LABORATORY ACTIVITY CLINICAL PATHOLOGY

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Subject : Routine analysis :
- Macroscopic & microscopic examination
- Chemistry analysis

Department : Clinical Pathology

A.	**Sequent**			
	I	Introduction	:	30 minutes
	II	Pretest	:	10 minutes
	III	Lab Activities	:	60 minutes
B.	**Topic**			
		1. Macroscopic examination 2. WBC counting 3. WBC differential counting 4. Chemistry analysis : - Nonne Apelt test - Pandy tes	: : : 	10 minutes 30 minutes 10 minutes 20 minutes
C.	**Venue**			
	Biomedical Laboratory, Faculty of Medicine, Unisba, Jl. Tamansari No.22 Bandung 40116			
D.	**Equipment**			

1	Routine analysis	Cerebrospinal fluid Refraktometer (for spesific gravity)		
2	WBC Counting	Cerebrospinal fluid Turk reagen Improve neubeuer Haemocytometer		
3.	Nonne Apelt tes	Cerebrospinal fluid Ammonium sulfat reagen Acetic acid 10% Glass for boil Bunzen		
4.	Pandy tes	Cerebrospinal fluid Phenol raegen		

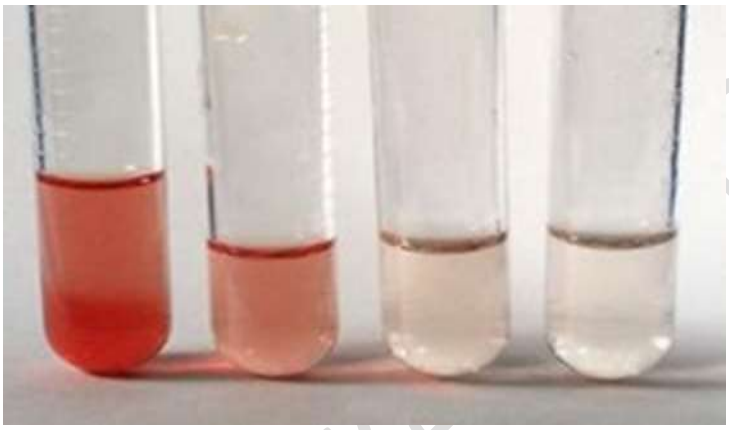
E.	**Pre-Test/Post test**			
F.	**Implementation**			
	1. The Students were divided into 14 group 2. Each group do lab. Activities accompanied by tutor			
	CEREBROSIPNAL FLUID **The CSF is produced by ultra filtration from blood plasma and secreted by cells of the choroids plexus, the amount is ±500 mL/day. It contains with water, circulates nutrients, and has functions as cushions and lubricates the central nervous system (CNS).** **Increased amount of CSF can be found in acute and chronic congestion of the meningens due to increased transudation; and in acute and chronic infections.** **The CSF pressure is around 100-150 mm of water. It is collected by lumbar puncture in the location between VL3-4 and VL4-5. It is only done by definite indications: DIAGNOSTIC or THERAPEUTIC. Informed consent must be done first to the patient and his/her family/ parents and signed.** **DIAGNOSTIC :** **1. To study the CSF** **2. To estimate intracranial pressure** **3. To test the spinal block** **4. To introduce air or lipoidal substance** **THERAPEUTIC:** **1. To introduce penicillin, streptomycin, or an anesthetic** **2. To remove blood or irritative substance** **CONTRAINDICATIONS:** **1. Subtentorial tumors** **2. Greatly increased intracranial pressure caused by any conditions** **SAMPLE COLLECTION AND HANDLING** **The CSF must be freshly collected (within 30 minutes), and the amount: 8-10 ml. The first drop may contain blood from the puncture and should not be used in laboratory examinations.** **The specimen should be divided into three sterile tubes, numbered 1, 2, and 3 :** **1. chemistry and immunological examination** **2. microbiological examination (must be in sterile container)** **3. cell count and differential count** **When xanthochromic CSF is obtained, add a trace of Lithium oxalate to prevent clotting.** **The cell count, bacteria and glucose examinations must be done at once, whilst the others can be delayed for several hours and the CSF sample must be put in the refrigerator.**			

ROUTINE ANALYSIS

MACROSCOPIC EXAMINATION:

- **Color, Normal: clear and colorless**
- **Turbidity, Normal: clear**
- **Reaction, Normal : alkaline**
- **Specific Gravity, Normal : 1.003-1.008**
- **Coagulation, Normal: does not coagulate**

If the blood in the specimen is due to a traumatic puncture, the CSF in the third tube should be clearer than those in tube 1 or 2, after centrifugation, the super-natant should be clear



STUDENT TASK:

RESULT

Describe macrsocopic the sample!

MICROSCOPIC EXAMINATION

- **LEUKOCYTE CELL COUNT**
- **DIFFERENTIAL COUNTING**

Normally only mononuclear (MN) cells (lymphocytes or an occasional monocyte) are found

LEUKOCYTE CELL COUNT

OBJECTIVE :

At the end of the activity the students will understand and can describe about:

1. How to perform the CSF leukocyte count
2. The interpretation of the results
3. The interfering factors which can affect the result

PREPARATION:

1. Turk
2. Sample:
   Shake the CSF well, place ± 0.4cc in a small test tube or a watch glass; so the remaining CSF may not be contaminated by the reagent

PROCEDURE:

- Draw up the reagent in the leukocyte pipette (Thoma) to the mark 1, and fill with CSF to the mark 11
- Mix by (rotatory) shaking the pipette well and then discard first 2-3 drops
- Place a drop on each side of double counting chamber of Improved Neubauer and wait for 2 minutes for the cells to settle
- Count the cells in the whole areas of the counting chamber 9 x 1 x 1 x 0.1 mm3
- The number of cells counted is then multiplied by 1/9 = the number of cells/mm3

Calculation

$$\frac{\text{Number of calculation (N) x dilution}}{\text{Number of square counted x vol of squares}}$$

With dilution

$$= \frac{N \times 0.1}{0.9}$$

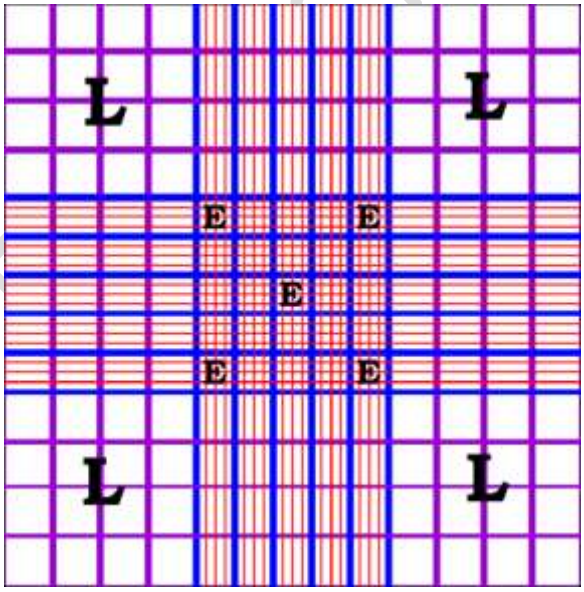
$$= 0.25N$$

No dilution

$$= \frac{N}{0.9}$$

$$= 10/9N$$

IMPROVED NEUBAUER CELL COUNTING CHAMBER



LEUKOCYTES COUNT : ALL 9 AREAS OF = 1 X 1 X 0.1 mm3;

THE VOLUME TO BE EXAMINED : 9 X 1 X 1 X 0.1 mm3 = 0.9 mm3

STUDENT TASK:

RESULT

Normal : 0-5 cell/uL

Result :

CONCLUSION:

LEUKOCYTE DIFFERENTIAL COUNT

METHODOLOGY:

Thin smear of the CSF sediment on glass slide

PREPARATIONS:

- Reagent: Giemsa stain
- Differential count sheet

PROCEDURES:

Centrifuge CSF for 10-15 minutes at mode-rate speed (1500 -3000 rpm).

Pour off the supernatant, and make thin smears of sediment on an object glass/slide

- Dry the slide in the air without heating it
- Stain with Giemsa`s reagent, let it dry
- Count and tabulate up to 100 leukocyte (or all of the amount of leukocyte when the amount is <100 cells).

NOTE:

When CSF is xanthochromic, from patient <2 months old, examine for toxoplasma in the PMN or monocytes

CSF FINDINGS IN DISEASES

DISEASE	PRESSURE (mm of water)	APPEARANCE	CELLS	QUALITATIVE PROTEIN (globulin)	QUANTITATIVE PROTEIN (mg/dl)	GLUCOSE (mg/dl)
Normal	100-150	Clear, colorless, no clot	0-8 MN	0	25-45 (A/Gratio1:5)	40-70 60% of blood glucose
Pneumo-coccus meningitis	Greatly increased	Turbid *to* yellow Clot (+)	***Acute*** : sl increase ***Less acute***: 100-5000 95% PMN	**++** *to* **+++**	100-400	0-10

TBC Meningitis	500-1000	Clear, opalescent, *or* white. Fibrin web	***Children*** **Early** 10-100 **Late**100-1000 70-90% MN ***Adult*** fewer cells	**±** *to* **+++**	30-400	15-20
Acute Polio-myelitis	Usually increased	Clear-milky *occ.* fibrin clot	***Pre-paralytic*** 15-2000 PMN ***paralytic*** 10-100 MN	± *to* **++**	***Pre-paralytic*** 25-60, ***paralytic*** 60-300	Normal
Epidemic encephalitis	Usually 200 *or* more	Normal, *occ*. Fibrin clot	10-200 all monocytes; <10 in 30-50% cases	±	25-60	Above normal 65-120
Brain abscess	Usually increased	Normal *or* sl. turbid	***Ruptured*** 10-100; 70-75% PMN; ***unruptured*** 5-30; 90-95% MN	± *to* **+**	30-100	Normal *or* increased
Brain tumors	Usually increased	Normal or xantho-chromic	Normal or 10-80	± *to* **++**	50-200	40-100
Spinal cord compres sion	Normal or decreased	Clear or xantho-chromic	Normal or increase in MN	**+** *to* **++++**	Complete block 300-2000; Partial block 45-300	Normal
Multiple sclerosis	Normal or decreased	Normal	70-90% cases: normal; others: 5-50 MN	0 *to* **+**	30-80 (10-49% cases : above normal)	Normal
Influenzal meningitis	Greatly increased	Sl.cloudy to turbid. Clots	200-3000 60-70% PMN	**++** *to* **++++**	Markedly increased	Diminished, but not absent
Subarach-noid hemor-rhage or cerebral injury	Slightly increased	Bloody or xantho-chromic	Cells increa-sed due to blood	**+** *to* **++**	45-200 or higher	Normal or increased

REFERENCE VALUES:

Normally: only mononuclear cells (MN) i.e. lymphocyte or an occasional monocyte and an occasional endothelial cells from the lining of the pia-arachnoidal spaces are found

RESULT :

CHEMISTRY EXAMINATION

PROTEIN :

- Qualitative
- Quantitative

GLUCOSE

TOTAL PROTEIN (QUANTITATIVE)

OBJECTIVE:

At the end of the lab activity the students will understand and can describe about:

1. The principle of the CSF total protein (quantitative) examination
2. The interpretation of the results
3. The interfering factors which can affect the result

INTRODUCTON:

Quantitative total protein examination in CSF used the same method with the method used in serum/plasma protein measurement. Because of the relatively small amount of protein in the CSF, certain procedure must be performed first in pre-analytical step in the laboratory.

PRINCIPLE OF THE TEST:

Protein in the presence of copper ions form a violet blue color complex in alkaline solu-tion (Biuret method)

The reagents used are same with reagents in serum/plasma protein measurement

REAGENTS:

R1: Sodium hydroxide
Potassium sodium tartrate

R2: Sodium hydroxide
Potassium sodium tartrate
Potassium iodide
Copper sulphate

THE CRUCIAL THINGS ARE:

1. The standard solution must be diluted 100x, to get the unit mg/dL
2. The amount of reagent and sample : inversion from serum/plasma measurement (i.e. 20 µL reagent + 1000 µL CSF)

- The photometer has to be programmed first, then get the fixed FACTOR to calculate the amount of protein

REFERENCE VALUE AND INTERPRETATION;

NORMAL : 15-45 mg/dL

INTERFERING FACTORS:

- Ascorbic acid > 30 mg/dl
- Bilirubin > 40 mg/dl
- Hemoglobin> 500 mg/dl

- Triglyceride > 1000 mg/dl
- IV polydextrans : cause falsely too high levels

ALBUMIN AND GLOBULIN EXAMINATION (QUALITATIVE)

OBJECTIVE

At the end of the lab activity the students will understand and can describe about:

1. How to perform the CSF examination (qualitative: albumin, and globulin: Nonne-Apelt, and Pandy`s test)
2. The interpretation of the results
3. The interfering factors which can affect the result

1) NONNE APELT TEST

This test is used to detect globulin and albumin. The presence of blood in the CSF sample can affect the result (falsely high). If the CSF is cloudy, it must be centri-fuged first, and the clear supernatant can be used for the test. These simple tests are still used in the ER or in the ward, because of its simplicity, but sensitive and specific enough to support the diagnosis.

REAGENT:

Ammonium sulfate (saturated)

EQUIPMENTS:

- Gloves
- Glass tubes 0.5 x 7cm
- Pasteur pipette

PROCEDURE:

GLOBULIN

1cc CSF + 1cc ammonium sulfate (saturated)

1. Place the tip of pipette containing ammonium sulfate to the bottom of the test tube which already contains with CSF
2. Let the ammonium sulfate solution layers underneath the CSF

Positive result:

A clear-cut, grayish-white ring appears at the contact zone between two fluids

Observe for 3 minutes. If a ring is formed, then shake the tube to mix the fluids.

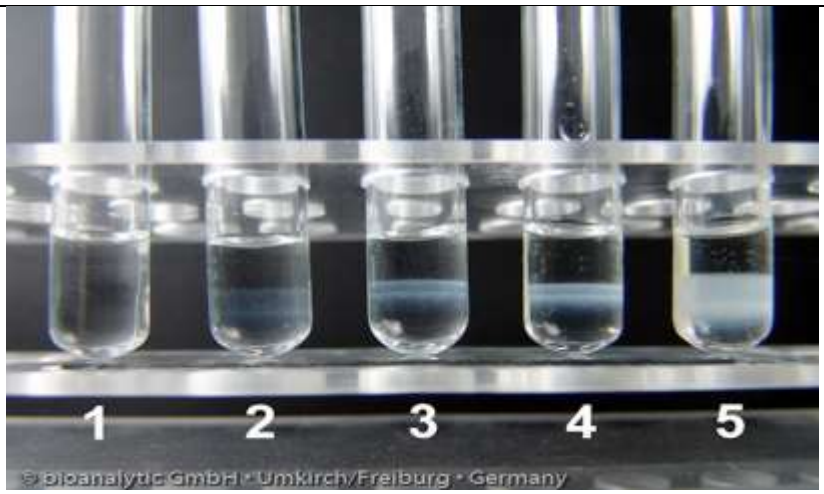
Result should be report as follows:

+ = a ring appears within 3 min, only visible against dark background, leaving no trace on mixing

++ = a faint, opalescence after mixing

+++ = a definite cloud after mixing

++++ = a heavy cloud after mixing



ALBUMIN

Shake the tube used in globulin test, and then add 1 drop of 10% acetic acid, boil

Report as follows:

Negative = a slight cloudiness

\+ = a definite cloudiness, with a fine precipitate

++ = a flocculate precipitate in a slightly cloudy fluid

+++ = a heavy flocculate precipitate in a clear fluid

2) PANDY'S TEST

PROCEDURE:

1cc phenol (saturated aqueous solution) + 1 large drop of CSF

Normal CSF may show a faint trace, and should be report as NEGATIVE

Positive:

A bluish-white cloud forming immediately around the drop of CSF: abnormal amount of globulin.

STUDENT TASK

RESULT :

NONNE APELT TEST :

PANDY TEST :

G.	**CLINICAL CASES**

1.	Seorang anak laki-laki 10 th datang ke EMG anak RS Al-Islam dengan keluhan demam, lethargi dan kaku kuduk. Dilakukan pemeriksaan lumbal punksi dengan menggunakan 3 tabung, dan didapatkan hasilnya sbb : Makroskopis : keruh, berawan Glukosa : 10 mg/dL Protein : 150 mg/dL A. Diagnosis apa yang paling mungkin pada pasien ini? B. Pemeriksaan lanjutan apa yang anda usulkan untuk menunjang diagnosis anda?
2.	Spesimen LCS yang jernih didapatkan dari seorang pasien anak laki-laki 5 th dengan keluhan kaku kuduk, tidak sadar disertai adanya gangguan neurologis, memberikan hasil pemeriksaan kimia sbb : Protein : 50 mg/dl Glukosa : 20 mg/dl WBC diff.count : Netrofil 5% Limfosit 43% Monosit 52% Diketahui ayahnya penderita batuk berdarah. A. Diagnosis apa yang paling mungkin pada pasien ini? B. Pemeriksaan lanjutan apakah yang anda pikirkan untuk menegakkan diagnosis
3.	Seorang pasien laki-laki, 30 th, mengalami kecelakaan lalulintas, datang dengan tidak sadarkan diri, kaku kuduk. Dilakukan pemeriksaan lumbal punksi dan didapatkan ketiga tabung semua berwarna xantochrom, dengan hasil sebagai berikut : Glukosa LCS : 120 mg/dL (Glukosa darah : 200 mg/dL) Protein : 150 mg/dL A. Diagnosis apa yang paling mungkin pada pasien ini? B. Sebutkan cara untuk memastikan penyebab LCS berwarna xantochrom (1 saja)
H.	**Reference**
	1. Karcher D, McPherson R. Cerebrospinal, Synovial, Serous Body Fluids, And Alternative Specimens in Henry JB; Clinical Diagnosis and Management by Laboratory Method; 23th 2. Mundt L. Shanahan K. Graff's Textbook of Urinalysis and Body fluid, 3th ed. Wolters Kluwer, 2016; page 184-198. ed 2017; W.B. Saunders Co; page 481–491.
I.	**Homework**
	1. Z, 4 yrs old boy, was suspected had meningeal TB, but the results of CSF examinations were normal; why? 2. Why do we have to count all leukocyte cells in the whole area of the counting chamber? 3. What are the differences between Improved Neubauer and Fuchs-Rosenthal counting chamber? 4. In brain tumor case, the protein level in the CSF is increased, why?