



Cerebrospinal Fluid

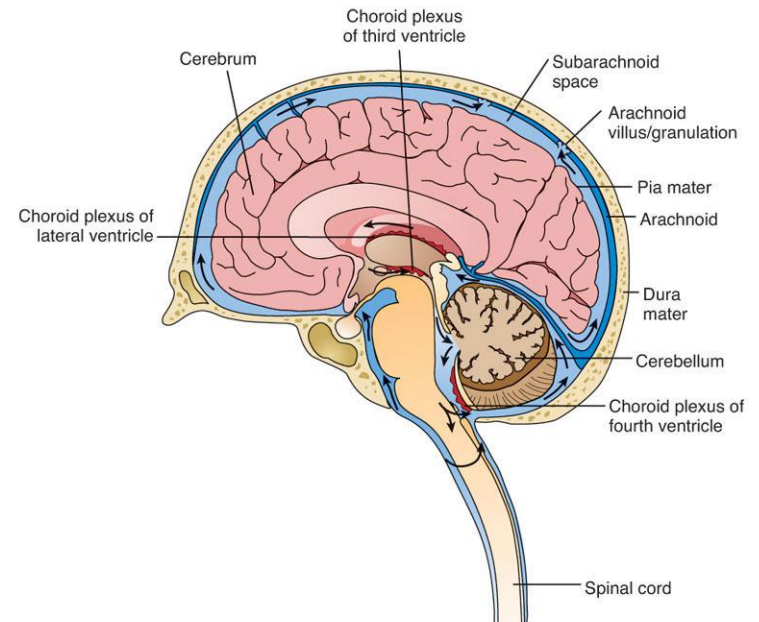
Cerebrospinal Fluid (CSF)



- Composition and formation
 - CSF is the 3rd major fluid of the body
 - Adult volume 120-180 mL
 - Neonate volume 10-60 mL

Cerebrospinal Fluid (CSF)

- Produced at the Choroid plexus of the 4 ventricles by modified Ependymal cells
 - At rate @20 ml / hr (adults)
 - Med training says @ 150 ml/day is produced
- CSF flows through the Subarachnoid space
 - Where a volume of 90 - 150 ml is maintained (adults)
- Reabsorbed at the Arachnoid villus / granulation
 - to be eventually reabsorbed into the blood



Cerebrospinal Fluid (CSF)



- Blood Brain Barrier
 - Occurs due to tight fitting endothelial cells that prevent filtration of larger molecules.
 - Controls / restricts / filters blood components
 - Restricts entry of large molecules, cells, etc.
 - Therefore CSF composition is unlike blood's
 - ** CSF is NOT an ultrafiltrate

Cerebrospinal Fluid (CSF)



- Blood Brain Barrier
 - Essential to protect the brain
 - Blocks chemicals, harmful substances
 - Antibodies and medications also blocked
- Tests for those substances normally blocked can indicate level of disruption by diseases: ie meningitis and multiple sclerosis.

Cerebrospinal Fluid (CSF)



- CSF functions
 - Supplies nutrients to nervous tissues
 - Removes metabolic wastes
 - Protects / cushions against trauma

Cerebrospinal Fluid (CSF)



- Four major categories of disease
 - Meningeal infections
 - Subarachnoid hemorrhage
 - CNS malignancy
 - Demyelinating disease

Cerebrospinal Fluid (CSF)



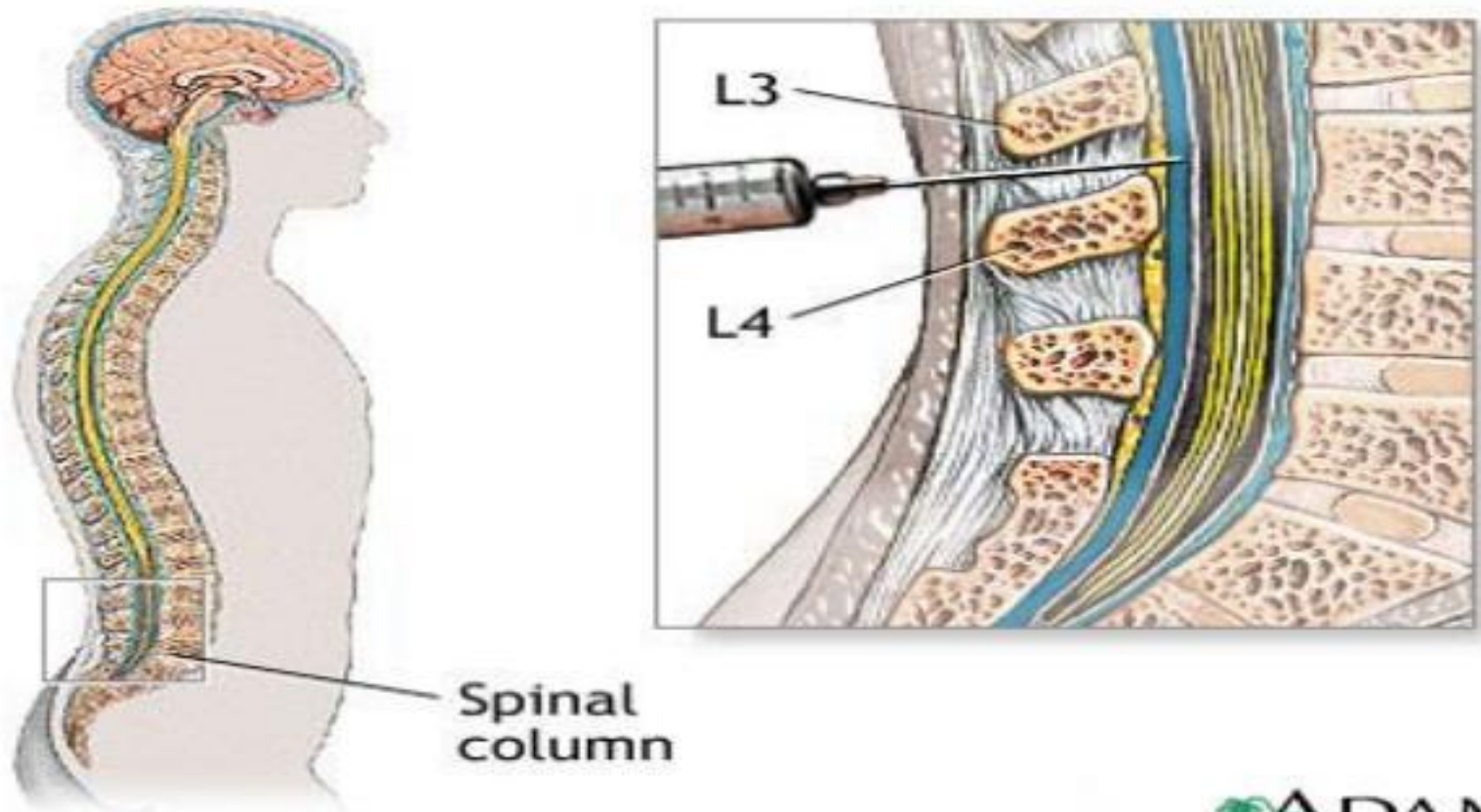
- Indications for analysis
 - To confirm diagnosis of meningitis
 - Evaluate for intracranial hemorrhage
 - Diagnose malignancies, leukemia
 - Investigate central nervous system disorders

Cerebrospinal Fluid (CSF)



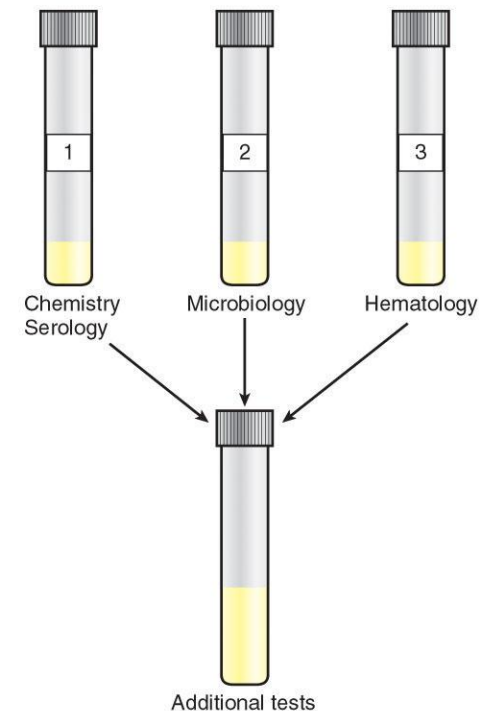
- Specimen collection and handling
 - Routinely collected via lumbar puncture between 3rd & 4th, or 4th & 5th lumbar vertebrae under sterile conditions
 - Intracranial pressure measurement taken before fluid is withdrawn.

Cerebrospinal Fluid (CSF)



Cerebrospinal Fluid (CSF)

- Specimen collection and handling
 - Tube 1 - chemistries and serology
 - Tube 2 - microbiology cultures
 - Tube 3 - hematology



Cerebrospinal Fluid (CSF)

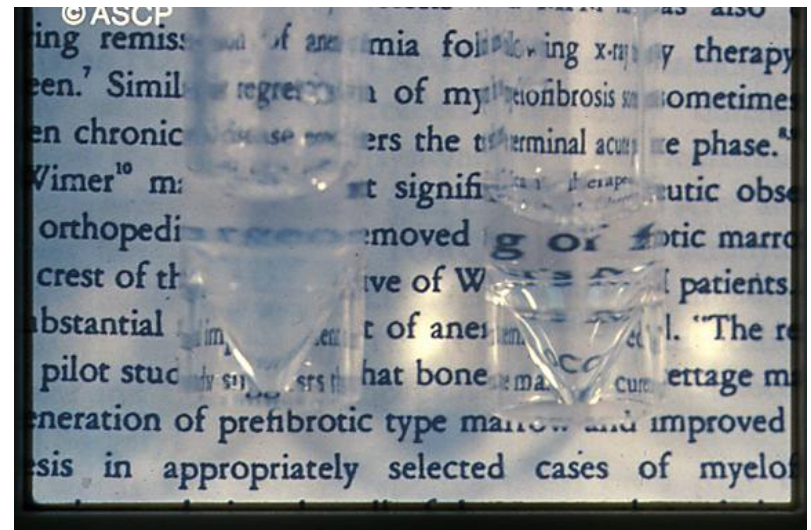


- Specimen collection and handling
 - If immediate processing not possible
 - Tube 1 (chem-sero) frozen
 - Tube 2 (micro) room temp
 - Tube 3 (hemo) refrigerated

Cerebrospinal Fluid (CSF)

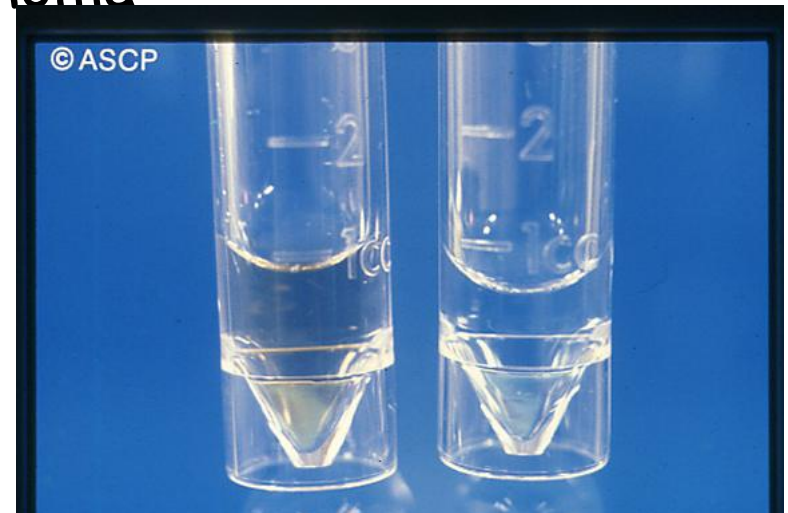
- Appearance

- Normal - Crystal clear, colorless
- Descriptive Terms - hazy, cloudy, turbid, milky, bloody, xanthochromic
- Often are quantitated - slight, moderate, marked, or grossly.
- Unclear specimens may contain increased lipids, proteins, cells or bacteria. Use precautions.
- Clots indicate traumatic tap
- Milky - increased lipids
- Oily - contaminated with x-ray media



Cerebrospinal Fluid (CSF)

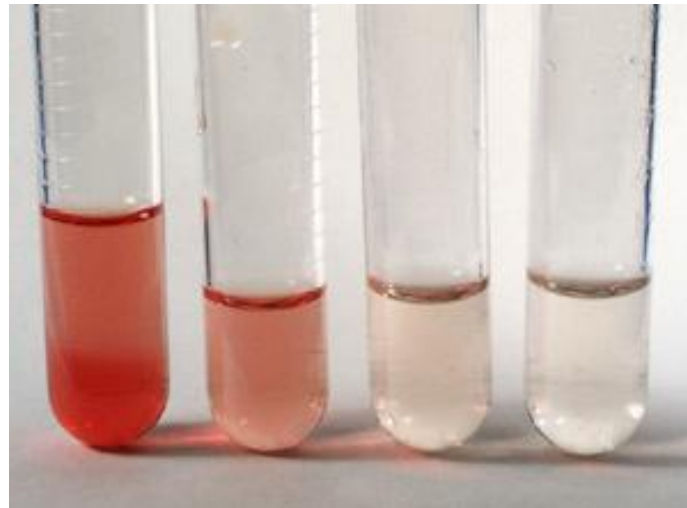
- Appearance
 - Yellowing discoloration of supernatant (may be pinkish, or orange).
 - Most commonly due to presence of 'old' blood.
 - Other causes include increased bilirubin, carotene, proteins, melanoma



Cerebrospinal Fluid (CSF)

- ## Appearance

- Clots - indicates increased fibrinogen & usually due to traumatic tap, but may indicate damage to blood-brain barrier. **(see below)**
- Pellicle formation in refrigerated specimen associated with tubercular meningitis.
 - Pellicle formation - picture at right **(pellicle in L. tube, R is normal)**
- Milky - increased lipids
- Oily - contaminated with x-ray media



Traumatic collection vs cerebral hemorrhage

- Cerebral hemorrhage
 - Even distribution of blood in the numbered tubes
 - Clot formation possible
 - Xanthochromic supernatant
 - - RBCs must have been in CSF @ 2+ hours
 - - D-dimer, fibrin degradation product from hemorrhage site
 - Microscopic presence of erythrophages, or siderophages, Hemosiderin granules



Cerebrospinal Fluid (CSF)



- Expected results
- Normally 0 RBCs/uL regardless of age
- WBCs
 - Adult - up to 5 mononuclear WBCs/uL
 - Newborn - up to 30 mononuclear WBCs/uL
 - Children (1-4) - up to 20 mononuclear /uL
 - Children (5+) - up to 10 mononuclear / uL
 - Increased numbers = Pleocytosis

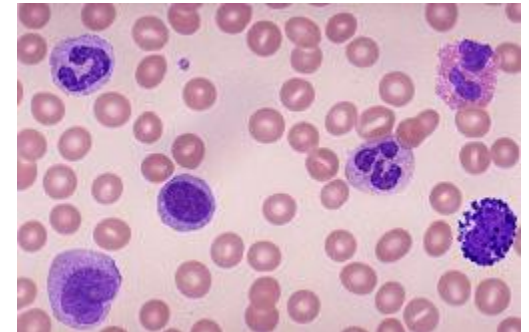
Cerebrospinal Fluid (CSF)



- WBC counts
 - 3% acetic acid can be used to lyse RBC
 - Methylene blue staining will improve visibility

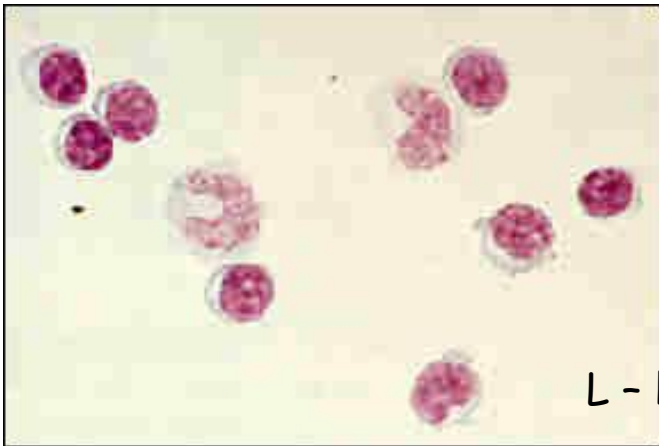
Cerebrospinal Fluid (CSF)

- Count and differentiate 100 nucleated cells.
- Any cell found in peripheral blood may be seen in CSF, other nucleated cells and malignant cells can also be found.
- Entire smear should be evaluated for
 - abnormal cells, inclusions within cells, Clusters, Presence of intracellular organisms
- Normal differential values
 - Adults: 70% lymps, 30% monos.
 - Children / newborns: monocyte
- Types of cells
 - Neutrophils - occasionally (with normal count)
 - Macrophages - increase following CVA
 - Ependymal cells, and normal lining cells can also be seen.



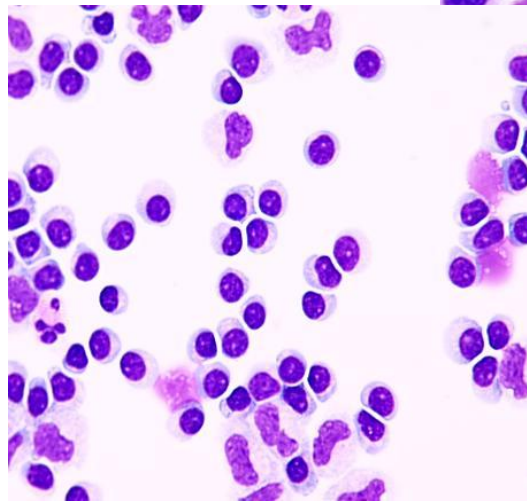
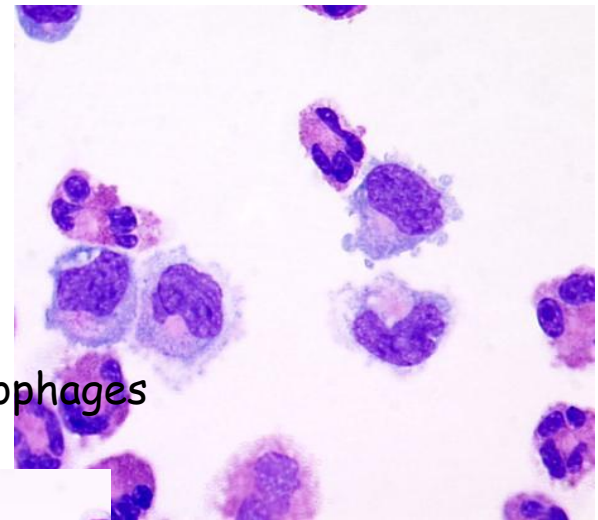
Cerebrospinal Fluid (CSF)

Lymphocytes & monocytes / macrophages



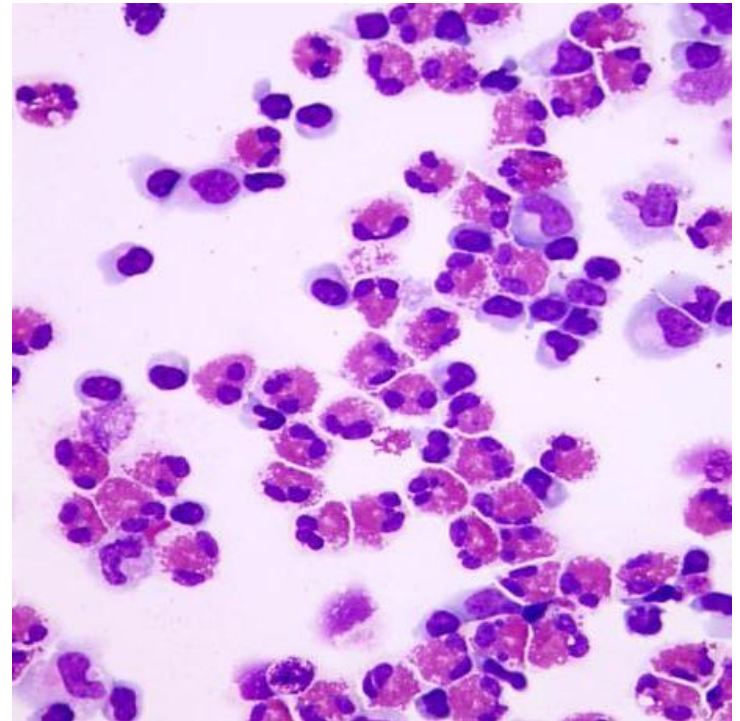
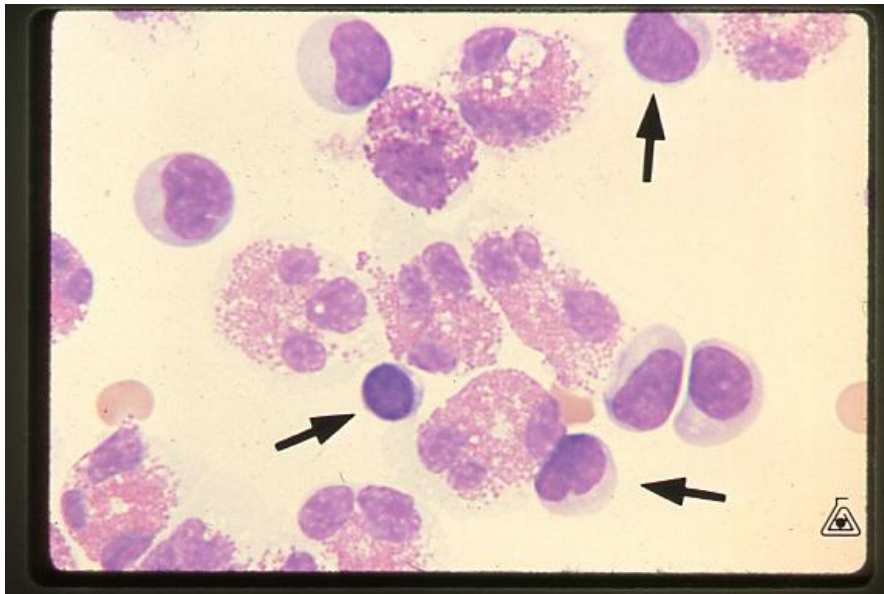
L - lymphocytes & macrophages

Mono / macro, segs and lymph



Cerebrospinal Fluid (CSF)

- Eosinophils
 - Often associated with parasitic / fungal infections, allergic reactions including reaction to shunts and other foreign objects.



Cerebrospinal Fluid (CSF) - protein

- Normal 15 - 45 mg/dL .
- Albumin fraction. If IgG - from damaged B-B, or CNS produced? Can electrophoresis to evaluate oligoclonal / malignant bands.
- Decreased levels not significant
- Increases levels
 - Damaged B-B (as in meningitis or hemorrhage)
 - Production of immunoglobulins within CNS (MS)
- Degeneration of neural tissue
- Dye-binding methods - preferred
 - Alkaline biuret
 - Coomassie brilliant blue - a blue color produced is proportional to the amount of protein present (Beers Law)

Cerebrospinal Fluid (CSF) - glucose



- Selectively transported across blood-brain barrier
- Normal values: 60-70% of blood glucose
- STAT procedure, glycolysis reduces level quickly.
- Procedure performed as for blood specimen
- Decreased levels seen in bacterial & fungal meningitis
 - Hypoglycemia
 - Brain tumors
 - Leukemias
 - Damage to CNS

Cerebrospinal Fluid (CSF)

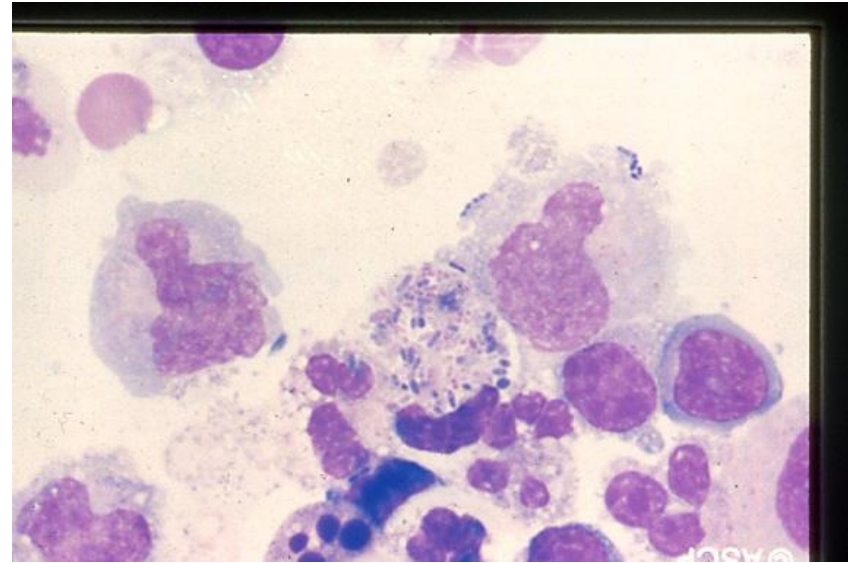
- CSF Lactate
 - Normal values = 11-22 mg/dL
 - Increase as result of hypoxia
 - Bacterial meningitis. Head injury
- CSF Glutamine
 - Normal 8-18 mg/dL
 - Increased levels associated with increases in ammonia (toxin)
- CSF Enzymes
 - Lactate dehydrogenase (LDH or LD)
 - 5 isoenzyme types; LD1&LD2 are in brain tissue
 - Creatine kinase (CPK or CK)
 - Isoenzyme CK3/ CK-BB from brain tissue
 - Following cardiac arrest, patients with CSF levels <17 mg/dL have favorable outcome.

Differential Diagnosis of Meningitis by Laboratory Results

Tests	Normal	Bacterial	Viral	Fungal	Tuberculous
Opening pressure	90 - 180 mmH ₂ O	Elevated	Usually normal	Variable	Variable
Total WBC count	0 - 5 cells/ μ L	>1 000 cells/ μ L	<1 000 cells/ μ L	<500 cells/ μ L	Variable
Differential count	Mononuclear cells, no neutrophils	>90% neutrophils	Lymphocytes	Lymphocytes/ monocytes	Lymphocytes
CSF glucose	2.8 - 4.4 mmol/L	Usually <2.2 mmol/L	Usually normal	Decreased	Decreased: may be <2.5 mmol/L
CSF protein	<40 mg/dL	Mild-marked increase	Normal-mild increase	Increased	Increased
Lactic acid	1.0 - 2.9 mmol/L	Mild-marked increase	Normal-mild increase	Mild-moderate increase	Mild-moderate increase

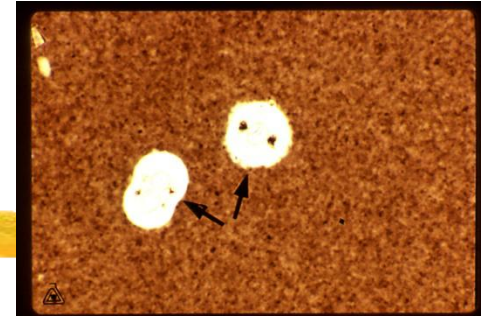
Cerebrospinal Fluid (CSF)- microbiology

- Gram stain - Extremely important for early diagnosis of bacterial meningitis
 - Even when well performed, 10% false negatives occur
 - Use of Cytospin to concentrate specimen increases sensitivity
- Cultures- Aerobic & Anaerobic. Culture blood at same time
- Organisms
 - Newborns
 - E. coli & group B Strep.
 - Children
 - Streptococcus pneumoniae
 - Hemophilus influenzae
 - Neisseria meningitidis
 - Adults -
 - Neisseria meningitidis
 - Streptococcus pneumoniae
 - Staph. aureus (if a shunt is present)
 - Immunocompromised
 - Cryptococcus neoformans,
 - Candida albicans, Coccidioides, or
 - any opportunistic organism



Mixed cells and intracellular bacteria

Cerebrospinal Fluid (CSF)



- India-ink / nigrosin preparation
 - Negative stain to view the encapsulated *Cryptococcus neoformans* (often AIDs /immunocompromised complication)
 - Instead of stain, can also use dark field microscopy for same effect.
- These direct procedures have @ 25-50% sensitivity
 - Prefer latex agglutination tests, better results