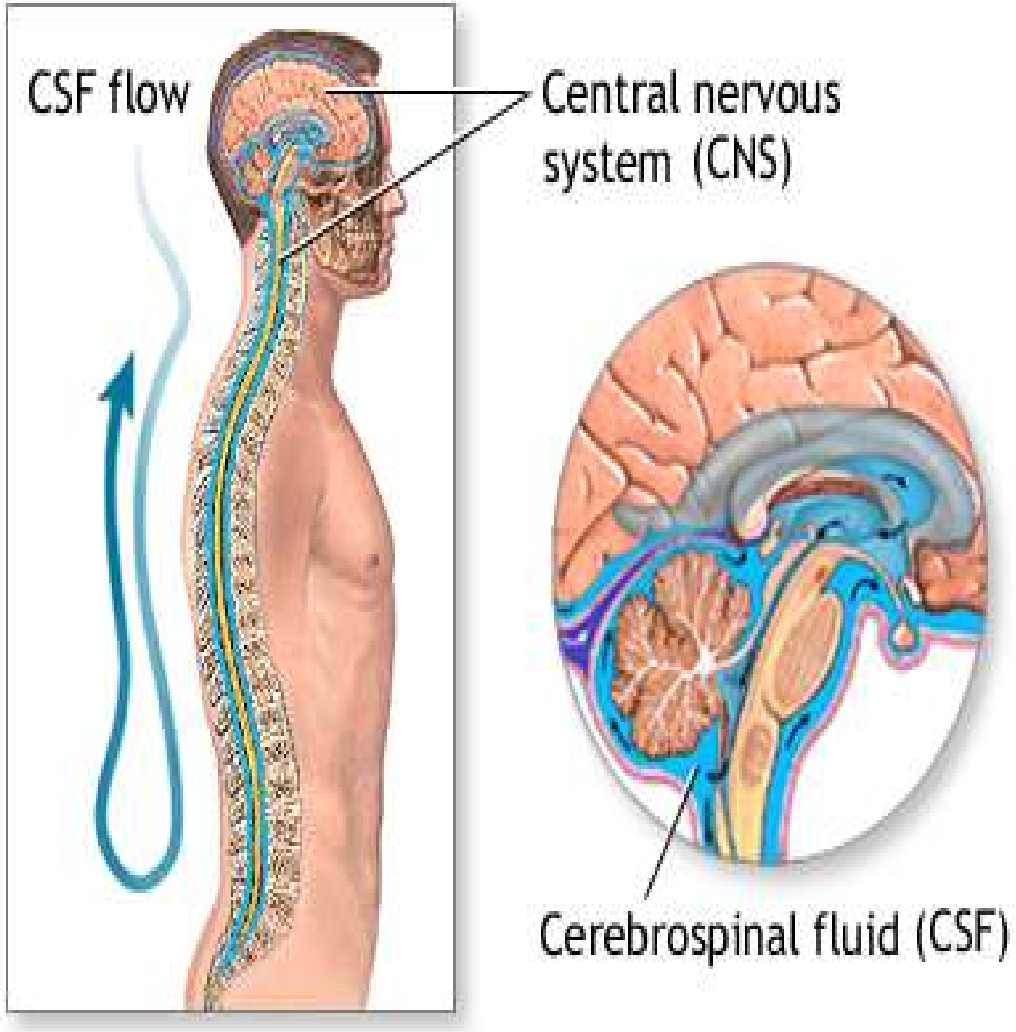
LAB DIAGNOSIS

- CSF EXAMINATION
- HISTOPATHOLOGY
- LATEX AGGLUTNATION
- POLYMERASE CHAIN REACTION
- VIRAL CULTURE
- RAPID DIAGNOSTIC TESTS (RDT)
- SEROLOGIC STUDIES
- OTHER LAB STUDIES

CEREBROSPINAL FLUID ANALYSIS

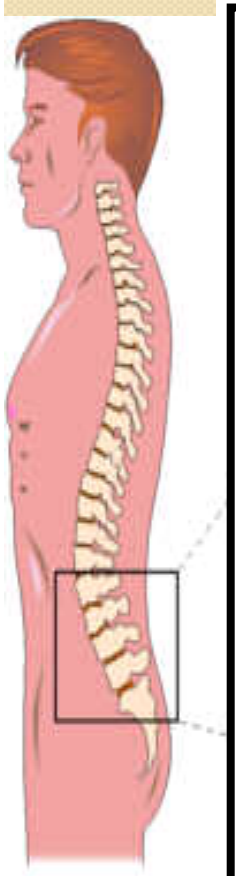


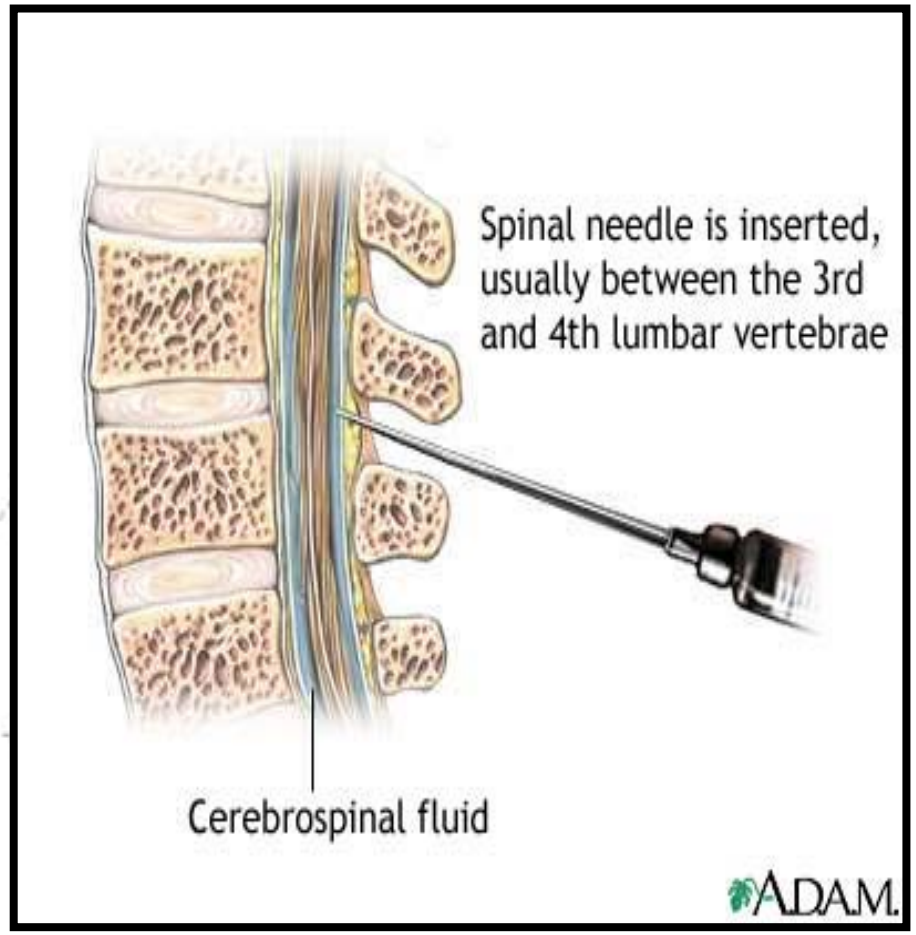
CSF Formation & Circulation



- *Liquor cerebrospinalis*: clear, colorless fluid
- CSF is formed at the *choroid* plexuses & by the cells lining the *ventricles*.
- occupies the subarachnoid space and the ventricular system around and inside the brain and spinal cord.

Method of CSF Sampling





- Obtained by ***lumbar puncture*** (At the interspace L3-4, or lower)
- Using ***aseptic*** technique

Traumatic tap (damage to blood vessel during specimen collection) → blood in CSF

CSF Specimen Collectio

- CSF is ***separated*** 3 aliquots:
  - for chemistry &
  - for microbiology
  - for cell count
- ***Immediate*** anal
- It's a ***precious*** sample: Preserve any remaining sample

Must always be ***centrifuged*** prior to analysis in order to precipitate any cells → falsely high values for CSF protein.

Normal composition of CSF

Clear ,Colorless	Appearance
<5/mm³	Lymphocytes
Nil	Polymorphs
7.4	pH
100 - 150 ml	Total Volume
450 - 500 ml	Daily Secretion
1.006 - 1.007	Specific Gravity
15 – 45 mg/dL	Protein
50 - 80 mg/dL (2.8-4.2 mmol/L) (60 -80% plasma level)	Glucose
115 - 130 mmol /L	Chloride
1.0 - 1.40 mmol/L	Calcium
0.4 - 0.7 mmol/L	Phosphorus
1.2 - 1.5 mmol/L	Magnesium
2.6 - 3.0 mmol/L	Potassium

Examination of CSF:

1- Physical examination

- **Normal CSF is:**
  - *Colorless*
  - *Clear*
  - *Free of clots*
  - *Free of blood*

Blood & Hemoglobin pigments in CSF

Subarachnoid hemorrhage (SAH)

- → Xanthochromia (hemoglobin breakdown pigments) = RBCs lysis & metabolism previously occurred (at least 2 hr earlier)

When would Xanthochromia indicate hemorrhage?

- If you exclude:
  1. Prior traumatic tap
  2. Hyperbilirubinemia (*bilirubin > 20 mg/dL*)

Compare CSF with a similar volume of water in an identical tube; look down the longitudinal axis of the tube, against a white background; .

Turbidity

- CSF is cloudy (turbid) →
  - is usually due to leucocytes
  - may be due to micro-organisms

- Meningitis – coccal forms
- 400-500 polymorphs per cu.mm

Coagulum

- Considerable rise in protein – fibrinogen – fibrin clots → Coagulum
- Spinal tumors

- Tuberculous meningitis – cob web- like coagulum (tubercle bacilli)

Examination of CSF:
2- Biochemical analysis of CSF

- Tests of interest:
  - √ Glucose
  - √ Protein
    - Total
    - Specific:
      - Albumin
      - Immunoglobulin
      - Others (e.g. myelin basic protein; MBP)
  - Chlorides
  - Lactate
  - Enzymes

The most reliable parameters diagnostically & accessible analytically

Glucose in CSF

- Glucose enters CSF via facilitative transporter (*GLUT*)
- CSF [glucose] is ~ 2/3 that of plasma
  - ***50 - 80 mg/dl***
- A ***plasma sample*** must be obtained ~ 2-4 hr before CSF sample
- Measure CSF [Glucose]:
  - ***immediately***
  - or preserve the specimen with an ***antiglycolytic agent*** e.g. fluoride ion

Abnormal CSF [Glucose]

- ↑ CSF [glucose](*hyperglycorrhachia*):
  - Not clinically informative
  - Provides only confirmation of hyperglycemia

- ↓CSF [glucose] (*hypoglycorrhachia*):
  1. Disorder in carrier-mediated transport
     - e.g. TB meningitis, sarcoidosis
  2. Active metabolism of glucose by cells or organisms:
     - e.g. acute purulent amoebic

Protein in CSF

- Source of CSF proteins:
  - 80% from plasma by ultrafiltration
  - 20% from intrathecal synthesis
    - serum to CSF protein: 200:1

- Ventricular fluid – 5-15 mg/dl
- Cisternal fluid – 15 - 25 mg/dl
- Lumbar fluid – 15 – 45 mg/dl

- *Premature and full term neonates – considerably higher (130mg/dl)*

Abnormal CSF [total proteins]

- Must be compared to the serum [protein]

- Examination of CSF protein is done mainly to detect:

  - a. Increased blood-brain barrier permeability to plasma protein

  - b. Increased intrathecal IgG secretion

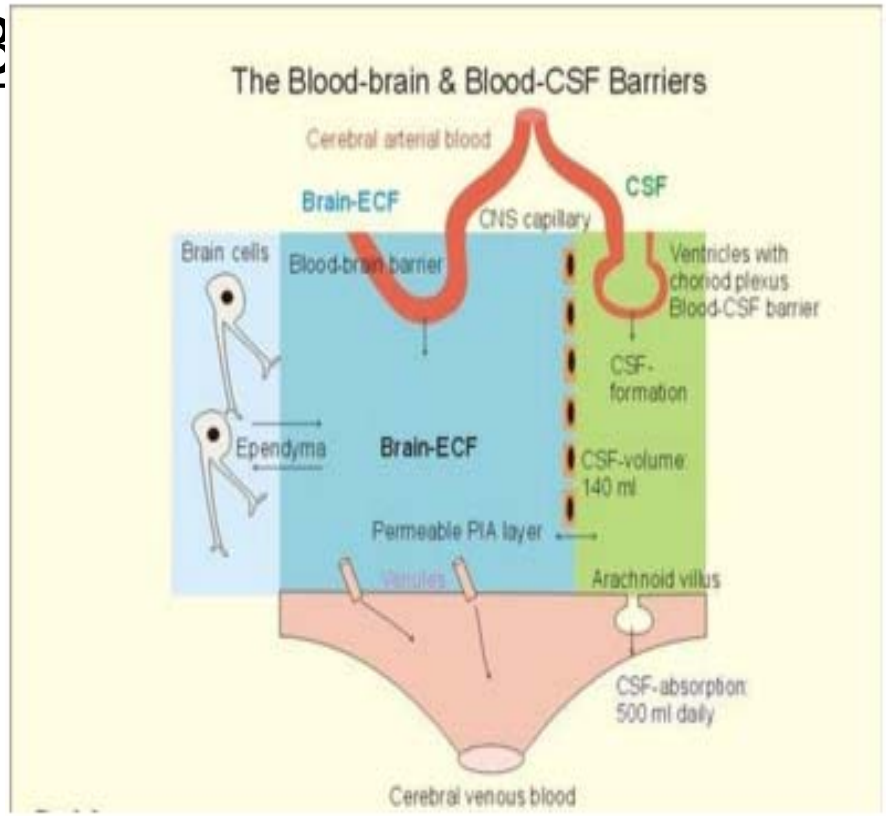
CSF Albumin

$$\frac{\text{CSF Albumin mg/dl}}{\text{Serum Albumin g/dl}} = \text{CSF serum albumin index:}$$

If < 0.9 = intact BBB

- Albumin – suitable indicator protein
- Its presence in CSF must occur through BBB

INCREASED BLOOD-BRAIN BARRIER PERMEAB



1) High intracrani
   -Brain tumor
   -Intracerebral
2) Inflammation
   - Bacterial meni
     striking eleva
   - encephalitis and poliomyelitis

INCREASED INTRATHECAL SYNTHESIS OF IMMUNOGLOBULINS

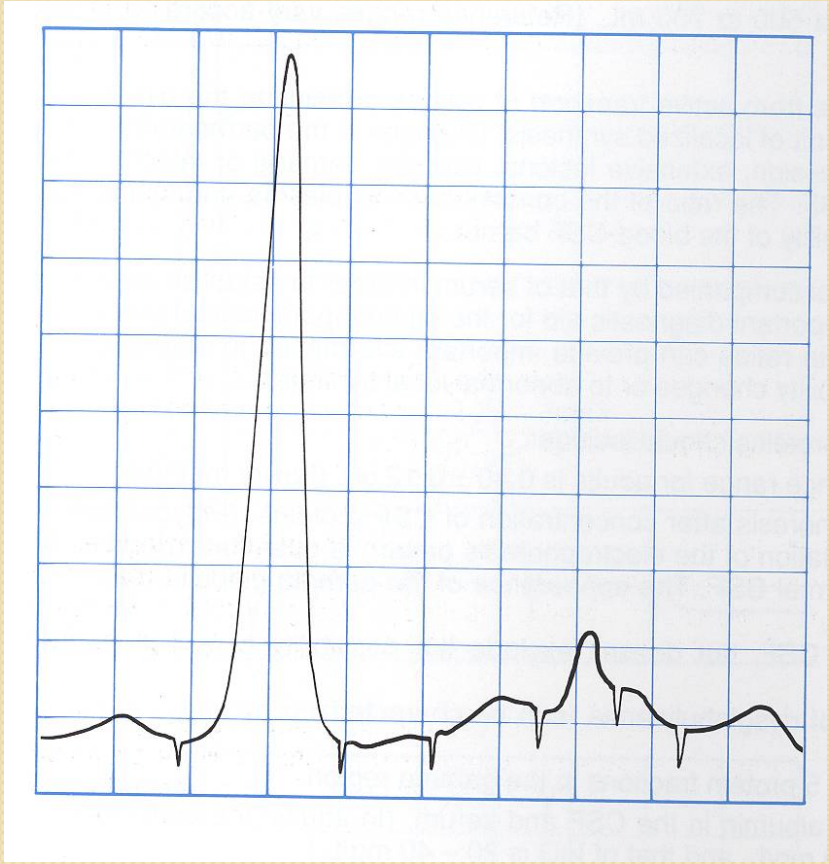
- IgG – **Demyelinating diseases**
  - Multiple sclerosis (MS)
  - Subacute Sclerosing Panencephalitis (SSPE)
- B lymphocytes infiltrating the leisions synthesize IgG

What to do if ↑ CSF [protein] was detected?

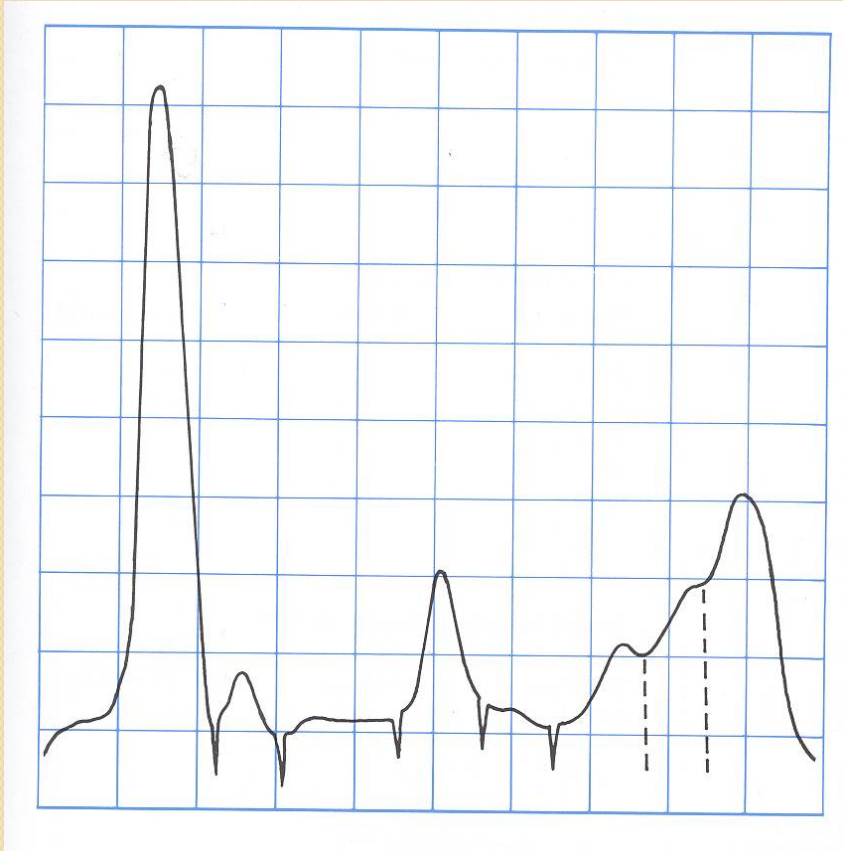
- Perform electrophoretic separation

- If multiple banding of the IgG band is detected (*oligoclonal bands*):
  - MS
  - SSPE
  - Inflammatory diseases

CSF Electrophoresis: Normal Pattern



CSF Electrophoresis: Oligoclonal Banding



Abnormal CSF Chloride

- 120 – 130 meq per litre
- **Higher than the plasma chloride**
  - marked ✵✵ in acute bacterial meningitis
  - slight ✵ in viral meningitis & brain tumors

Abnormal findings of CSF in meningitis

Condition			Parameter
Viral Meningitis	**Tuberculous Meningitis**	**Bacterial Meningitis (pyogenic)**	
Usually clear	Often fibrin web	Often turbid	Appearance
Mononuclear	Mononuclear	Polymorphs	Predominant cell
50-1000	10-1000	90-1000+	Cell count/mm^3
None seen or cultured	Often none in smear	In smear & culture	Bacteria

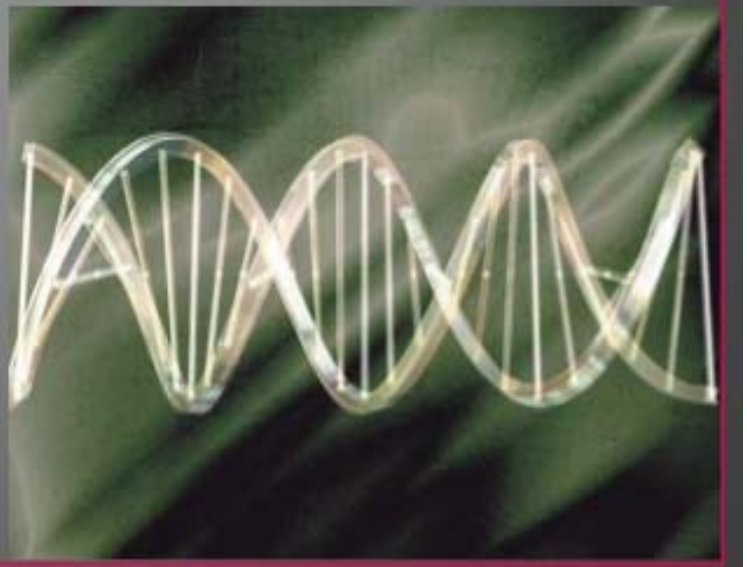
Abnormal findings of CSF in meningitis

Condition			Parameter (reference range)
Viral Meningitis	**Tuberculous Meningitis**	**Bacterial Meningitis (pyogenic)**	
< 100 Normal or ↑	50-400 (↑ ↑)	80-500 (↑ ↑↑)	Protein (15-45 mg/dL)
>1/2 plasma (Normal or slightly ↓)	<1/2 plasma (↓ ↓)	<1/2 plasma (↓ ↓)	Glucose (50-80 mg/dl)
Normal	↓ ↓	↓ ↓	Chlorides (120 - 130 meq/L)

- *1. Increased **lactate** → Bacterial meningitis*
- *2. Increased **LDH** → Bacterial meningitis*
- *3. Increased **adenosine deaminase** → Tuberculous meningitis*

POLYMERASE CHAIN REACTION

- Amplification of virus specific DNA or RNA from CSF using PCR amplification has become the single most effective method for diagnosing CSF viral infections.
- It is a highly sensitive and specific test since only trace amounts of the infecting agent's DNA is required.
- It may identify bacteria in bacterial meningitis and may assist in distinguishing the various causes of viral meningitis.



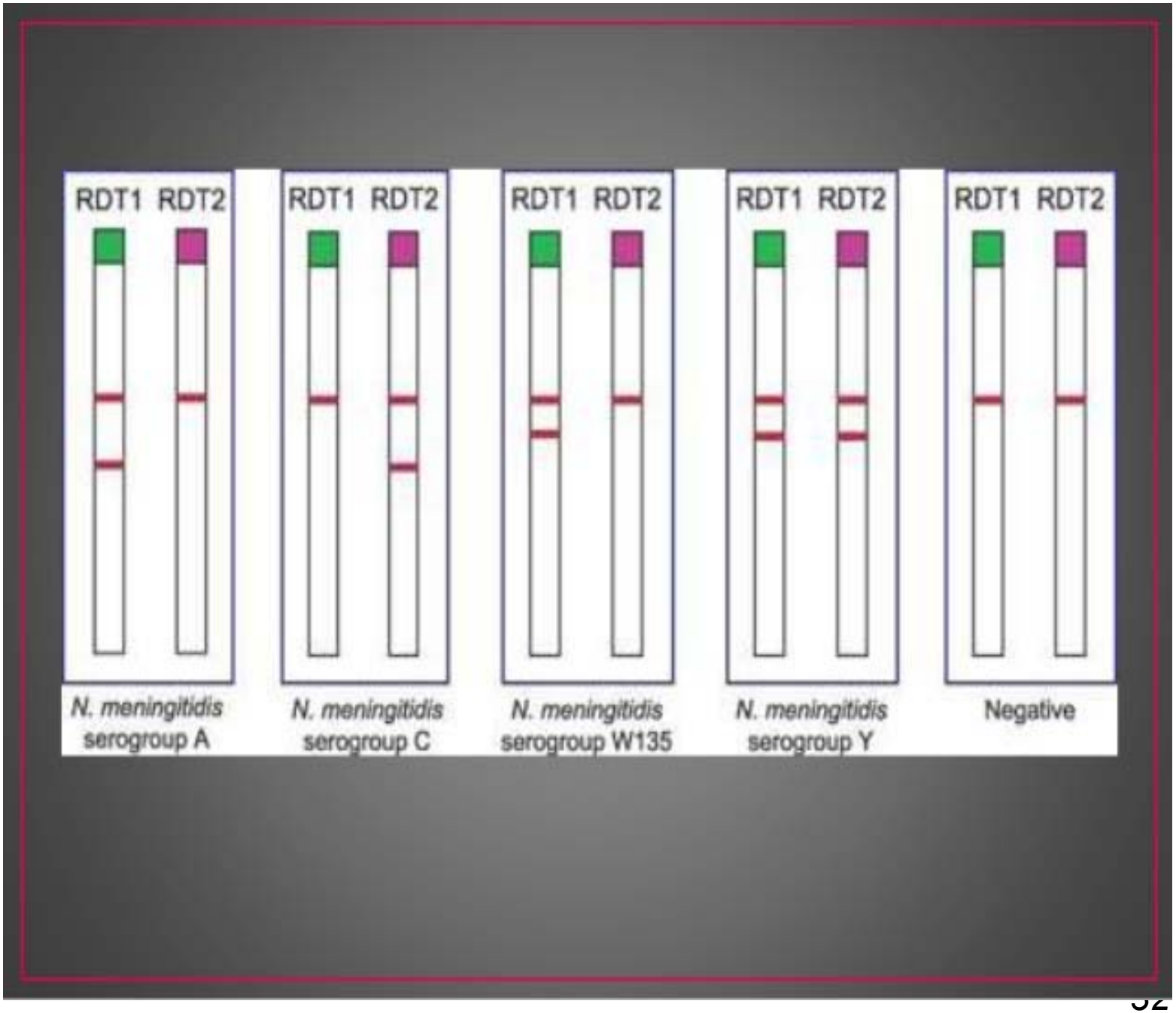
Thank You

RAPID DIAGNOSTIC TESTS (RDT)

- RDTs have been developed for direct testing of CSF specimens without prior heat or centrifugation.
- The test is based on the principle of vertical flow immunochromatography.
- Gold particles and nitrocellulose membranes are coated with monoclonal antibodies to capture soluble serogroup-specific polysaccharide antigens in the CSF.

READING THE RDT RESULTS

- Appearance of red lines on the dipsticks will indicate whether one of the four meningococcal serogroups has been detected in the CSF.
- The upper line on the dipstick is the positive control and should always be present.
- If the CSF is positive for one of the serogroups, a lower red line will also be present. The position of that red line indicates the specific serogroup based on the RDT that was tested.
- A negative result consists of a single upper pink control line only.


RDT1 RDT2
RDT1 RDT2
RDT1 RDT2
RDT1 RDT2
RDT1 RDT2
N. meningitidis serogroup A
N. meningitidis serogroup C
N. meningitidis serogroup W135
N. meningitidis serogroup Y
Negative

OTHER LABORATORY STUDIES

- CBC (complete blood count) & DLC (differential leucocyte count)
- Liver and Renal function tests
- ESR (erythrocyte sedimentation rate)
- C- Reactive protein
- Electrolytes etc
- MRI and CT are not necessary in patients with uncomplicated meningitis.
- They may be performed in patients with altered consciousness, seizures etc