

# ***Body Fluids***

## I. OVERVIEW OF BODY FLUID ANALYSIS

- A. In addition to accurate and timely test results, the laboratory must be prepared to inform the physician or other medical staff on normal values, reliability of test results including medications or other substances that could interfere and advise on proper specimen collection.
- B. Lab exam of body fluids
1. Physical characteristics
  2. Chemical constituents
  3. Morphologic elements
  4. Culture for microorganisms
  5. Ancillary studies

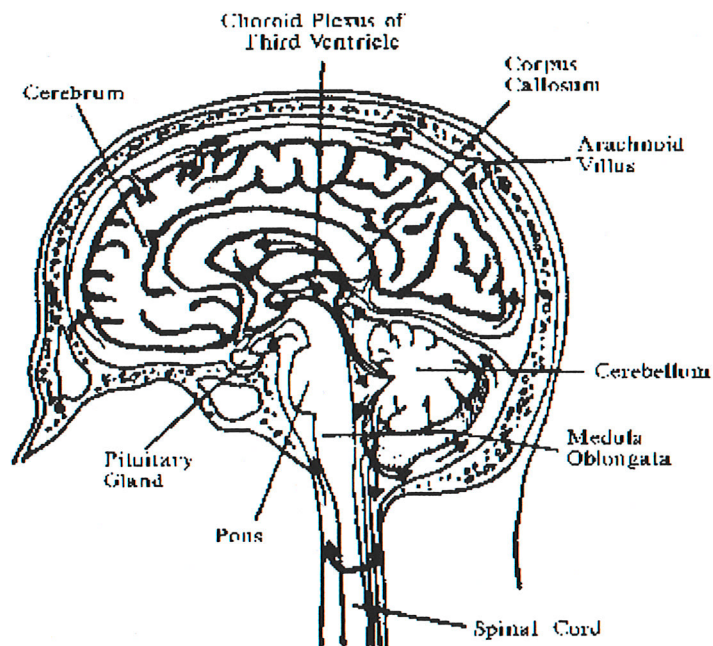
## II. CEREBROSPINAL FLUID (CSF)

### A. Composition and formation

1. CSF is the third major fluid of the body
  - a. Adult total volume 140-170 ml
  - b. Neonate total volume 10-60 ml
2. Subarachnoid space - area that lies between the arachnoid membrane and pia mater. Cerebrospinal fluid formed by the choroid plexus cells and ependymal cells occupies this space.

Blood Brain Barrier - restricts entry of macromolecules such as blood cells, large proteins, lipids. Therefore, the composition of CSF does **not** resemble plasma.

3. Absorbed by the arachnoid villus



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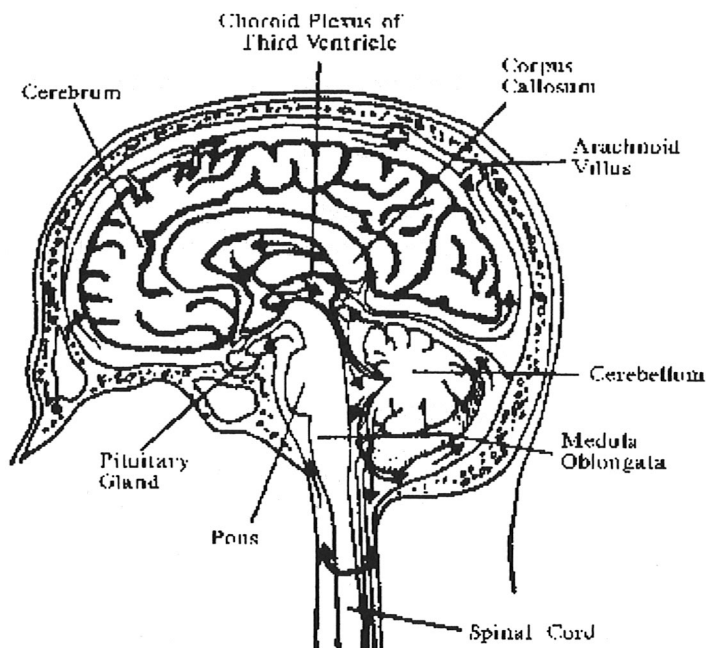
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**B. Functions**

1. To supply nutrients to the nervous tissue
2. To remove metabolic wastes
3. Serves as a mechanical barrier to cushion the brain and spinal cord against trauma

**C. Indications**

CSF analysis is performed to diagnose meningitis, intracranial hemorrhage (CVA), leukemias, malignancies, and central nervous system disorders.

**D. Specimen Collection**

1. Routinely via lumbar puncture under sterile conditions
2. Intracranial pressure measured
3. Three sterile tubes collected
  - a. Tube 1 — Chemistry and serology
  - b. Tube 2 — Microbiology
  - c. Tube 3 — Hematology
4. All CSF should be treated with extreme caution as they can be highly infectious. They should always be considered STAT.

**E. CSF Physical Characteristics /Appearance, and Gross Examination**

1. Normally crystal clear and colorless
2. Descriptive terms used: clear, hazy, cloudy, turbid, bloody, xanthochromic. These terms should also be quantitated as slight, moderate, marked, or grossly.
3. Other terms
  - a. Xanthochromic
    - 1) xanthochromia — term used only for CSF to describe a yellowing discoloration of the supernatant

## 2) Causes

- a) Usually an indication of the presence of old blood. RBCs have been present in the CSF for an extended period of time, have broken down, released their hemoglobin, and the Hgb has been converted to bilirubin
- b) Elevated serum bilirubin
- c) Carotene pigment, merthiolate contamination and increased proteins

## b. Clotted

## 1) Clot formation is abnormal and indicates increased fibrinogen due to

- a) traumatic tap
- b) diseases causing damage to the blood brain barrier resulting in increased protein (meningitis, Froin's syndrome, and blockage of CSF circulation)

## 2) Clot formation is not seen as a result of intracranial hemorrhage, as not enough fibrinogen present to clot

- c. Pellicle formation — a web-like pellicle formation in a refrigerated specimen is associated with tubercular meningitis
- d. Milky — due to increased lipids
- e. oily — due to x-ray material

**F. Traumatic tap vs. cerebral/intracranial hemorrhage** - It is very important for the doctor to differentiate for proper diagnosis and treatment.

## 1. Uneven distribution of blood in specimen tubes

- a. In cerebral/intracranial hemorrhage blood is evenly distributed in all tubes
- b. A traumatic tap will result in the heaviest concentration of blood being seen in the first tube, with diminishing amounts in subsequent tubes

## 2. Clot formation

- a. Cerebral hemorrhage will not clot as fibrinogen is not present in high enough concentration.
- b. Traumatic tap may clot due to contamination of peripheral blood which contains fibrinogen

## Body Fluids

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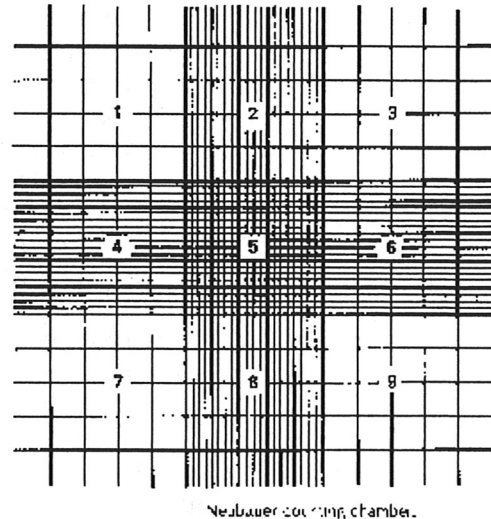
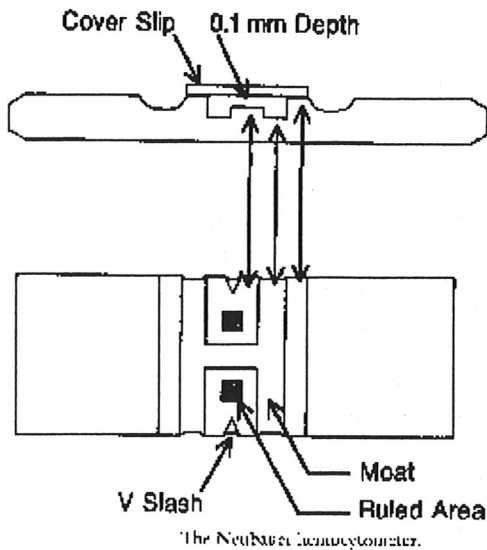
3. Xanthochromic (supernatant)
  - a. Cerebral hemorrhage will be xanthochromic due to RBC degradation
  - b. Traumatic tap will not be xanthochromic. The RBCs are too fresh and intact. They haven't had time to breakdown yet.
  
4. Inclusions in macrophages on the differential slide
  - a. Cerebral hemorrhage will show inclusions representing RBC degradation. For example: erythrophages, siderophages, hematoidin bilirubin crystals
  - b. Traumatic tap will not show RBC degradation signs

### G. Laboratory Procedures (Spinal fluids are always considered STAT!)

1. Hematology
  - a. Color/appearance - normally colorless and clear
  - b. Cell counts – both WBC and RBC are always performed
    - 1) WBC Normals
      - a) Adult = up to 5 mononuclear WBC cells / $\mu$ L
      - b) Newborn = up to 30 mononuclear WBC cells / $\mu$ L
      - c) Children (1 - 4 yrs) = up to 20 mononuclear WBC cells / $\mu$ L  
Children (5 yrs - puberty) = up to 10 mononuclear WBC cells

**0 RBC/ $\mu$ L** should be seen in normal CSF specimens, regardless of patient's age.

- 2) Methodology
  - a) Cannot use electronic counters
  
  - b) Usually no dilution required. If needed, may use saline or Unopettes specific for WBC and RBC counts.
  
  - c) Must perform a manual WBC and RBC using a standard Neubauer hemacytometer. The number of squares counted depends on the amount of cells present in the fluid. You may or may not need to use different squares for the WBC and RBC count.



- d) Basic formula for calculation of manual cell counts

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

c. CSF slide differential

- 1) Wrights stained smear made from a concentration of the sediment
  - a) Cytocentrifuge technique
  - b) Centrifuge an aliquot of the specimen and make a smear from the sediment.
  - c) If count is high enough, do regular push smear
- 2) Count and differentiate 100 WBCs. Any cell seen in the peripheral blood may be seen in body fluids. Other cells unique to each body cavity may be seen, as well as malignant cells.

If the WBC count is very low, you may not be able to find a total of 100 WBCs on the slide to do your differential. In this case, count as many as you can, report the results out in per cent (%) and make a note of how many WBCs were counted for the differential

## Body Fluids

- 3) After performing the differential, the entire smear must be scanned for abnormalities.
  - abnormal cells
  - inclusions
  - clusters, or clumps of cells
  - *intracellular* bacteria, yeast, or parasites (Do not try to classify or identify the kind of bacteria or yeast seen.) Extracellular organisms cannot be reported from a hematological slide, since testing not performed under sterile conditions.
- 4) The predominant cell seen on the *normal* differential varies with patient's age. Adults have @ 70% lymphocytes and 30% monocytes.; children and neonates have a predominance of monocytes.
- 5) Neutrophils rarely seen unless there is contamination by traumatic tap.
- 6) Few macrophages may be seen in normal CSF, and are increased following subarachnoid hemorrhage.
- 7) Both normal and abnormal CSFs may contain cells from the lining of the ventricles, choroid plexus, and ependymal cells.

Cells seen in CSF and their major significance are as follows:

| Type of Cell                 | Major Clinical Significance                    | Additional Information  |
|------------------------------|--|---|
| 1. Lymphocyte                | * normal cell in low numbers. Viral meningitis | May appear as plasma cells.   |
| 2. Neutrophil                | Bacterial meningitis                           | May also contain visible bacteria or yeast.   |
| 3. Eosinophil                | Parasitic meningitis<br>Allergic reactions     | Allergic reaction usually due to a plastic shunt inserted into the patient's cranial cavity.  |
| 4. Basophil                  | rare   | A few may be seen along with eosinophil conditions and CML.   |
| 5. Monocyte                  | * a normal cell in low numbers. non-specific   | Seen in many conditions where CNS damage has occurred.  |
| 6. Macrophage                | depends on number & type of inclusion          | Identify the inclusion to determine significance.   |
| 7. Ependymal (unique to CSF) | normal lining cells of CNS                     | Large cell with distinct round nucleus, large amount of cytoplasm with distinct cell walls. May be seen in sheets.                            |
| 8. Blasts                    | Acute leukemias                                | Appearance similar to leukemic blasts on peripheral smear.  |
| 9. Malignant cells           | carcinomas                                     | Often seen in balled up clusters, deeply basophilic with fusing of cell borders and nuclei. May be heavily vacuolated and irregular staining. |

- 8) Any suspicious, unclassified, or malignant cell must be referred to a pathologist for identification and/or confirmation. On our report form we will call them "other" or "unclassified" cells.
- 9) Cellular inclusions (refer to Plates 48, 49, & 50)



## 2. Cytology

An unstained slide of the CSF sediment may also be prepared and sent to the cytology department. Special stains will be used to identify various cells (especially malignant cells and inclusions).

## 3. Chemistry - There are few clinically significant CSF chemistry tests since the blood:brain barrier causes selective filtration from the plasma. Abnormal values result from alterations in the permeability of the blood:brain barrier.

a. CSF protein

- 1) Normal values 15-45 mg/dL (plasma protein reported in g/dL)
- 2) Majority of CSF protein is of the albumin fraction
- 3) Significance of elevated protein (a decrease is not significant)
  - a) Damage to blood/brain barrier as seen in meningitis and hemorrhaging conditions
  - b) Production of immunoglobulins within the CNS as seen in multiple sclerosis
  - c) Degeneration of neural tissue
- 4) Determination CSF protein
  - a) Turbidity methods
    - (1) Sulfosalicylic acid
    - (2) Trichloroacetic acid
  - b) Dye-binding methods use a smaller sample size and are influenced less by external factors
    - (1) Coomassie brilliant blue
    - (2) Alkaline biuret
- 5) Albumin & IgG
  - a) CSF/serum albumin ratio used to evaluate the blood-brain barrier ratio  
$$\text{CSF / serum albumin ratio} = \frac{\text{CSF albumin gm/dL}}{\text{serum albumin gm/dL}} \quad \text{NV} = 1/230$$
  - index  
$$\text{CSF / serum albumin index} = \frac{\text{CSF albumin mg/dL}}{\text{serum albumin gm/dL}} \quad \text{NV} = 4-8$$
  - b) CSF/serum IgG ratio and index calculations

- 6) CSF protein electrophoresis
  
- b. CSF glucose - perform this test STAT since cells and organisms will utilize glucose causing levels to decrease fairly rapidly.
  - 1) Glucose is selectively transported across the blood/brain barrier
  - 2) Normally the CSF glucose will be 60-70% of the patient's plasma glucose value. \* Therefore, a plasma glucose level should be performed concurrently.
  - 3) CSF glucose procedure is the same as for blood glucose
  - 4) Decreased CSF glucose concentration are significant and are seen in
    - a) Bacterial or fungal meningitis
    - b) Hypoglycemia
    - c) Brain tumors
    - d) Leukemias
  - 5) An increase CSF glucose is always a result of increase plasma levels.
  
- c. Other Chemistry tests
  - 1) Multiple sclerosis panel / Basic Myelin Protein
  - 2) CSF lactate
  - 3) CSF glutamine
  - 4) CSF lactate dehydrogenase (LDH or LD)
  
4. Microbiology – CSF
  - a. Gram stain
  
  - b. Culture
    - 1) Aerobic and Anaerobic

- 2) \*Blood cultures are usually done concurrently
- 3) May be bacterial, viral, fungal, or parasitic
- 4) Certain types of CSF infections tend to be seen in certain populations of patients.

| Patient Population                   | Organism   |
|--------------------------------------|--|
| Neonates                             | E Coli or Group B Strep  |
| Children > 6 mo.<br>and young adults | Streptococcus pneumoniae,<br>Haemophilus influenza or Neisseria meningitidis                 |
| Adults                               | Neisseria meningitidis or<br>Streptococcus pneumoniae<br>Staph aureus if a shunt is present  |
| Immunocompromised patient            | Cryptococcus neoformans,<br>Candida albicans, Coccidioides, or<br>any opportunistic organism |

- c. Acid-fast or fluorescent antibody stains – TB, Mycobacterium
- d. India-ink preparation (nigrosin) – *Cryptococcus neoformans*

5. Serology

- a. VDRL – tertiary syphilis (neuro syphilis), Treponema pallidum
- b. Latex agglutination
- c. ELISA
- d. CIE
- e. Lymus lysate

H. **Clinical Correlations and QC**

III. **SEROUS FLUIDS**

A. **Composition and formation**

Serous fluid is the small amount of fluid that lies between the membranes lining the body cavities (parietal) and those covering the organs within the cavities (visceral).

1. It **is** considered an “ultrafiltrate” of the plasma and **closely** resembles it.

## Body Fluids

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2. Production and reabsorption are normally at a constant rate. They are influenced by
  - a. Changes in osmotic and hydrostatic pressure in the blood
  - b. Concentration of chemical constituents in the plasma
  - c. Permeability of blood vessels and the membranes
3. There are three types of serous fluids
  - a. Peritoneal fluid (ascitic fluid) - from the abdominal cavity - paracentesis
  - b. Pleural fluid (thoracic fluid) - lung - thoracentesis
  - c. Pericardial fluid - heart - pericardiocentesis

B. **Indications** – infections, hemorrhages, malignancies, other disorders.

C. **Collection**

1. Needle aspiration – paracentesis, thoracentesis, pericardiocentesis  
3 sterile tubes, often in EDTA to prevent clotting.
2. Lavage – peritoneal

D. **Specimen**

E. **Transudates vs. exudates** – A build up of serous fluid is called an **effusion**. Classifying a cause can be aided by determining if it is a transudate or exudate. Diagnosis and treatment depend on this.

1. Transudate
  - a. Due to a systemic disorder, ex. congestive heart failure
  - b. Disruption in the balanced regulation of fluid filtration (formation) and its reabsorption
  - c. Thought of as a mechanical process
2. Exudate
  - a. Produced by conditions that **directly** involve the membranes of the particular cavity, ex. infections, inflammation, and malignancies

- b. Thought of as an inflammatory process

|                                   | Transudate | Exudate   |
|-----------------------------------|------------|-----------|
| Appearance                        | Clear      | Cloudy    |
| Specific Gravity                  | <1.015     | >1.015    |
| Total Protein                     | <3.0 g/dl  | >3.0 g/dl |
| Fluid Protein:Serum Protein Ratio | <0.5       | >0.5      |
| Lactic Dehydrogenase (LDH)        | <200 IU    | >200 IU   |
| Fluid LDH:Serum LD Ratio          | <0.6       | >0.6      |
| WBC Count                         | <1000/ul   | >1000/ul  |
| RBC Count                         | 0-low      | high      |
| Spontaneous Clotting              | No         | Possible  |

- F. Laboratory procedures performed on all serous fluids:

1. Hematology / Gross Examination

- a. Color/clarity – Normally yellow and clear.  
Otherwise use the same descriptives as CSF (*except for the term Xanthochromic which is only used for CSF*)
- b. Cell count (performed same as for CSF)
- c. Differential (performed same as for CSF) May see any peripheral blood cell and/or organisms plus:
  - 1) Mesothelial cells
    - a) Unique to serous fluids, they form the lining of peritoneal, pleural, and pericardial cavities
    - b) Normally are large round cell, abundant cytoplasm, large very round nucleus. Sometimes described as having a "fried egg" appearance.
    - c) "Reactive" mesos undergo morphological changes during inflammation. Cytoplasm becomes deeply basophilic, may be vacuolated, but nucleus is still distinct and round. Uniform appearance from one meso to the next. Uniform staining characteristics. A cluster of reactive mesos may resemble malignant cell clusters, but the mesos display "cell windows."
  - 2) Malignant cells
    - a) A frequent concern in any serous fluid due to possibility of cancer of any organ and/or metastasis of CA from one location to another.

## Body Fluids

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- b) Cells have irregular size, shape, and staining characteristics of nucleus and cytoplasm. Usually deeply basophilic, molded or balled up clusters of cells with little distinction from one cell to the next. May be vacuolated.
- 3) LE cells
  - a) Seen in patients with Systemic Lupus Erythmatosis (SLE) a systemic disease in which an autoantibody attacks the patients organs and body systems
  - b) LE cell is a neutrophil that has engulfed a homogeneous mass of purple staining nuclear material
- 2. Chemistry
  - a. Total protein, and ratio to serum protein
  - b. LDH, and ratio to serum LDH
  - c. Glucose
  - d. Amylase & Lipase evaluation for pancreatic disorders
  - e. Bilirubin and alkaline phosphatase – occasionally on peritoneal fluid
  - f. pH and ammonia
- 3. Microbiology
  - a. Gram stain and acid fast
  - b. Culture (aerobic and anaerobic)
- 4. Serology – rarely done
- 5. Cytology – fluid and/or biopsy
- 6. QC - No prepared/commercial controls available for serous fluids, usually run chemistry serum controls.

**IV. SYNOVIAL FLUID****A. Composition and formation**

1. Secreted by the cells of the synovial membrane
2. Very viscous fluid containing
  - a. Hyaluronic acid
  - b. Mucopolysaccharides
  - c. Small amount of plasma protein

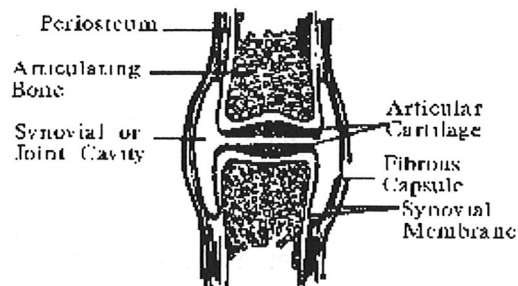


Diagram of a Synovial Joint.

**B. Functions**

1. Supplies nutrients to the cartilage
2. Acts as a lubricant

**C. Indications** – infections, hemorrhage, degenerative disorders (arthritis), inflammatory disease (SLE)**D. Collection**

1. Arthrocentesis — needle aspiration of joint fluid
2. Volume — normal knee = approximately 3.5 mL
3. Tubes
  - a. Heparin — chemical and immunological testing
  - b. Plain sterile tube — microbiological culturing and crystal examination
  - c. EDTA — cell counts and differential

**E. Laboratory Procedures**






1. Hematology / Gross Examination
  - a. Color/clarity
    - 1) Normal = yellow and clear (tho viscous)
    - 2) Abnormal = same descriptives as for other serous fluids

- 3) Bloody
  - a) Hemarthrosis
  - b) Traumatic Tap - differentiate the same as for CSF
  
- b. Viscosity - this test is unique to synovial fluid. It is essential for lubrication of joints, is the result of polymerization of the hyaluronic acid
  - 1) Screening test - release a drop from a pipette  
Normal = 2 in. or more stretch  
Decrease viscosity is abnormal
  
  - 2) Rope's test for mucin clot
    - a) Evaluates the degree of hyaluronate polymerization
    - b) Mix fluid with 2-5% acetic acid and observe for clot
    - c) Grading
      - (1) Good - solid clot
      - (2) Fair - soft clot
      - (3) Poor - friable clot
      - (4) Very poor - no clot
  
- c. Cell counts
  - 1) Both WBC and RBC are counted same as other body fluids –  
Normals < 200 WBC/• L; 0 RBC/• L
  
  - 2) Synovial fluid is so viscous it may be difficult to load the hemacytometer. Once loaded, it may need to sit for 30 minutes in order for all the cells to settle into the same plane. They may never settle so constant focusing up and down may be needed to see all of the cells on all of the planes.
  
  - 3) Synovial fluid may need to be diluted using saline. \* Do **not** use Unopette WBC diluent (acetic acid) or it will cause the fluid to clot.
  
- d. Cell differential – prepare and read same as other body/fluids. May see any of the cells of the peripheral blood plus
  - 1) Synovial lining cells (similar to mesothelials)
  - 2) LE cells
  - 3) Malignant cells



4) Organisms

- e. Microscopic examination for crystals – use both regular light microscope and a polarizing filter to visualize crystals. May be both intracellular and extracellular.

| Synovial Fluid Crystals* |   |   |
|--------------------------|---|---|
| Crystal                  | Shape   | Condition                                   |
| Monosodium Urate         | Needles<br>                        | Gout  |
| Calcium Pyrophosphate    | Rods<br>Needles<br>Rhombics<br>    | Pseudo-gout                                 |
| Cholesterol              | Notched rhombic plates<br>        | Nonspecific; chronic inflammatory disorders |
| Apatite                  | Small needles<br>                  | calcific arthritis                          |
| Corticosteroid           | Flat, variable shaped plates<br> | Drug injections                             |

\*Adapted from Samuelson and Ward.

2. Chemistry - few are clinically important, non specific. Values are approximately the same as serum values. Should test serum concurrently.
  - a. Total protein
  - b. Glucose
  - c. Lactate
  - d. Uric acid
3. Microbiology
  - a. Gram stain and acid fast
  - b. Culture (aerobic and anaerobic)
    - 1) Infections are usually bacterial but can be viral, fungal or tubercular
    - 2) Certain organisms tend to be seen in certain age groups

| Age Group       | Organism                            |
|-----------------|-------------------------------------|
| Children        | Haemophilus influenzae              |
| Adults 16-50 yr | Staph, Strep, Neisseria gonorrhoeae |
| Adults > 50 yrs | Staphylococcus aureus               |

## Body Fluids

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4. Synovial fluid serology - rarely done
  - a. Autoantibodies
  - b. Complement levels
5. QC - No prepared/commercial controls available for serous fluids, usually run chemistry serum controls

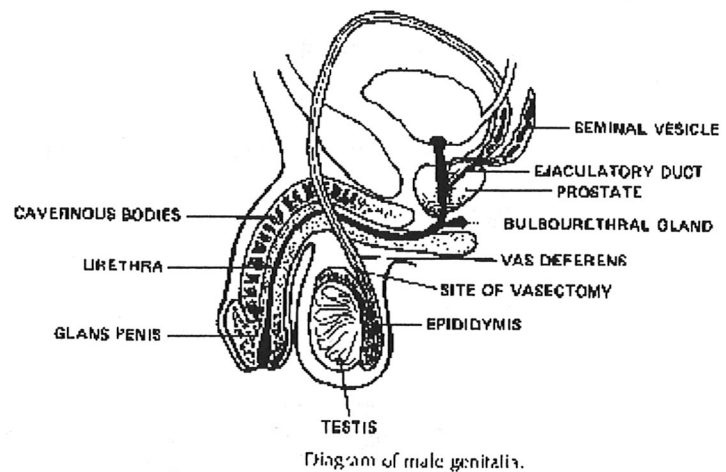
## V. SEMINAL FLUID

### A. Composition and formation

1. Spermatozoa
2. Fluid from seminal vesicles — a viscous fluid that provides fructose and other nutrients to maintain the spermatozoa. Provides the major volume of the seminal fluid.
3. Prostate fluid — a milky fluid that contains acid phosphatase and other enzymes that act on the fluid from the seminal vesicles resulting in coagulation and liquification of the semen.

### B. Indications / Reasons for Testing

1. Infertility
2. Post-vasectomy
3. Identification of a fluid as semen (forensic medicine)
4. Artificial insemination programs – sperm donors



### C. Collection

1. Sterile container
2. 3-day period of sexual abstinence required
3. Kept at room temperature and delivered to lab within one hour  
\* The time of collection must be recorded.

### D. Laboratory Procedures

1. Macroscopic
  - a. Physical characteristics

- 1) Liquification
    - a) A freshly collected specimen will gel (clot) and should liquefy within 30 minutes (time of collection is essential)
    - b) Persistence of the gel is abnormal
    - c) Further examination of semen should not be undertaken until liquification has occurred
  - 2) Volume — 2-5 mL
  - 3) Viscosity — pours in droplets, not too stringy
  - 4) pH — 7.3-7.8
2. Microscopic examination - done 30 - 60 min after collection
- a. Motility
    - 1) Using a hemocytometer, examine a drop of undiluted specimen using high dry objective
    - 2) Determine the percentage of motile (making forward progressive movement) spermatozoa – Normal = >50-60%
    - 3) Rate motility from 0 (no motility) to 4 (rapid motility).
    - 4) May be examined at timed intervals starting 30 - 60 min after collection, at 1 hr, and at 8 hrs.
  - b. Morphology – evaluate both head and tail morphology for abnormalities
    - 1) Normal sperm have an oval head measuring approximately  $3 \times 5 \mu\text{m}$  and a long tapering tail
    - 2) A smear of the semen is made (usually on frosted slides) and stained with Papanicolaou stain. At least 200 sperm are evaluated under oil immersion and the percentage of abnormal forms reported
    - 3) Normal — <30% abnormal forms
  - c. Also note the presence of WBC, RBC or bacteria
  - d. Sperm count
    - 1) Normal values — 20-160 million/mL

- 2) Procedure
  - a) Dilution — usually 1:20 with normal saline or saline plus sodium carbonate and formalin
  - b) Neubauer hemacytometer (same method as other body fluids)
  - c) Calculation: same calculation on hemacytometer except this will give a result of # sperm/uL. Most labs instead report the result as # sperm/mL. \* Therefore, to convert uL units to mL units you must multiply your result by 1000.
  
- e. Other tests (infertility)
  - 1) Sperm viability - Normal = >50% alive
  - 2) Seminal fluid fructose level
  - 3) Sperm agglutinins - antibodies
  - 4) pH – normal = 7.5-8.0
  
- f. Other tests - determining the fluid to be semen. May be performed on vaginal scrapings, clothes, skin, hair, etc.
  - 1) Acid phosphatase - a highly sensitive method for identification of a fluid as semen. For example: semen contains approx 2500 units of Acid Phos/mL. Other body fluids contain only about 5 units/mL.
  - 2) ABO, HLA typing
  - 3) DNA analysis
  - 4) UV light scan - semen fluoresces

## VI. AMNIOTIC FLUID

### A. Composition and Formation

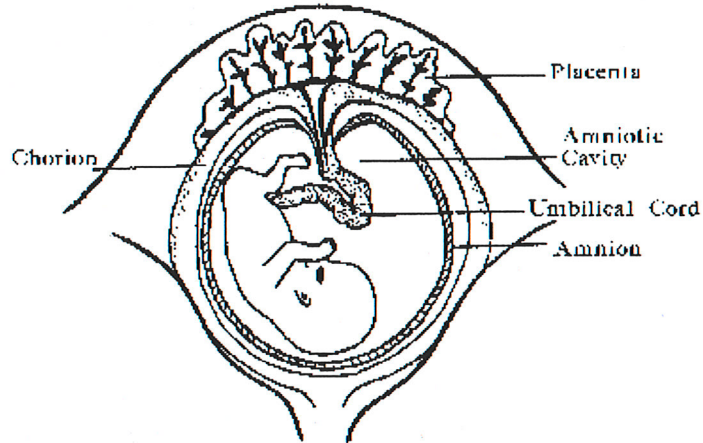
1. Found in membranous sac surrounding fetus
2. Formed by
  - a. Metabolism of fetal cells
  - b. Transfer of water across placental membrane
  - c. Fetal urine (later stages of development)
3. Provides cushion to protect fetus
4. Volume 500-2500 mL term

- B. **Indications** – suspected chromosomal abnormalities, metabolic disorders, neural tube defects, HDN, gestational age, infections, fetal maturity

**C. Collection & Handling**

Use Ultrasound (sonography) to locate placenta and fetal position.

1. Amniocentesis – 16-42 weeks gestation



2. Specimen – collection of no more than 20 ml or may cause premature labor or rupture membranes
3. Special precautions – protect from light, process immediately

**D. Laboratory Procedures**

1. L/S Ratio (lecithin/sphingomyelin) – L/S ratio routinely used to determine fetal lung maturity and to determine if it is safe to deliver a premature baby. L and S are the phospholipids that make up the majority of the alveolar lining and provides for alveolar stability, preventing the baby's lungs from collapsing on expiration. Hyaline membrane disease is the most common cause of death of a newborn.
  - a. Lecithin – produced at constant rate, equal to sphingomyelin until 33-35th week of gestation. Then there is a sharp increase in lecithin production.
  - b. Sphingomyelin – produced at a constant rate throughout pregnancy
  - c. Ration of 2:1 (lecithin:sphingomyelin) or greater indicates that the lungs are mature enough for delivery.
  - d. Performed by TLC - thin layer chromatography
  - e. Interferences = blood, meconium, vaginal mucous
2. Phosphatidyl glycerol – another surface lipid that increases greatly after 35 weeks gestation. More sensitive method than L/S

3. "Foam test" or "Shake test"
  - a. Developed before TLC, for "bedside" test
  - b. Amniotic fluid mixed with 95% ethyl alcohol
    - 1) Shake for 15 seconds
    - 2) Let sit undisturbed for 15 minutes
    - 3) Observe for presence of a continuous line of bubbles around outside edge
4. Alpha fetal protein – detects neural tube defects. The AFP test is performed on both the amniotic fluid and the mother's blood.
5. Cytogenetic analysis – determines chromosome abnormalities and metabolic defects. Indications for this test include:
  - a. Mothers: > 35 yrs, or those with a history of abortions, or a woman with an increase AFP test.
  - b. Parents: who are known carriers of chromosome abnormalities, or a family history of Down's babies.
6. Other chemistry tests
  - a. Bilirubin – HDN
  - b. Creatinine – fetal age

## VII. SWEAT

- A. **Indication** – Sweat test is used to confirm the diagnosis of **Cystic Fibrosis**. This is the most common fatal inherited disease of Americans.
  1. This is an incurable metabolic disease affecting the mucous secreting glands of the body, especially the lungs.
  2. Inherited as an autosomal recessive disease affecting 1 out of every 1500-2000 Caucasian births.
  3. Since multiple organs are affected it has many symptoms. The newborn will show respiratory distress, GI obstructions, pancreatic insufficiencies and failure to thrive.

**B. Laboratory Procedure**

**Pilocarpine iontophoresis**

1. Sweat glands on the cleansed forearm are subject to the sweat inducing alkaloid pilocarpine in the presence of a mild electrical current for 5-10 min.
2. Sweat is then collected for 25-30 minutes onto filter paper or a tubing apparatus.
3. Following elution off the filter paper, or removal from the tubing, the chloride content of the sweat is measured.
4. May also measure sodium levels in conjunction with chloride.
5. Results
  - a. Sweat chloride (and sodium) values over 50 mEq/L are seen consistently in 98% of patients with Cystic Fibrosis
  - b. Sweat chloride levels 40-70 mEq/L are considered borderline



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**VIII. GASTRIC FLUID****A. Composition and formation**

1. Gastric acidity results from the secretion of HCl by the parietal cells in the stomach
2. The hormone **gastrin** stimulates the parietal cells to produce HCl
3. HCl converts pepsinogen to pepsin, which aids in the digestion of protein
4. Gastric fluid contains: HCl, pepsin, saliva, mucous, acid neutralizing chemicals, as well as secretions from the intestines, biliary tract and pancreas

**B. Indications – Peptic ulcers, drug analysis****C. Specimen collection**

1. Nasal or oral intubation and aspiration
2. Patient should be fasting and avoid swallowing saliva (neutralizes gastric acidity)

**D. Laboratory procedures**

1. Gastric acidity – litmus paper used to determine pH
  - a. Peptic ulcers
  - b. Zollinger-Ellison Syndrome – tumor of pancreas causing gastric over secretion
  - c. Decreased gastric secretions: gastric carcinoma, pernicious anemia
  - d. Anacidity = the inability to produce proper gastric acidity
2. Drug screen – Analysis of gastric fluid for drugs may be done in conjunction with blood and urine testing

**IX. FECES**

**A. Composition and formation** – The normal specimen contains bacteria, cellulose and other undigested foodstuffs, GI secretions, enzymes, bile pigments, cells from GI walls, electrolytes and water.

**B. Indications** – GI bleeding, malabsorption syndrome, pathogenic bacterial or parasitic infections, liver and biliary duct disorders

**C. Collection**

1. Clean container, avoid contamination with urine or toilet water.
2. For most qualitative tests, a small random specimen is all that is needed.
3. Certain quantitative tests may require timed specimens over several days. Ova and Parasite analysis also requires collection over several days.

4. Restricted or controlled diets prior to collection are necessary for certain tests such as occult blood and fecal fats.

**D. Laboratory procedures**

1. Color and appearance – macroscopic changes may be the first indication of GI problems. See Table 10-1, p. 200, Strasinger.
2. Fecal leukocytes – methylene blue, Wrights or Gram stain may be used to visualize WBCs. Can indicate pathogenic bacterial infection or ulcerative colitis
3. Microbiology tests:
  - a. Gram stains: are not much help since the feces is full of bacteria, the most common normal flora being E. coli.
  - b. Cultures: used to determine infections by pathogenic organisms such as Salmonella, Shigella, Campylobacter, C. difficile, and certain pathogenic strains of E. coli.
  - c. Ova and Parasites (O & P): Giardia, Enterobius vermicularis (pinworm), tapeworms, and various other parasitic infections.
4. Occult blood
  - a. Detects GI bleeding, commonly associated with colorectal cancer
  - b. Guaiac and ortho-toluidine are methods used to detect presence of blood. These reagents produce a blue color change in the presence of blood due to the pseudoperoxidase activity of hemoglobin.
  - c. Used for mass screening.
  - d. False positives – unrestricted diets that had included red meat and certain vegetables, as well as patients on iron therapy.
  - e. False negatives are seen in patients taking large doses of vitamin C.
5. Fecal fats – steatorrhea
  - a. Presence of excess fat in the stool caused by fat malabsorption.
  - b. Qualitative methods – Oil red O or Sudan IV - stain feces and examine microscopically for large red fat droplets.
  - c. Quantitative method – Na hydroxide to chemically titrate the amount of fat
6. Other laboratory tests
  - a. APT – when newborn has bloody stool or vomitus. Tests for maternal blood in the newborn's stool that he/she may have swallowed during delivery

- b. Electrolytes and/or osmolality – aid in diagnosis of malabsorption syndromes

**X. Bronchial Washings & Bronchoalveolar Lavage**

A. Specimen Collection and Clinical Correlations

1. Fiberoptic bronchoscope - used to enter lung passages for the diagnosis / evaluation of immunocompromised patients, interstitial lung diseases, and airway diseases.
2. Sterile saline infused, then retrieved to be analyzed.

B. Testing

1. Fluid measurement, manual cell counts & differential on cytocentrifuged specimen.

macrophages  
lymphs,  
neutrophils  
eosinophils  
bronchial epithelial cells,  
squamous cells

2. Cultures

**XI. OTHER FLUIDS**

- A. Nasal Smears
- B. Cyst Fluid
- C. Tears
- D. Breast Milk

