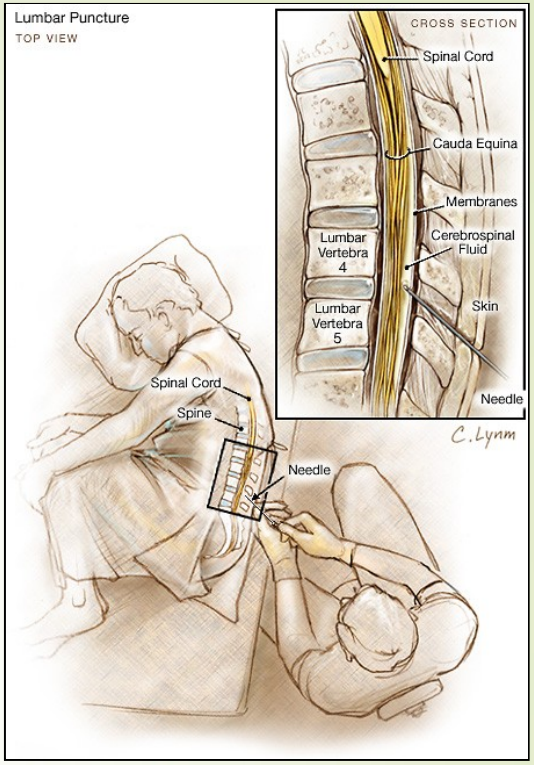
Cerebrospinal Fluid (routine and microscopy)



MODERATOR : Dr. Ramu Thakur

Speaker: Dr. Gaurav Shelgaonkar
MGMMC,Indore

Graphic accessed http://www.medem.com/medem/images/jamaarchives/JAMA_MedicalTests_Tests_lev20_LumbarPuncture_JPP_01.jpg, 2006.

CSF - Liquour Cerebrospinalis

The cerebrospinal Fluid [CSF] is a clear, colourless transparent fluid present in the cerebral ventricles, spinal canal, and subarachnoid spaces.

CEREBROSPINAL FLUID [FORMATION]

CSF is largely formed by the choroid plexus of the lateral ventricle and remainder in the third and fourth ventricles.

CSF is a selective ultrafiltrate of plasma.

Small amount of the CSF is also formed from the ependymal cells lining the ventricles and other brain capillaries.

Rate of formation:

About 20 ml/hour (0.3 – 0.4 ml/min)

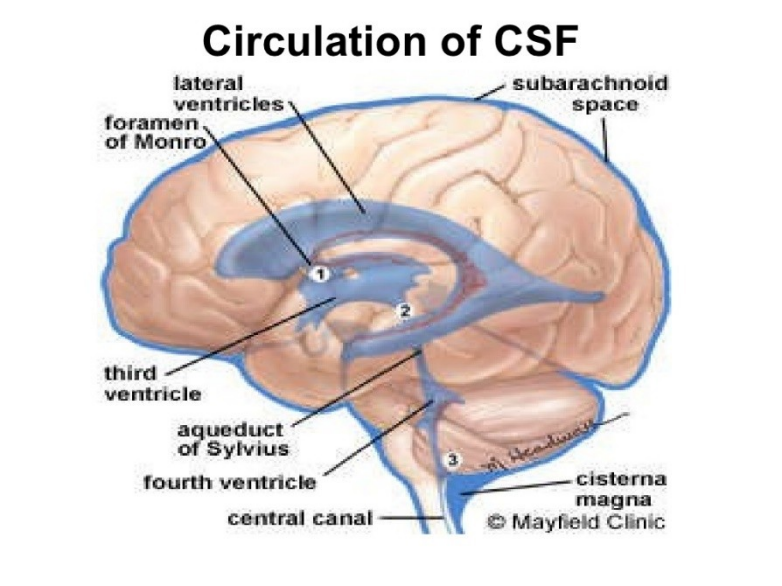
500 ml/day in adults. Turns over 3.7 times a day

Total quantity: 90 - 150 ml in adults

30 - 40 ml within the ventricles

About 110-120 ml in the subarachnoid space [of which 75-80 ml in spinal part and 25-30 ml in the cranial part].

CIRCULATION OF CSF



Lateral ventricle

↓ Foramen of Monroe [Interventricular foramen]

Third ventricle:

↓ Cerebral aqueduct

Fourth ventricle:

↓ Foramen of magendie and formen of Luschka

Subarachnoid space of Brain and Spinal cord

ABSORPTION OF CSF THROUGH ARACHNOID VILLI

The arachnoid villi are finger like inward projections of the arachnoid membrane through the walls into venous sinuses.

Villi form arachnoid granulations protruding into the sinuses.

CHARACTERISTICS OF CSF

Nature :

Color - Clear, transparent fluid

Specific gravity - 1.004-1.007

Reaction - Alkaline and does not coagulate

Cells - Adults : 0-5 cells/cumm
Infants : 0-30 cells/cumm
1-4 years : 0-20 cells/cumm
5-18 years : 0-10 cells/cumm

Pressure - 60-180 mm of H2O (adult)
10-100 mm of H2O (newborn)

COMPOSITION OF CSF

Proteins	-	15-45 mg/dl
Glucose	-	45-80 mg/dl
Na+	-	147 meq/L ↑
Chloride	-	120-130 mEq/L ↑
Ca+	-	2.3 meq/dL ↓
Urea	-	12.0 mg/100 ml
Creatinine	-	1.5 mg/100 ml
Lactic acid	-	18.0 mg/100 ml
Bilirubin	-	Absent

FUNCTIONS OF CSF

- Protection (Buoyancy)
- Nutrition
- Removal of waste
- Lubrication

INDICATIONS OF CSF EXAMINATION

1. Infections: meningitis, encephalitis.

2. Inflammatory conditions: Sarcoidosis, Neurosyphilis, SLE.

3. Infiltrative conditions: Leukemia, lymphoma

4. Administration of drugs in CSF (Therapeutic aim):
   Antibiotics
   Anticancer drugs
   Anesthetic drugs

LUMBAR PUNCTURE

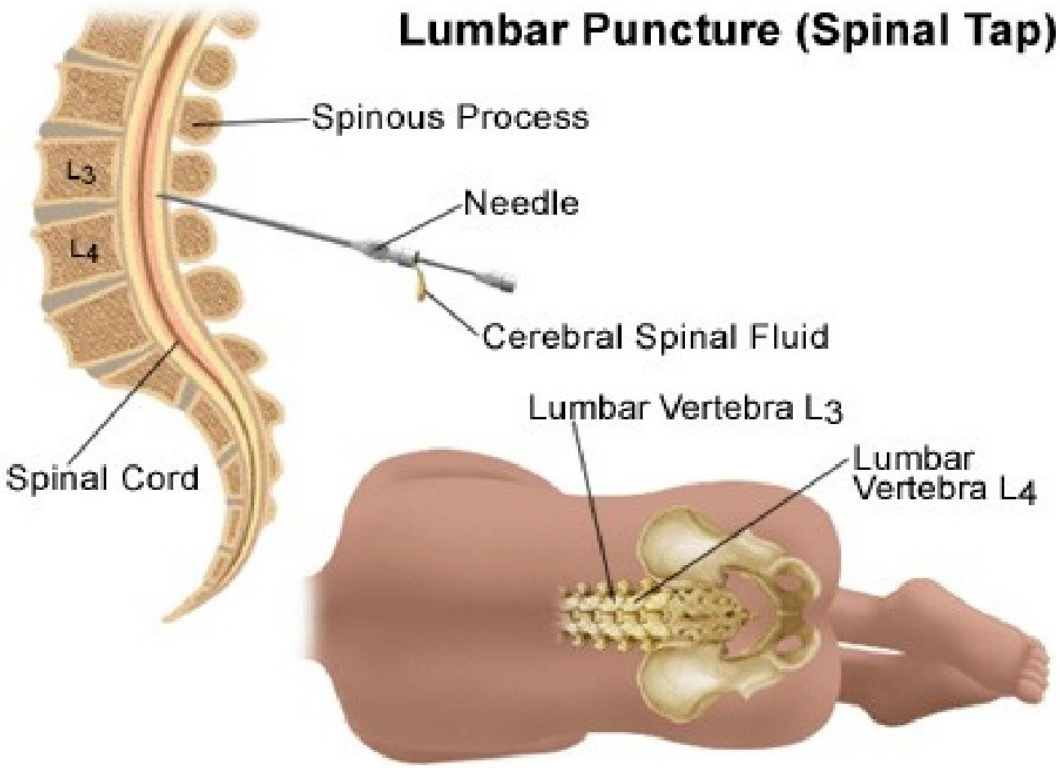
- A lumbar puncture also called a spinal tap is a procedure where a sample of cerebrospinal fluid is taken for examination.

- First performed by Quincke in 1891.

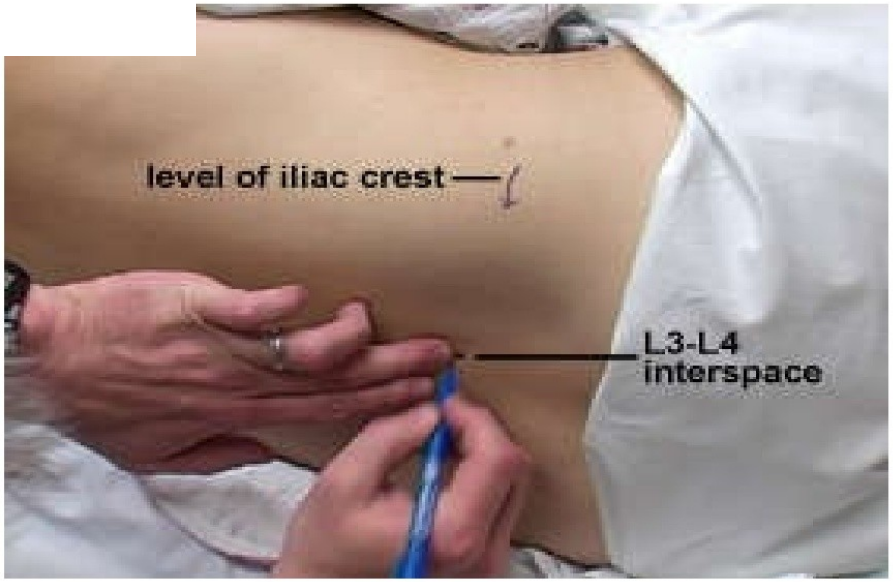
LUMBAR PUNCTURE PROCEDURE

- Patient usually lie on a bed on side (lateral recumbent position) with knees pulled up against the chest.
- It may also done with sitting up and leaning forward on some pillows. Sterilize the area.
- Push a LP needle through the skin and tissues between two vertebra into the space around the spinal cord which is filled with CSF.
- CSF leaks back through the needle and is collected in three tubes.

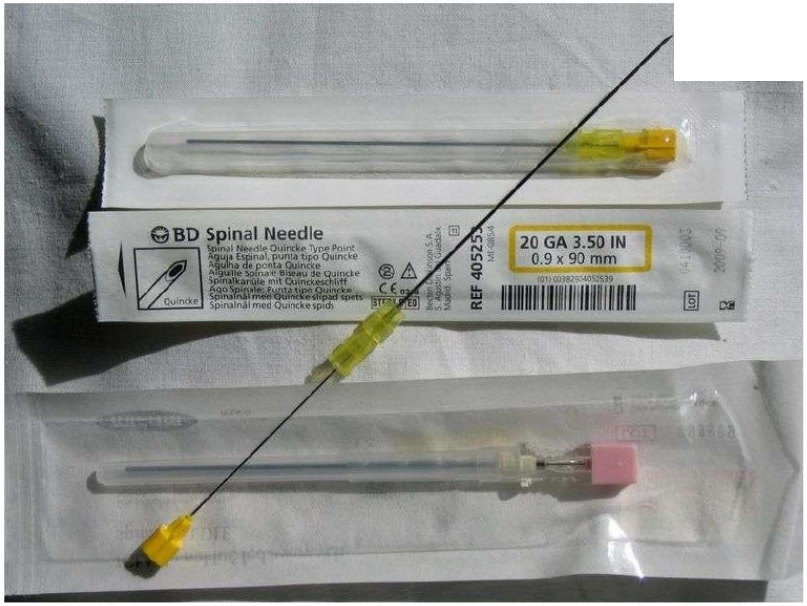
Generally up to 6-7 ml can be taken from an adult, if pressure is normal (50-180 mm H2O).

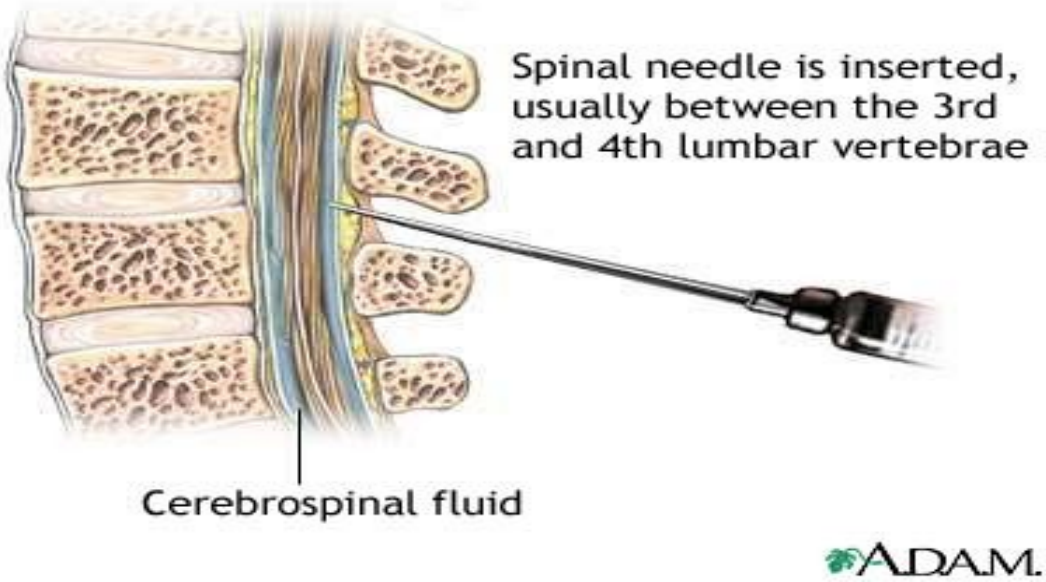


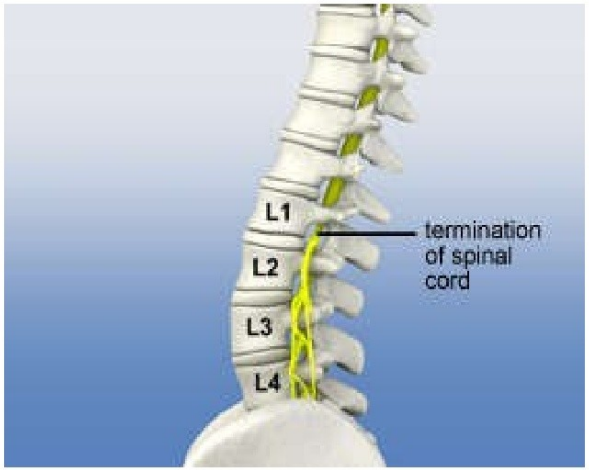
Level of entry



Spinal needle







Spinal cord terminates at L1; needle entry must occur distal to this location

LUMBAR PUNCTURE [Complications]

- Post lumbar puncture headache
- Introduction of infection in the spinal canal
- Subdural hematoma
- Failure to obtain CSF (dry tap)
- Herniation of brain
- Subarachnoidal epidermal cyst

CONTRA-INDICATIONS for Lumbar Puncture

ABSOLUTE	RELATIVE
➢ Local skin infections over proposed puncture site	➢ Raised intracranial pressure (ICP); exception is pseudo-tumor cerebri.
➢ Intracranial mass lesion (based on lateralizing neurological findings) with raised ICT	➢ Uncontrolled bleeding diathesis
	➢ Spinal column deformities (may require fluoroscopic assistance)
	➢ Lack of patient cooperation

Tube 1 - Chemistry

Tube 2 - Haematology

Tube 3 - Microbiology

Glass tubes – X

Refrigeration - X

Routine

- Gross examination
- Cell Counts + Differential
- Glucose [60-70% plasma]
- Protein [15 - 40 mg/dL]

When Indicated

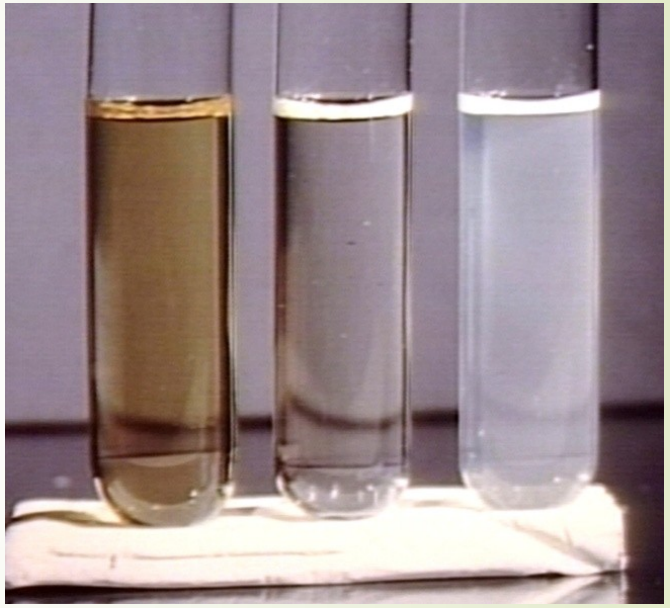
- Cultures
- Stains [Gram, Acid Fast]
- Cytology
- Electrophoresis
- VDRL

Macroscopic Examination

- Normal CSF appearance is crystal clear and colourless
- Pathological processes can cause fluid to appear cloudy, turbid, bloody, viscous, or clotted.
- The clarity of the fluid is of little clinical use, except to provide an immediate indication of abnormality of the CSF. A very useful point to remember is that a large number of cells can be present without affecting the clarity.

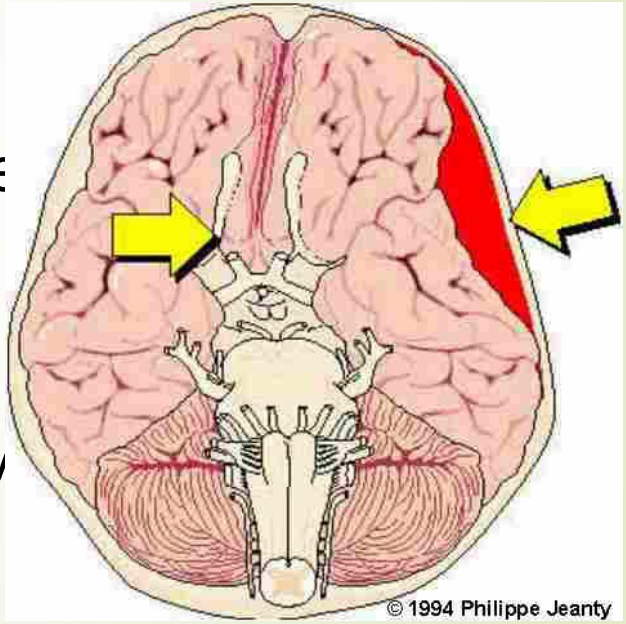
APPEARANCE

1. *Blood Mixed.*
2. *Xanthochromic.*
3. *Thick Viscous.*
4. *Clot/ Cob web.*



1. Traumatic Tap or CNS Hemorrhage

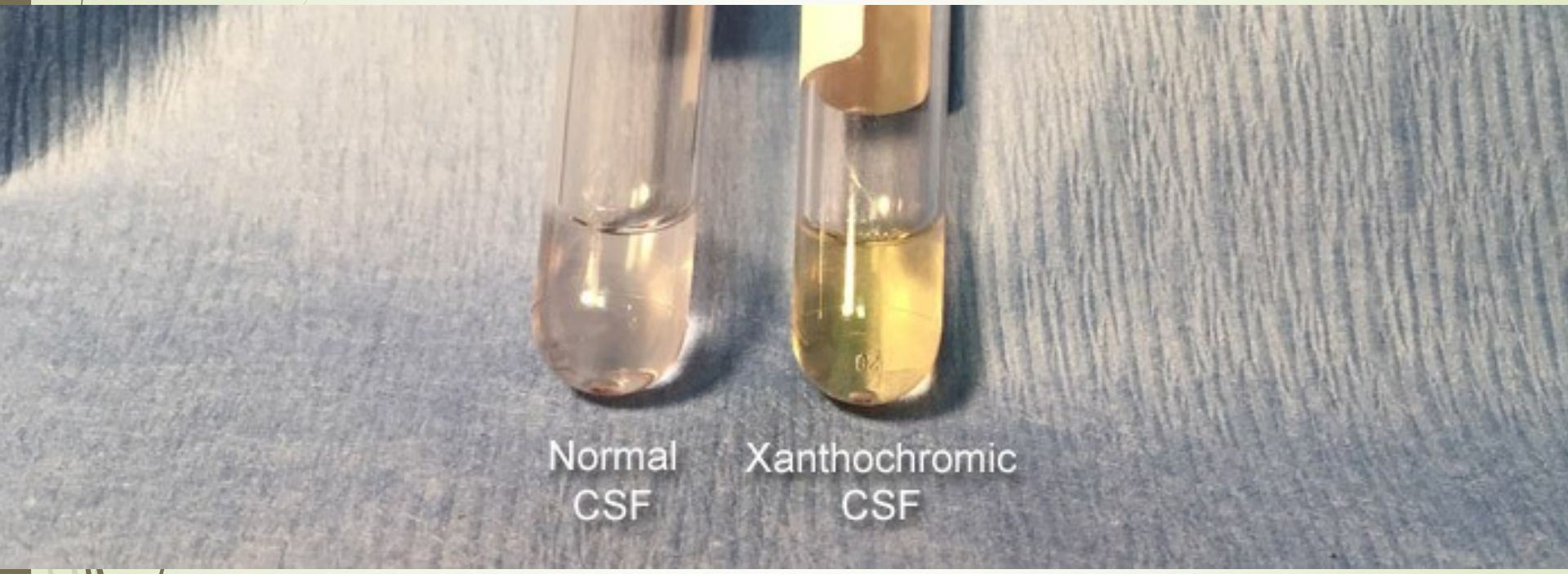
- ~20% of LPs result in bloody specimens.
- Pink-red CSF usually indicates the presence of blood.
- It is extremely important to identify the source of the blood



Graphic accessed URL http://www.thefetus.net/images/article-images/central_nervous_system/subdural_hematoma_files/image17.jpg, 2005.

2. Xanthochromia

- Subarachnoid and intracerebral hemorrhage.
- Traumatic tap.
- Jaundice.
- Elevated protein level (>150 mg/dl)
- Premature infants (with immature blood-CSF barrier & elevated bilirubin.
- Hypercarotenemia.
- Meningeal malignant melanoma.


Normal
CSF
Xanthochromic
CSF

CSF finding	Traumatic LP	Subarachnoid Hemorrhage
Gross appearance	Blood more in initial tubes, Blood clot on standing	Blood uniform in all tubes, Blood does not clot on standing
Centrifugation	Clear supernatant	Pink or yellow supernatent
Microscopy	Progrresive decrease in RBC count in later tubes	RBC count uniform in all tubes
CSF Pressure	Normal	Increased
CSF Protein	Normal	Increased

3. Thick viscous CSF.

- Severe meningitis.
- Cryptococcal meningitis.
- Metastatic mucinous adenocarcinoma.

4. Clot formation:(cob web)

- Increased proteins(>150 mg)
- Tuberculous meningitis.
- Spinal block

Differentiation on the basis of type of clot

- Delicate and fine clot - Tuberculous meningitis.
- Large clot - Purulent meningitis
- Complete and spontaneous clot - Spinal constriction.

Causative Organisms – Age Wise

- 0- 6 months - Group B streptococcus, E. coli. Listeria monocytogens.
- 6months- 6 years –

  Streptococcus pneumonia,
  Neisseria meningitidis,

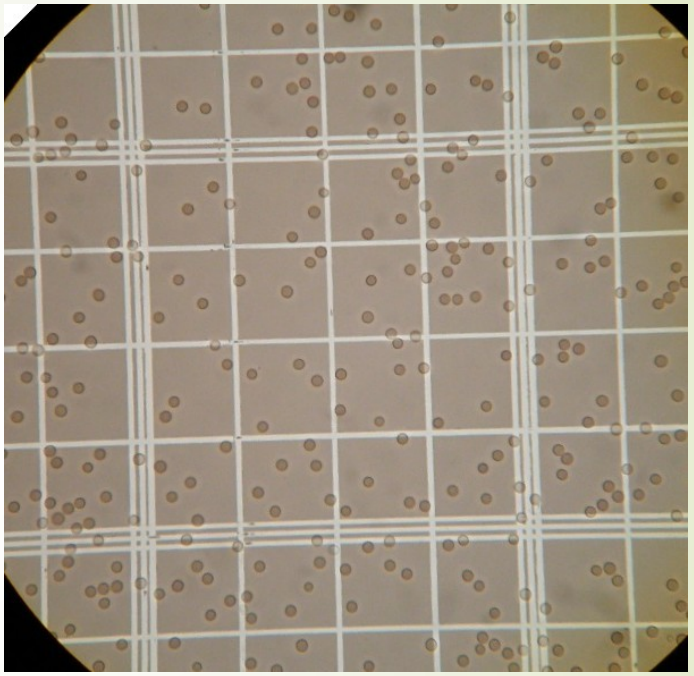
  Haemophilus influenzae type-b.
- 6-60 years - Neisseria meningitidis,

  Herpes simplex.
- >60 years - Streptococcus pneumoniae ,

  Listeria monocytogenes.

Microscopic Examinations

- Cell counts
  - Total
  - Leukocyte
  - RBC
- Differential
- Cytology



http://www.neuropat.dote.hu/jpeg/liquor/kmcarc1.jpg

METHOD(Total leukocyte count)

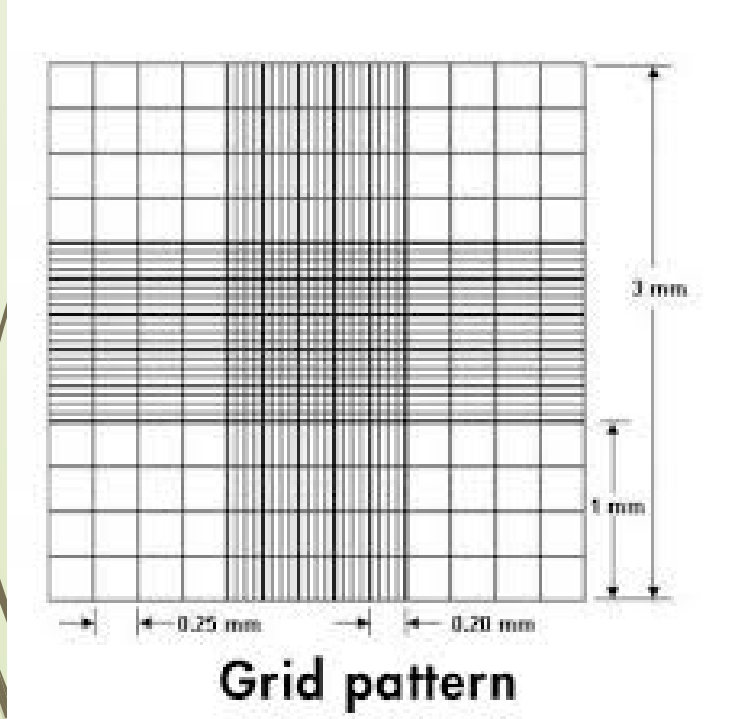
- Properly mix the CSF sample.
- Nine drops of CSF is diluted with one drop of CSF diluting fluid (in the ratio 9: 1)
- The counting chamber is covered with a cover slip.
- Charge the counting chamber with fluid and allowed to stand for 5 min for the cells to settle.
- Cells are counted in all the nine squares.

CSF Diluting Fluid: Add 10 ml of glacial acetic acid and 0.2 grams of crystal violet to a 100-ml volumetric flask. Dilute to the mark with distilled water.

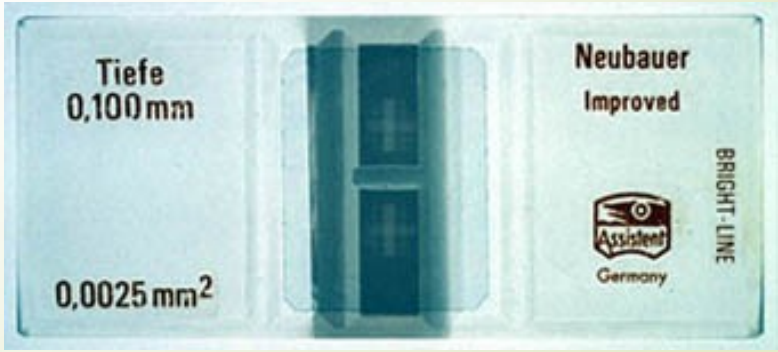
Calculation: Number of cells counted x 10

9

(as neubauer's chamber has a depth of 0.1 mm and total counting area is 9 sq. mm.)



Grid pattern

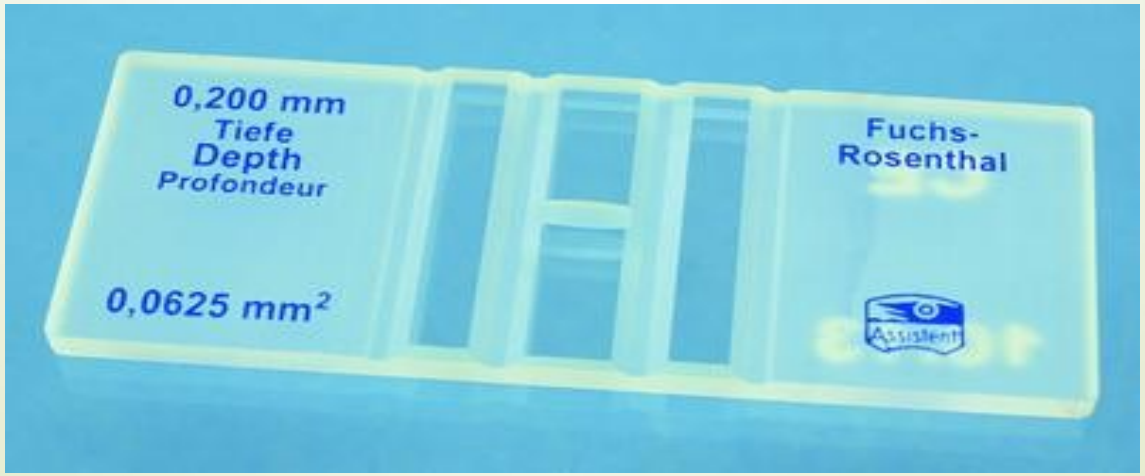


Fuch's rosenthal chamber: Cells are counted in five large squares.

Calculation = no. of cells counted x 10

5 x 2

(depth is 0.2 mm. and total counting area is 16 sq.mm.)



Cell Counts

- "Normal" adult CSF

  0-5 cells/ml

  - Lymphocytes.

- RBC count is of limited use, but can be used to correct CSF leukocyte counts* & CSF protein values of a traumatic tap CSF.

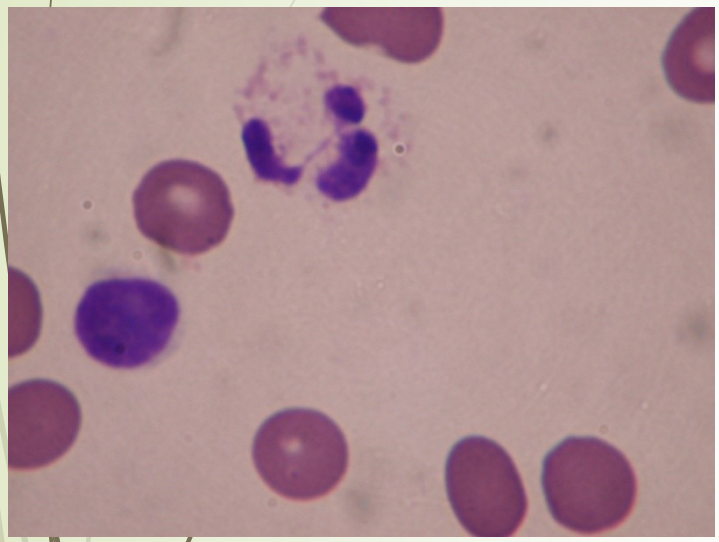
$$W^* = \frac{WBC_f - WBC_b \times RBC_f}{RBC_b}$$

http://www.tpub.com/content/medical/14295/img/14295_279_1.jpg

Causes of increased cell count :

- ✓ Meningitis.
- ✓ Intracranial hemorrhage.
- ✓ Meningeal infiltration by malignancy.
- ✓ Multiple sclerosis.

Differential

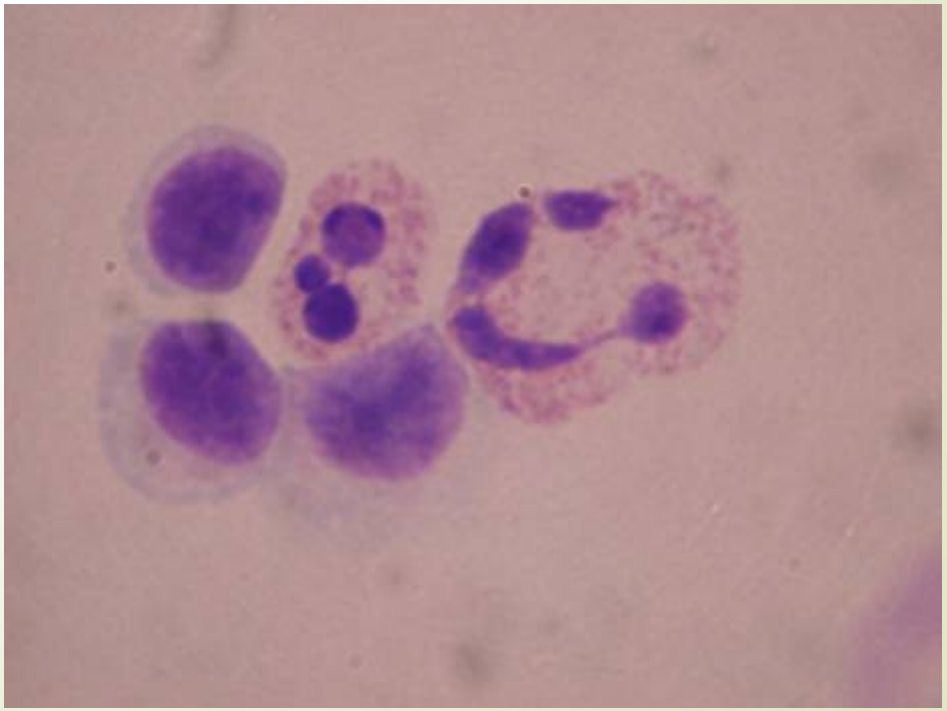


- Performed on a stained* smear made from CSF.
- It is recommended that stained smears be made even when the total cell count is within normal limits.
  - Count 100 cells in consecutive oil-power fields.
  - Report percentage of each type of cell present.

** usually Wright's stain.*

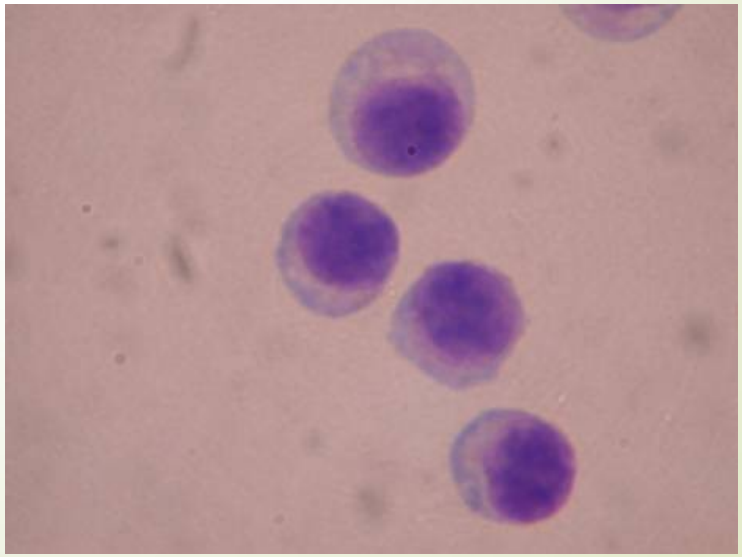
Predominant Neutrophils-

1. Meningitis(bacterial, early viral ,early tubercular and funga
2. Sub arachnoid hemorrhage.
3. Metastasis.

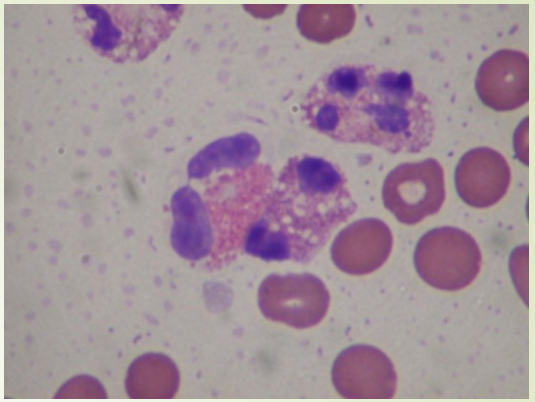


Predominant Lymphocytes-

I. Meningitis (viral or tubercular)
II. Incompletely treated bacterial meningitis.
III. Toxoplasmosis and cysticercosis.

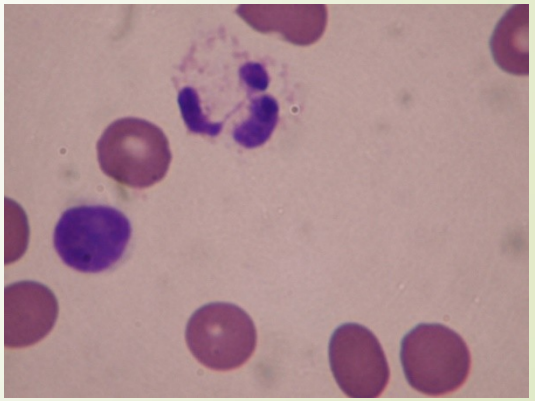


Predominant Eosinophils



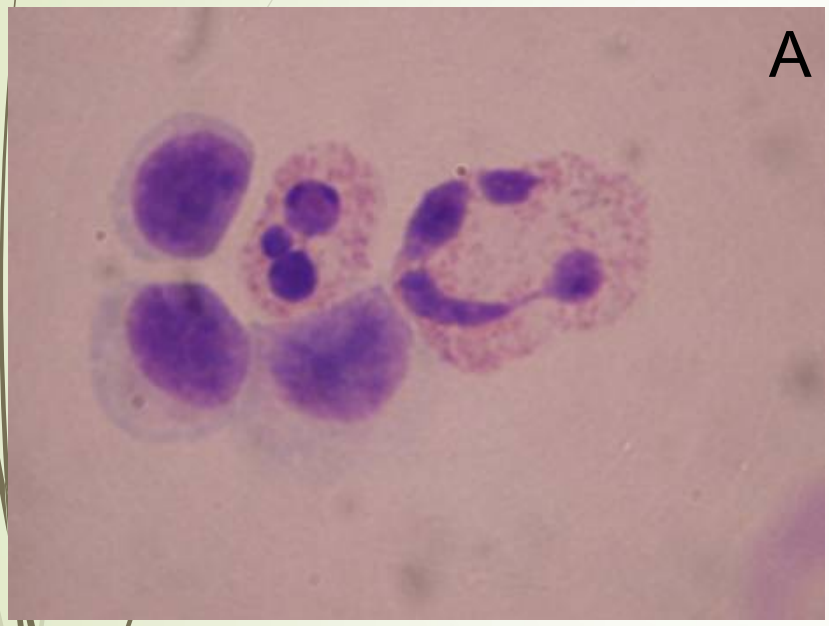
I. Parasitic and fungal infections.
II. Reaction to foreign material.

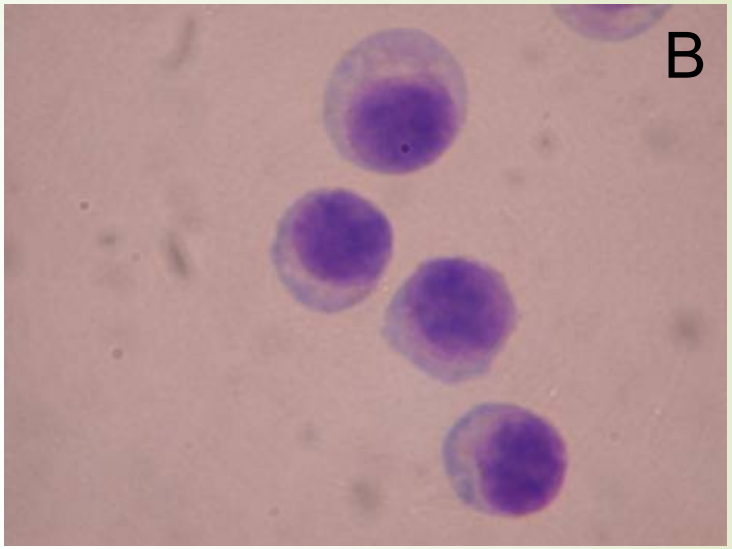
Mixed cell pattern

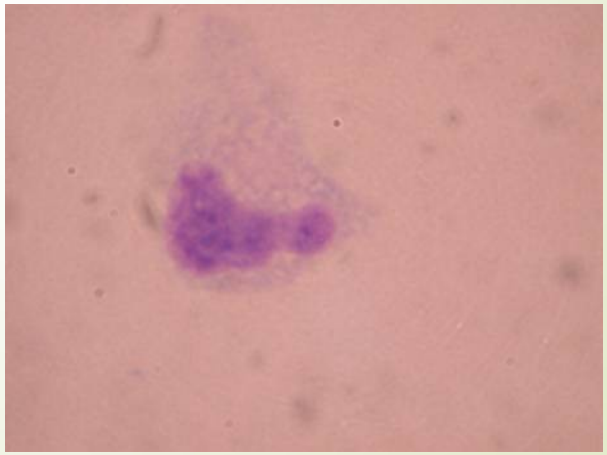


1. Tubercular meningitis.
2. Chronic bacterial meningitis.

Cells Observed in CSF





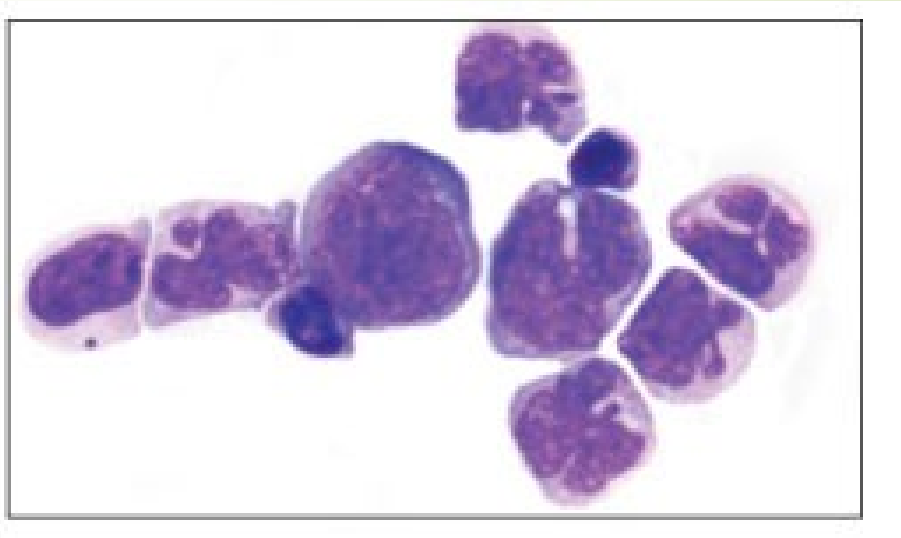


CSF cytoprep, Wright-Giemsa. 1000x

A – PMNs, Lymphocytes; B – Lymphocytes; C – Monocyte.

Features of Malignant Cells

- Multi-layered formations
- LARGE cells
- Irregular nuclear membrane
- Multi-nucleation
- Nuclear hyperchromasia
- Unevenly distributed chromatin
- Irregularly-sized/shaped nucleoli
- Prominent nucleoli
- High N:C ratio
- Bizarre vacuolization/inclusions
- Uneven staining of cytoplasm



Large cells with convoluted nuclei and moderate amounts of basophilic cytoplasm, intermixed with some small lymphocytes (cytospin preparation of fresh cerebrospinal fluid, stained with Diff-Quik, original magnification 3600). (Courtesy of Dr Andrew Schriner, department of cytopathology, New York-Presbyterian Hospital/Weill Cornell Medical Center.) URL accessed http://theaidsreader.consultantlive.com/display/article/1145619/1362837?verify=0, 2009.

Chemical Analysis of CSF

***Protein*(15-45mg/dl)**

- 80% plasma derived
  - LMW
    - Transthyretin (prealbumin)
      - Albumin
    - Transferrin
    - IgG – very small amount
- 20% intrathecal synthesis.

Glucose(45-80 mg/dl)

- Need to know plasma value
- Increased

  Hyperglycemia **2/3rd**

  Traumatic tap **OF**
- Decreased **PLASMA**

(Hypoglycorrhachia) **VALUE**

Hypoglycemia

Meningitis (bacterial, tuberculous & fungal)

Tumors(meningeal carcinomatous)

Note: CSF glucose normal in viral meningitis.

Glucose Estimation

Pipette in 3 test tubes labelled as Blank, Standard and Test.

Ingredients	Blank	Standard	Test
Glucose Working Solution	*1.5ml*	*1.5ml*	*1.5ml*
Distilled Water	*0.02ml*	-	-
Standard	-	*0.02ml*	-
Sample (CSF)	-	-	*0.02ml*
Incubate in water bath for 15mins and then add 1.5ml of distilled water to each tube.			

CSF glucose levels normalize before protein level and cell counts during recovery from meningitis, making it an useful parameter in assessing the response to the treatment.

Qualitative Test - Pandy's Test

CSF is added in concentrated solution of phenol, appearance of cloudiness indicates increased protein (globulins).

Pandy's Reagent –

30 gm phenol

500 ml distilled water.

Quantitative Test - Proteins

Turbidimetric method:

Principle : In the presence of sulphosalicylic acid and sodium sulphate, protein yields a uniform turbidity which absorbs maximum at 520 nm or green filter and is directly proportional to the concentration of proteins.

Composition:

CSF Protein Reagent - Sulphosalicylic acid-30 gms/lt.

Sodium sulphate-70 gms/lt.

Standard – Albumin fraction-100 mgs/ lt.

MANUAL METHOD:

Ingredient	Blank	Standard	Test
CSF Protein Reagent	*1.5 ml*	*1.5 ml*	*1.5 ml*
Distilled Water	*0.1 ml*	-	-
CSF Protein Standard	-	*0.1 ml*	-
Sample (CSF)	-	-	*0.1 ml*

Pipette in 3 test tubes labelled as Blank, Standard and Test.

Note :- False elevation of protein occurs if CSF is contaminated with blood, this can be corrected by deducing 1 mg/ dl of protein for every 1000 RBC’s.

Causes of Raised CSF Protein

- Lysis of contaminated blood from traumatic tap (each 1000 RBC/mm3 raise the CSF protein by 1mg/dl).
- Increased Permeability of epithelial membrane(Blood Brain Barrier) -
  - CNS Bacterial or fungal Infections(Meningitis)
  - Cerebral Hemorrhages
- Increased production by CNS tissue as in -
  - Multiple Sclerosis, Neurosyphilis (Increased production of local immunoglobulin)
  - Subacute sclerosing panencephalitis (SSPE) Guillain Barre syndrome.
- Obstruction as in cases of – Tumours or abscess.

Multiple sclerosis

1. Mononuclear cell pleocytosis.
2. Increased intrathecal production of IgG.
3. IgG index: ratio of IgG and albumin in the CSF to the ratio of IgG and albumin in the serum.
4. Measurement of oligoclonal band.
5. Paired serum samples studied to exclude peripheral i.e., non CSF production of oligoclonal bands.
6. Pleocytosis of >75 cells /microlt.with presence of neutrophils and protein >1 mg/dl excludes the diagnosis.

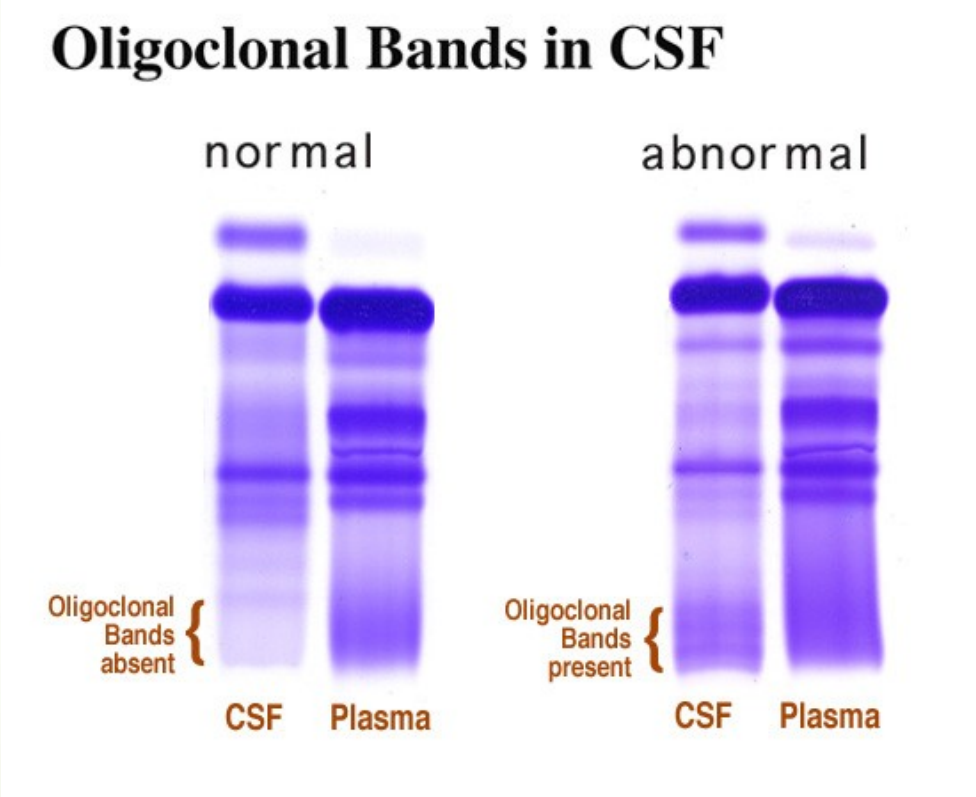
Albumin and IgG

- Albumin – neither synthesized, nor metabolized in CNS.
- ALB used to address blood-brain barrier integrity
- Evaluate CSF/serum ALB index
  - Index < 9 = normal
  - 9 – 14 minimal impairment
  - > 100 = not intact barrier

- IgG sourced from inside and outside.
- CSF IgG index = ratio IgG_{CSF}/IgG_{serum} X ALB_{serum}/ALB_{CSF}
  - Reference range 0.3 – 0.7
    - > 0.7 = CNS sourced
    - < 0.3 = compromised BBB

Electrophoresis

- Normal = 4 bands
  - ALB
  - Transthyretin
  - Transferrin
    - b1
    - t = unique to CSF
  - Oligoclonal bands ~ multiple sclerosis
  - Myelin basic protein
  - Monitoring disease progression



http://library.med.utah.edu/kw/ms/mml/ms_oligoclonal.jpg

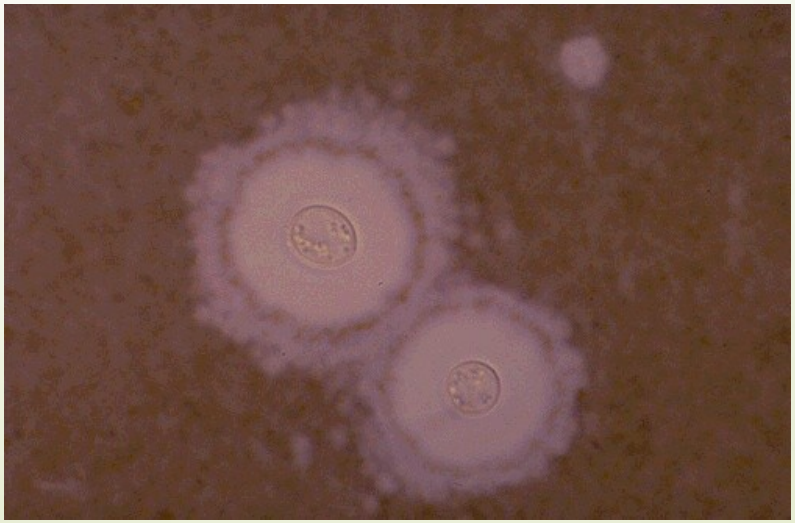
Guillan Barre Syndrome

1. Albumino cytological dissociation.
2. A sustained CSF pleocytosis suggestive of an alternative diagnosis i.e., viral myelitis.

Microbiological examintion:

- Direct wet mount- candida, crytococcus infection, amoebic encephalitis.

Indian ink preparation- *a drop of CSF and Indian ink is placed on a slide and covered with cover slip and observe it under 40x – crytococcus appears as budding yeast surrounded by unstained capsule.*



Gram's Stain-

Smear is made from the sediment and is air dried, stain it with gram's stain and observe it under oil immersion.

Streptococcus pneumococci – Diplococci, gram positive, lying end to end.

Neisseria meningitides - Diplococci gram negative, lying side by side.

Haemophilus influenza - coccobacilli gram negative

- Ziehl- Neelsen stain:

AFB smears are negative in 70% of cases however florescent auramine stain have better sensitivity.

- Serologic test for Neurosyphilis :

Combination of VDRL test in CSF and FTA-ABS test in serum is diagnostic.

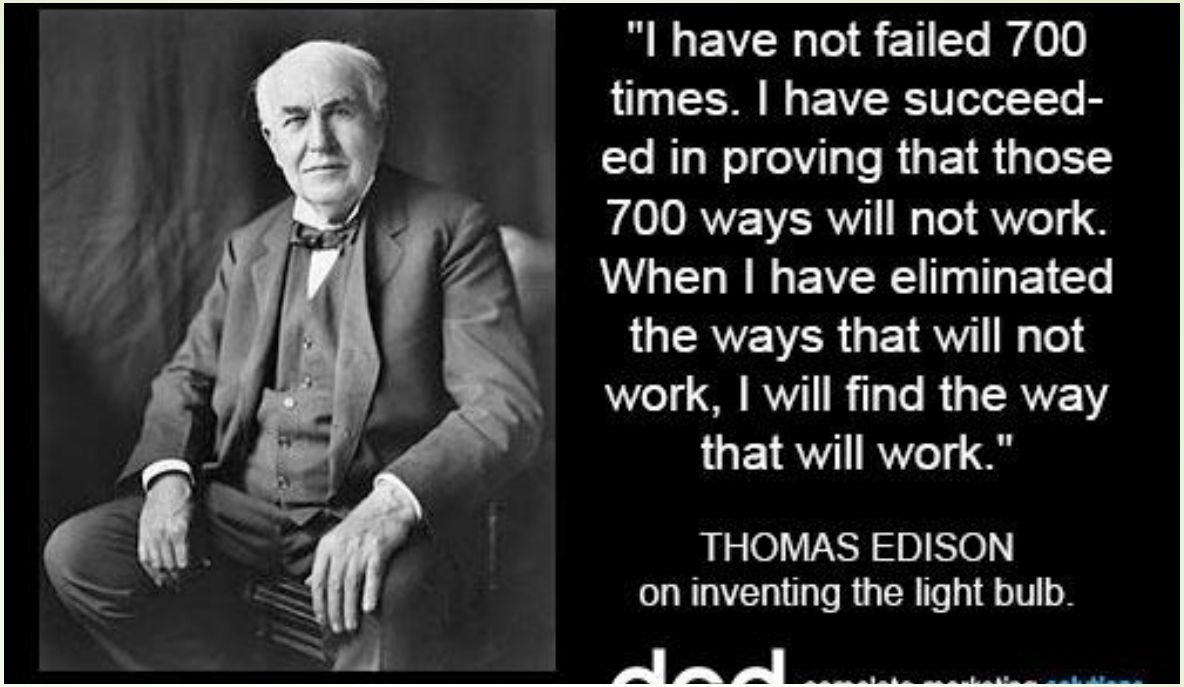
CSF CULTURE- Gold standard

1. Appearance of bacteria on gram stained smears.
2. Increased proteins or cell count.
3. Inoculated on chocolate agar.
4. Sensitivity -90%.

PCR :- Viral infection of CNS.

Requires only a drop of CSF.

Featur-es	Normal	Bacteri-al Mening-itis	Viral Mening-itis	TB Mening-itis	Fungal Mening-itis	Brain Tumour	SAH
Appear-ance	*Clear, Colurless No clot*	*Cloudy, Large clot* ★	*Clear, No clot*	*Slightly cloudy*	*Clear, No clot*	*Clear, No clot*	*Xantho-chromic*
WBC (cells/cumm)	*0 – 5 Lympho*	*>500 PMN* ★	*10 – 200 lympho +*	*200 – 500 lympho +*	*0 – 5 lympho +*	*0 – 5*	*0 – 5 lympho +*
Total Protein	*15 – 45 mg/dl*	+ + +	+ +	+ + +	*Normal* ★	+	+ + +
Globulin (mg/dl)	*low*	+	-	+	*Normal*	-	+
Glucose	*45 – 80 mg/dl*	- - -	*Normal* ★	- - -	- - -	+	-


"I have not failed 700 times. I have succeed-ed in proving that those 700 ways will not work. When I have eliminated the ways that will not work, I will find the way that will work."
THOMAS EDISON
on inventing the light bulb.


Thank You