Laboratory No.7

Cerebrospinal fluid

(CSF)

Cerebrospinal Fluid (CSF): - is a clear, watery liquid that flows around the brain and spinal cord, surrounding and protecting them. A CSF analysis is a group of tests that evaluate substances in CSF in order to diagnose conditions affecting the brain and spinal cord ([central nervous system](https://labtestsonline.org/glossary/centralns/)).

CSF:- is formed and secreted by the choroid plexus, a special tissue that has many blood vessels and that lines the small cavities or chambers (ventricles) in the brain. It is continually produced, circulated, and then absorbed into the blood. About 17 ounces (500 mL) are produced each day.

GENERAL INFORMATION:

Both the brain and spinal cord are covered by three protective membranes referred to as the meninges. The outermost layer is called the dura mater and is composed of tough connective tissue. The middle layer is the arachnoid named for it spider web like appearance.

The delicate innermost layer which is in direct contact with the brain and spinal cord is called the piamater. An inflammation of the meninges is referred to as meningitis.

Between the arachnoid layer and the piamater is a space called the subarachnoid space. It contains a clear, colorless fluid referred to as Cerebrospinal Fluid (CSF).

CSF:- is produced in the ventricles of the brain by a collection of rich vascular protrusions called the choroid plexus. Excess CSF is continuously reabsorbed by arachnoid villi and returned to the venous system thus maintaining a consistent amount of fluid under an intracranial pressure between 50 - 180 mmHg. Generally, the total volume of CSF circulating throughout the adult Central Nervous System (Brain and Spinal Cord) is approximately 90 - 150 ml. In newborns this volume is 10 -60 ml.



 THE TWO CHIEF FUNCTIONS OF CSF (SPINAL) FLUID ARE:

1-To protect and cushion the brain and the spinal cord against possible injury.

2-To deliver nutrients from the blood to the Central Nervous System and removes wastes.

The procedure used to obtain cerebrospinal fluid is referred to as Lumbar Puncture or a Spinal Tap. A needle is inserted aseptically between vertebral levels L3 and L4 or L4 and L5. Fluid is collected aseptically from the subarachnoid space and placed in 3 sterile tubes numbered in the order of collection (Tubes 1, 2, 3).

Why analyze CSF?

Spinal fluid is readily accessible and its examination is a very valuable diagnostic tool.

The composition of CSF reflects many biochemical and cell shedding

alterations in CNS diseases for example, it can reveal presence of infection,

tumors, neurological disease or leukemia.

**PROCEDURE FOR CSF EXAMINATION**

Generally, CSF is collected into three sterile tubes which do not contain anticoagulant.

The tubes are numbered in the order in which they were collected and are then distributed to the appropriate laboratory for testing. A description of each specimen and the clinical laboratory tests for which it is **most suitable** are listed below:

**TUBE #1**

• Contains debris from the puncture and occasionally blood in a "traumatic tap ".Since it is the most likely to be contaminated with microbes, tissue fluid and blood cells which could yield misleading results it should not be used for micro or hematology studies. It is best used for **chemistry** and **immunological**

determinations.

**TUBE #2**

• May contain some blood cell contaminants but is suitable for microbiological studies.

**TUBE #3**

• Has the least cellular or debris contamination and therefore is used for cell

counts, white cell differentials and the examination of abnormal cells e.g. tumor cells.

**PHYSICAL EXAMINATION of CSF**

All fluids are measured and the volume is recorded. The color and clarity before and after centrifugation is noted. The presence of a clot should also be reported.

 **Normally:-**CSF is perfectly clear, colorless, and transparent.

 **Pathologically:-** it may be turbid/cloudy, bloody, or xanthochromic.

♦ **Turbidity** or **cloudiness** may result from the accumulation of protein,

microorganisms or cells and generally suggests **infection**.

♦ **Bloody** fluids may result from a "traumatic tap" in which blood from vascular damage during the performance of the lumbar puncture has occurred or from ahemorrhage in the CNS. It is imperative that these two situations are accurately differentiated. Centrifuge and examine supernatant.

♦ **Xanthochromia = yellow color** - in the supernatant denotes pathological

bleeding and occurs as a result of hemoglobin degradation and bilirubin formation in the subarachnoid space. Two to 12 hours after a subarachnoid hemorrhage the supernatant is pale orange in color in 90% of patients.

Over time this turns to yellow as due to conversion of hemoglobin to bilirubin (2-4 days). Xanthochromia due to bilirubin typically persists for 12 to 40 days.

**In addition to CNS hemorrhage, xanthochromia may also be observed under the following conditions:**

1. CSF protein over 150 mg./dl. (Due to bilirubin complexed with albumin.)

2. Bilirubinemia...in adults conjugated bilirubin diffuses across the blood-

CSF barrier if the serum bilirubin exceeds 6 mg/dl, in neonates unconjugated bilirubin may also pass.

3. Contamination of the skin by merthiolate...used to disinfect the skin.

4. Carotemia; Melanin ...due to meningeal melanosarcoma.

NOTE: The need to differentiate grossly bloody CSF due to "traumatic tap" from subarachnoid hemorrhage occurs frequently. It is critical that the correct diagnosis be made as quickly as possible so that treatment, if necessary, can be initiated.

**MICROSCOPIC EXAMINATION OF CSF:-**

Normal CSF contains up to 10 mononuclear cells/ul (lymphs & monos). An increased cell count is usually indicative of meningitis. In this case a differential may not be required, however, a smear should be made, rapidly dried and stained and examined to make sure it correlates with the cell count.

Cell Types Which May be Encountered in CSF

1. Cells Found In **Normal** Cerebrospinal Fluid include:

♦ Lymphocytes

♦ Monocytes

In addition occasionally you may also encounter the following:

♦ ependymal cells - epithelium of the cerebral ventricles

♦ choroid plexus cells

♦ nRBCs or early myeloid cells (if bone marrow is inadvertently

aspirated along with the spinal fluid)

2. Cells Found In CSF Under **Abnormal** Conditions

• Neutrophils, lymphocytes, monocytes in large numbers. Occasionally

eosinophils. See table for specific information

• Malignant Cells (from 3 General sources)

o Hematopoietic - leukemia, lymphoma, and plasma cell myeloma are all

hematopoietic malignancies that shed cells into CSF.

\*Metastatic Carcinoma - about 40 to 50% of all carcinomas metastatic to

the CNS will shed cells into the CSF.

\* Primary Brain Tumors - can be diagnosed by CSF analysis, but only 10%

\*f them shed cells into the CSF.

**PROCEDURE FOR CSF CELL COUNT**

1. Mix the specimen thoroughly by gentle inversion at leat 10 times.

2. Using a pipet transfer the undiluted fluid to hemacytometer counting chamber.

Fill both sides of the chamber using proper technique. (See manual cell count

lab).

3. Allow the cells to settle.

4. Focus under low power (10X) and adjust condenser and diaphragm for

maximum visualization. Switch to high dry (45X), adjust if necessary, and

count cells. For an undiluted sample, usually all 9 squares are counted.

Average the results from both sides of the chamber.

**CSF PROTEIN DETERMINATION**

• Normal CSF protein concentration is 15 - 45 mg/dl. It is derived from the plasma.

• The general method used is a Turbidimetric Method...or precipitation of protein by TCA or SSA with Sodium Sulfate...Dye-binding techniques utilizing the dye Coomassie brilliant blue G-250

• Protein concentration is used as a nonspecific but reliable indicator of CNS

Pathology Increased levels are associated with:

- infection

- altered capillary permeability

- decreased absorption

- local biosynthesis of gamma globulin

• IGG...Primary Immunoglobulin is Increased in Multiple Sclerosis,viral

meningoencephalitis, neurosyphilis and subacute sclerosing panencephalitis.

The IGG protein is measured by Immunodiffusion or Nephelometry

• Characterisitc Oligoclonal Bands can be seen on electophoretic agarose gel

preparations in up to 95% of patients with Multiple Sclerosis. and some other

immune diseases. They represent specific antibodies.

• Myelin Basic Protein...as a result of the degradation of neural tissue...serologic

method of detection

• Normal CSF LACKS FIBRINOGEN.

**CSF GLUCOSE DETERMINATION**:

• Normal CSF glucose is: 60 to 70 % of the patient's serum glucose concentration.

• It is generally decreased in bacterial meningitis, as well as in meningitis

associated with a fungus, tuberculosis or syphilis.

**ADDITIONAL INFORMATION:**

• Normal Cerebrospinal Fluid is STERILE.

• The VDRL is the recommended serological test for Syphilis.

• INDIA INK should be used for the examination of CSF for Cryptococcus

• CSF Lactate levels above 35 mg / dl are found in bacterial, tuberculous, and

fungal meningitis...and rule out viral meningitis.