

# Urinalysis and Body Fluids CS3

## Unit 4

### Cerebrospinal Fluid

---

---

---

---

---

---

---

---

## Overview of Body Fluid Analysis

- **Laboratory responsibilities**
  - Accurate & timely results
  - Source of information
    - Normal values
    - Reliability of results, effects of medication, etc.
    - Proper specimen collection and handling
- **Laboratory exam of body fluids**
  - Physical characteristics
  - Chemical constituents
  - Morphologic elements
  - Culture for microorganisms
  - Ancillary studies

---

---

---

---

---

---

---

---

## Cerebrospinal Fluid (CSF)

- **Composition and formation**
  - CSF is the 3<sup>rd</sup> major fluid of the body
    - Adult volume 90-150 mL
    - Neonate volume 10-60 mL

---

---

---

---

---

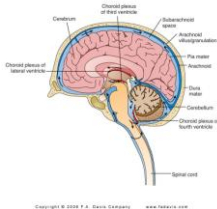
---

---

---

## 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 3<sup>rd</sup> & 4<sup>th</sup>, or 4<sup>th</sup> & 5<sup>th</sup> lumbar vertebrae under sterile conditions
  - Intracranial pressure measurement taken before fluid is withdrawn.

---

---

---

---

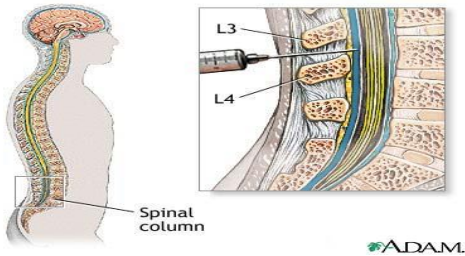
---

---

---

---

## Cerebrospinal Fluid (CSF)



---

---

---

---

---

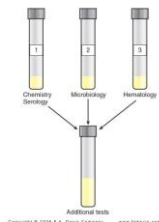
---

---

---

## Cerebrospinal Fluid (CSF)

- Specimen collection and handling
  - Tube 1 - chemistries and serology
  - Tube 2 - microbiology cultures
  - Tube 3 - hematology
- Testing considered STAT
- Specimen potentially infectious



---

---

---

---

---

---

---

---

## 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

---

---

---

---

---

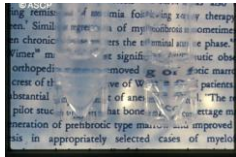
---

---

---

## 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
  - Xanthochromic - 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



---

---

---

---

---

---

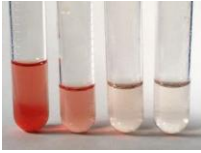
---

---

## 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



---

---

---

---

---

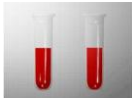
---

---

---

## 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



---

---

---

---

---

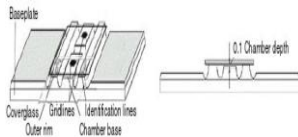
---

---

---

## Cerebrospinal Fluid (CSF) - procedures

- All specimens should be examined microscopically - hematology
- Stat priority, RBC lyse in 1 hour, WBC in 2 hrs. Refrigerate if not able to process immediately.
- Electronic counters generally unusable. Manual count
- No dilution usually required (use saline if needed)
- Standard Neubauer hemacytometer counting chamber



---

---

---

---

---

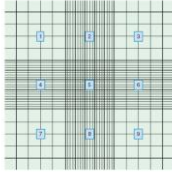
---

---

---

## Neubauer hemacytometer / counting chamber

- **Formula for calculations** - results in # cells / uL
  - Count and record cells from both sides of the chamber.
  - Average the two sides
  - Multiply by dilution factor (if no dilution is made, this number is 1)
- Divide by number of squares counted X volume of each square
  - Large squares, such as # 1-9 have volume of 0.1
  - Small squares - in center # 5 have volume of 0.004



$$\frac{\text{ave. \# cells counted} \times \text{dilution}}{\# \text{ squares counted} \times \text{volume of each square}}$$

---

---

---

---

---

---

---

---

## 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)

- Correction of WBC count for traumatic tap contamination.
- Uses ratio of WBCs to RBCs in blood and compares it to same ratio (WBC/RBC) in CSF
- If patient's peripheral cell counts are normal, can subtract 1 WBC for each 700 RBCs counted in CSF.
- Great chance for considerable error, makes this of little value.

---

---

---

---

---

---

---

---

## Cerebrospinal Fluid (CSF)

- QC
  - CSF controls
  - Check techniques
  - Check of reagents
  - Check of centrifuges
- Decontaminate all counting chambers in bleach water for @ 15 minutes. Rinse in water and cleaned again with alcohol.

---

---

---

---

---

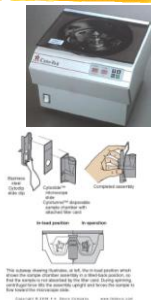
---

---

---

## Cerebrospinal Fluid (CSF)

- CSF Slide Differential
  - Wrights stained smear of concentrated sediment.
  - Cytocentrifuge - places cells on filter/membrane. Increases number of cells to evaluate, however, risk of cell distortion from the centrifugation process.
    - Use of albumin reduces cell distortion



---

---

---

---

---

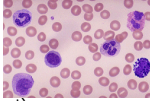
---

---

---

## 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.



---

---

---

---

---

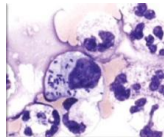
---

---

---

## Cerebrospinal Fluid (CSF)

- Entire smear should be evaluated for
  - abnormal cells
  - inclusions within cells
  - Clusters
  - Presence of intracellular organisms



---

---

---

---

---

---

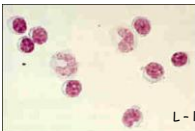
---

---

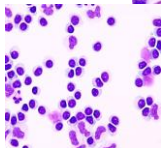
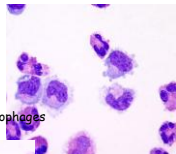
## Cerebrospinal Fluid (CSF)

Lymphocytes & monocytes / macrophages

Mono / macro, segs and lymph



L - lymphocytes & macrophages



---

---

---

---

---

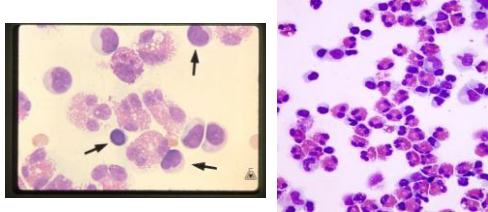
---

---

---

## Cerebrospinal Fluid (CSF)

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



---

---

---

---

---

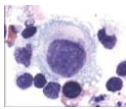
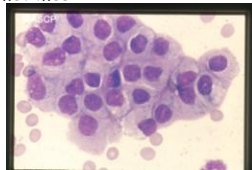
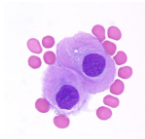
---

---

---

## Cerebrospinal Fluid (CSF)

- Ependymal cells
  - Normal cell, unique to CSF
  - Line the ventricles, produce CSF fluid
  - Large cell with distinct round/oval nucleus, sometimes found in sheets



---

---

---

---

---

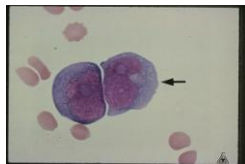
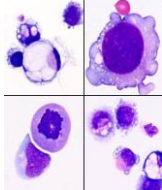
---

---

---

## Cerebrospinal Fluid (CSF)

- Suspicious / unclassified or malignant cells are reported as "other" or "unclassified" AND are sent to pathology. (as seen below)
- Cytology - send unstained slide to cytology / pathology
- 1986 CAP CM10 CSF - blasts (appearance similar to peripheral blood, always consult with hematology specialist / pathologist) (see below right)



---

---

---

---

---

---

---

---



## 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 -MS Panel

- Multiple Sclerosis
  - Diagnosis is difficult - no one specific test
  - CSF Protein electrophoresis
    - Looking for oligoclonal bands
  - Myelin Basic Protein
    - Abnormal protein that indicates demyelination of neuron axons
    - Measurement used to monitor course of disease and effectiveness of treatment
  - IgG levels (both serum and CSF)
    - $IgG \text{ index} = \frac{CSF \text{ IgG (mg/dl)} / \text{serum IgG (g/dl)}}{CSF \text{ albumin (mg/dl)} / \text{serum albumin (g/dl)}}$  NV < 0.77
  - Albumin (both serum and CSF)
  - IgG synthesis rate.



---

---

---

---

---

---

---

---

## 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

Bacterial	Viral	Tubercular	Fungal
Increased WBC count	Increased WBC count	Increased WBC count	Increased WBC count
Neutrophils	Lymphs	Lymps & Monos	Lymphs & Monos
Marked ↑ protein	Mod. ↑ protein	Mod-Marked ↑ protein	Mod-Marked ↑ protein
Marked ↓ glucose	↔ normal glucose	↓ glucose	Normal to ↓ glucose
Lactate > 35 mg/dL	Lactate normal	Lactate > 25 mg/dL	Lactate > 25 mg/dL
+ gram stains		Pellicle formation	+ India ink with <i>Cryptococcus neoformans</i>
+ bacterial antigen tests			+ immunological test for <i>C. neo.</i>

---

---

---

---

---

---

---

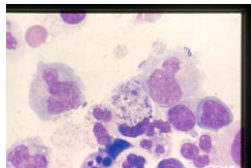
---

---

---

## 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

---

---

---

---

---

---

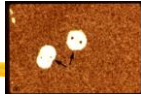
---

---

---

---

## 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

---

---

---

---

---

---

---

---

## Cerebrospinal Fluid (CSF)

- Serology
  - VDRL (Venereal Disease Research Laboratory)
    - For detection of neurosyphilis
    - On CSF test low sensitivity, but great specificity
    - FTA-Abs also used on CSF, more sensitive, but must prevent blood contamination.

---

---

---

---

---

---

---

---

## Differential Diagnosis of Meningitis by Laboratory Results

Bacterial	Viral	Tubercular	Fungal
Increased WBC count	Increased WBC count	Increased WBC count	Increased WBC count
Neutrophils	Lymphs	Lymphs & Monos	Lymphs & Monos
Marked ↑ protein	Mod. ↑ protein	Mod-Marked ↑ protein	Mod-Marked ↑ protein
Marked ↓ glucose	↔ normal glucose	↓ glucose	Normal to ↓ glucose
Lactate > 35 mg/dL	Lactate normal	Lactate > 25 mg/dL	Lactate > 25 mg/dL
+ gram stains		Pellicle formation	+ India ink with <i>Cryptococcus neoformans</i>
+ bacterial antigen tests			+ immunological test for <i>C. neo.</i>

---

---

---

---

---

---

---

---

## Cerebrospinal Fluid (CSF)

- CSF Quality Control
  - Commercial quality control samples available
    - END of CSF

---

---

---

---

---

---

---

---