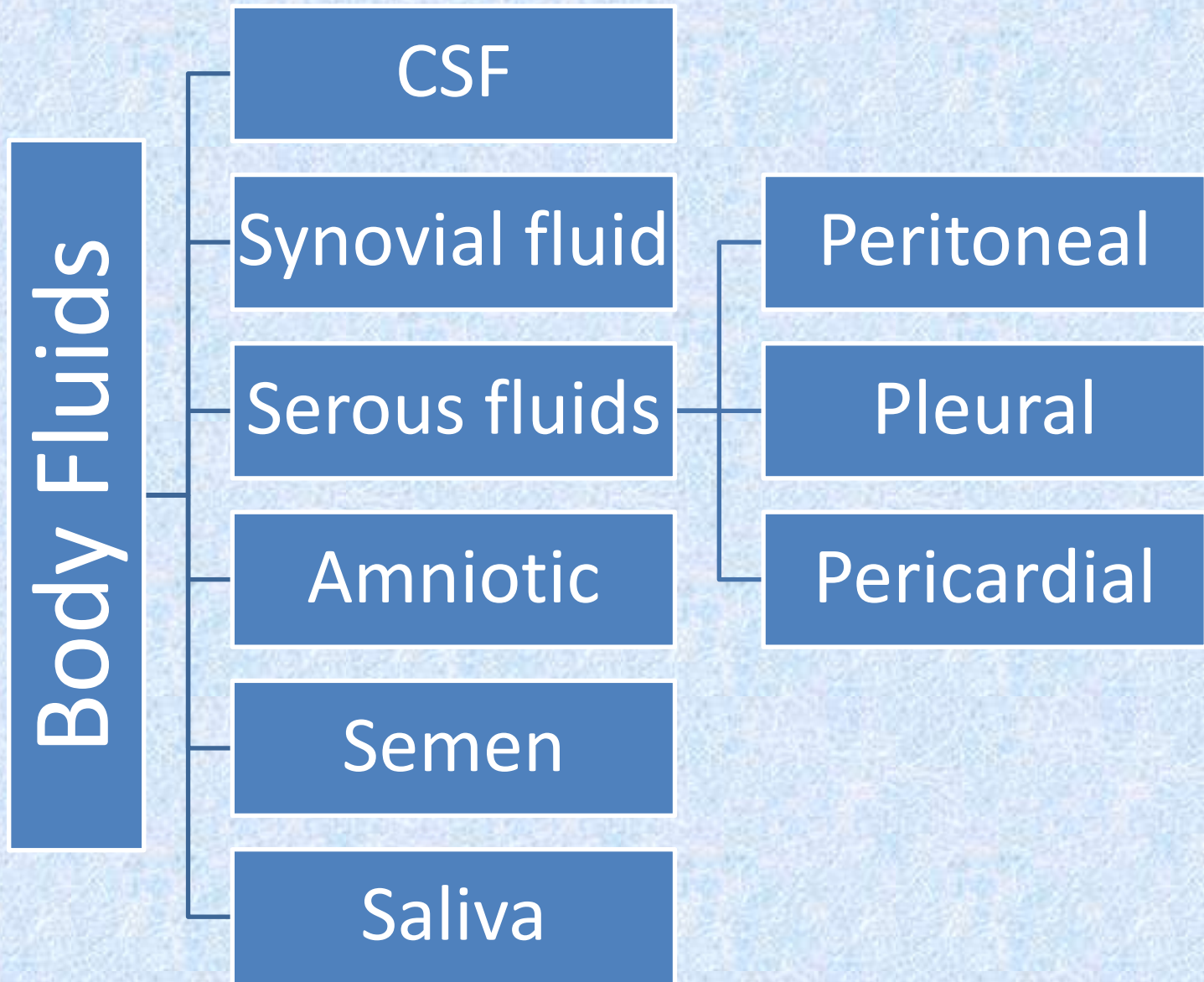


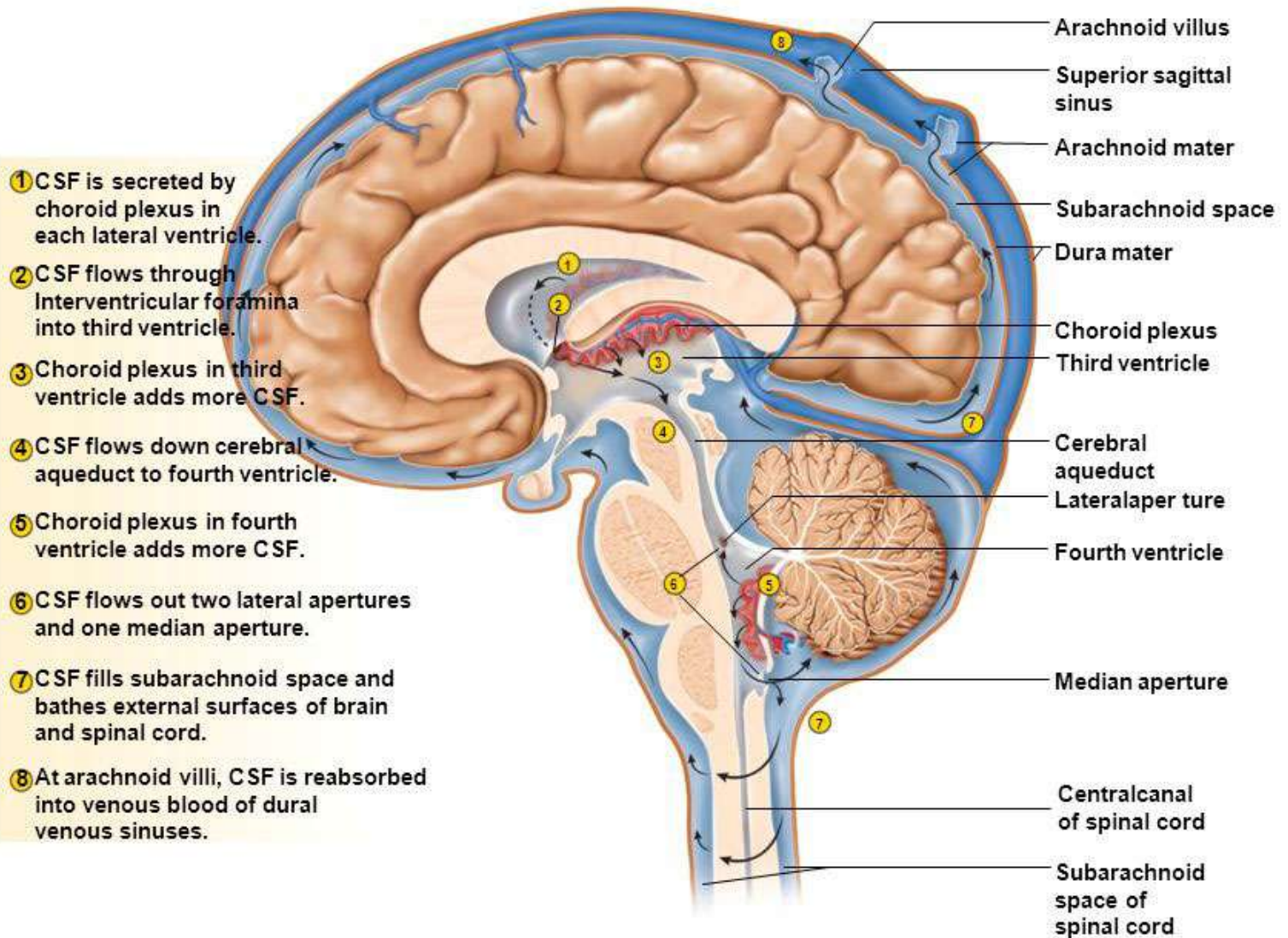
Body Fluids

By:

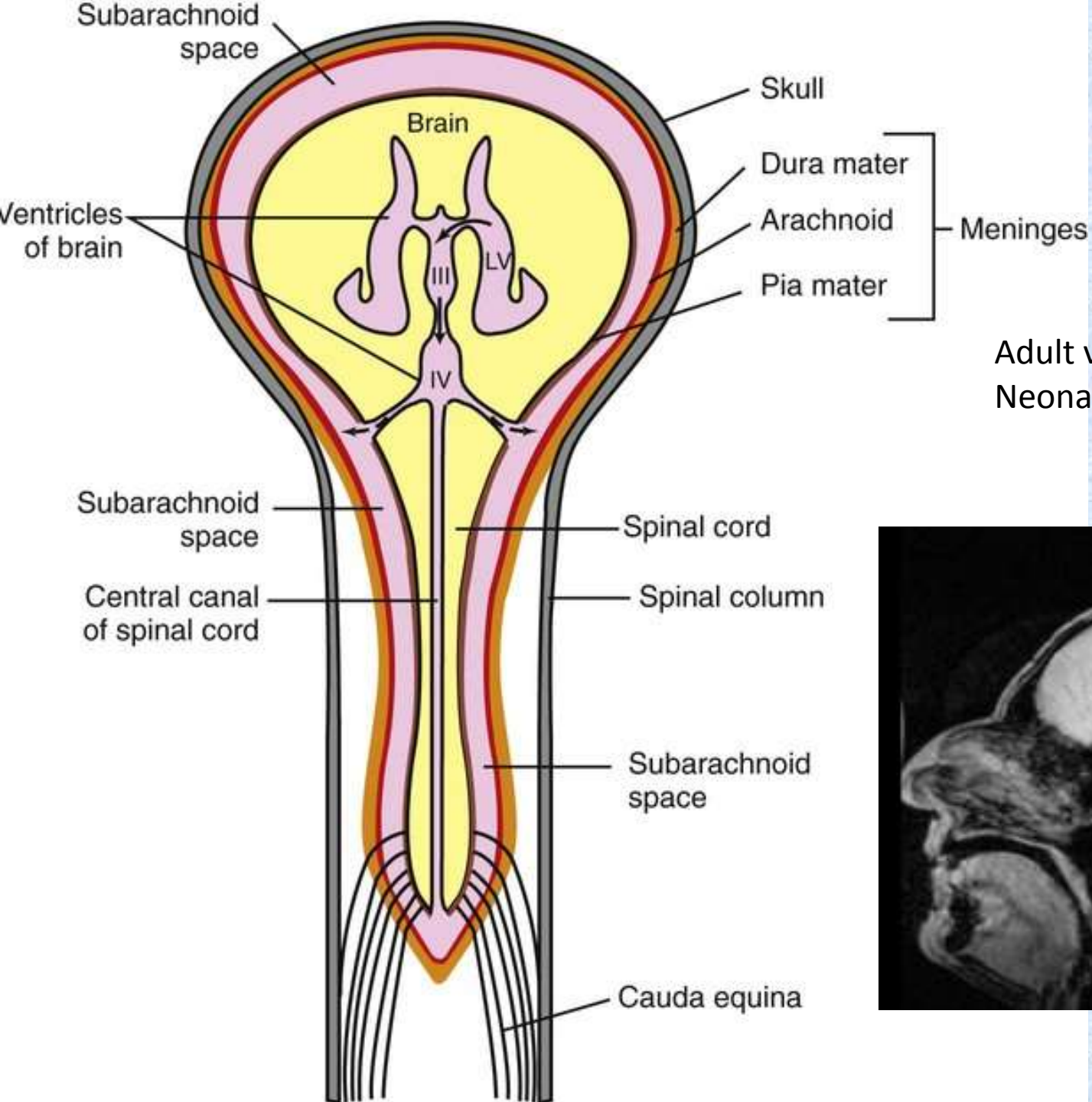
Dr. Mohammad Reza Haeri



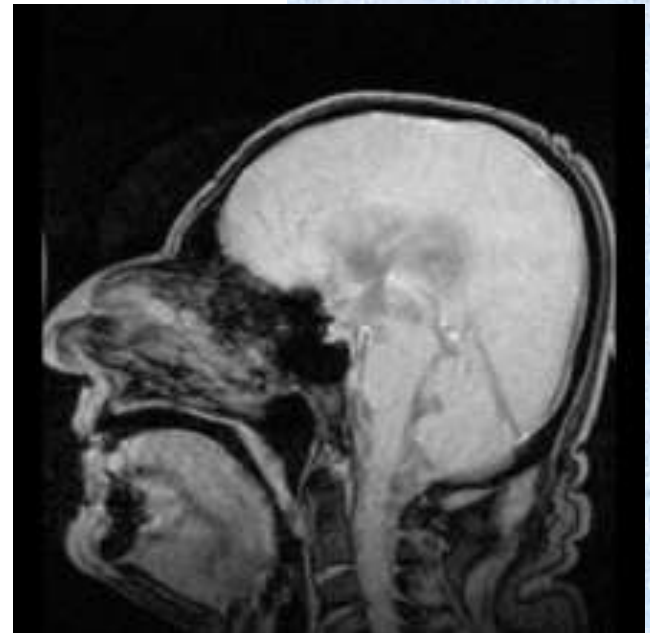
Flow of Cerebrospinal Fluid



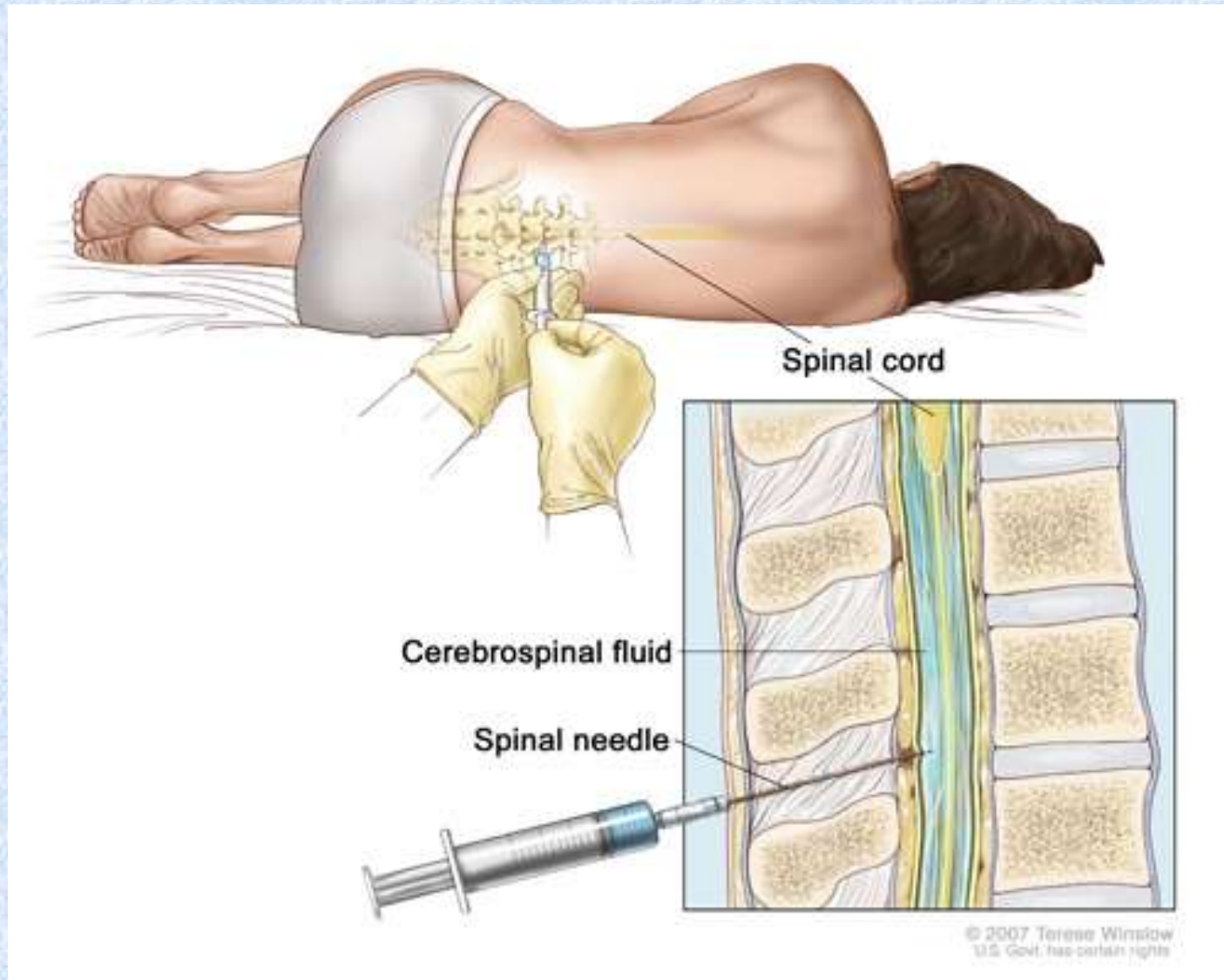
Produced at the Choroid plexus of the 4 ventricle and eventually reabsorbed into the blood



Adult volume 90-150 mL
Neonate volume 10-60 mL



Sample collection



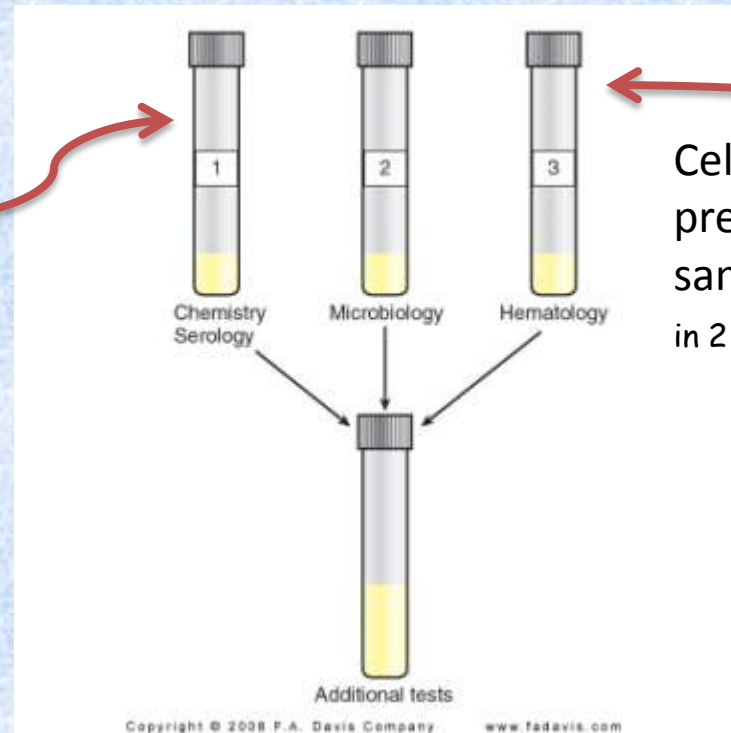
Why examination of CSF?

- To evaluate:
- Meningitis (viral, bacterial and fungal)
- Multiple sclerosis (MS)
- CNS bleeding (small and hidden bleeding)
- Malignancy

CSF sampling

- Three tube (**with no anticoagulant**):
- Tube 1 - chemistries and serology (frozen)
- Tube 2 - microbiology cultures (room temperature)
- Tube 3 - hematology, cytology (refrigerated)

should be **centrifuged** to avoid contamination by cellular elements
Glucose and protein measure as soon as possible



Cell counting should be preformed up to **1 hour** after sampling (RBC lyse in 1 hour, WBC in 2 hrs)

For protein

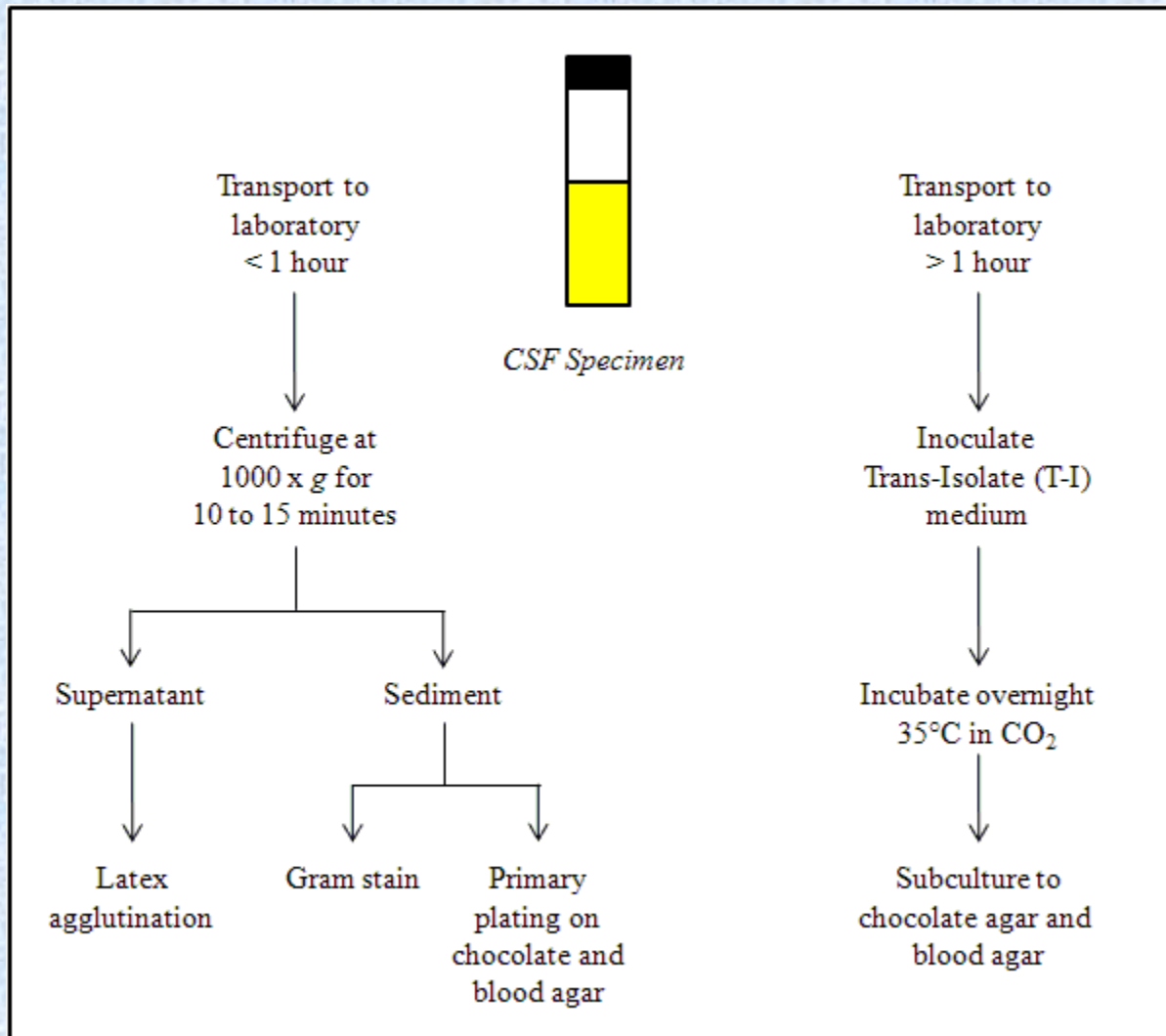


For glucose



CSF sample

- immediately deliver to the laboratory
- Cell counting should be preformed up to **1 hour** after sampling (RBC lyse in 1 hour, WBC in 2 hrs)
- Glucose and protein estimations should be performed as soon as possible after drawing the CSF specimen
- If testing is to be delayed, the specimen should be frozen at – 20°C.



CSF should be processed in a microbiology laboratory within 1 hour after collection or inoculated into Trans-Isolate (T-I) medium for transport to the laboratory if processing within 1 hour is not feasible

CSF analysis

```
graph TD; A[CSF analysis] --> B[Microbiological]; A --> C[cellular]; A --> D[Biochemical studies]; A --> E[Gross];
```

Microbiological

cellular

Biochemical
studies

Gross

Gross Examination

⌘ Turbidity

- ☒ Clear → normal
- ☒ Cloudy/ turbid → presence of WBC, or RBC, microorganisms, or an increase in protein level

⌘ Color

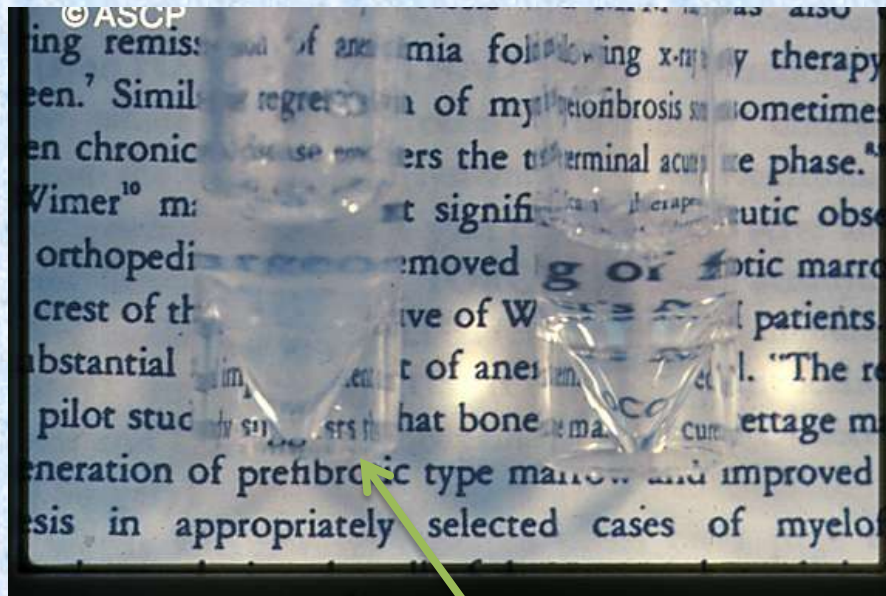
- ☒ Colorless → normal
- ☒ Yellow, orange-brown, or red → presence blood

⌘ Viscosity

- ☒ Normal CSF should have the same consistency as water
- ☒ Thicker CSF → mucin producing carcinoma, or bacterial (Cryptococci) meningitis

⌘ Clotting

- ☒ CSF is not coagulated normally, except in trauma and Tuberculous meningitis (Hemorrhagic)



- Unclear specimens may contain lipids, proteins, cells or bacteria.
- Clots indicate traumatic tap
- Milky - increased lipids
- Oily - contaminated with x-ray



- pinkish, orange due to presence of 'old' blood



Clots - indicates increased fibrinogen due to traumatic tap or damage to blood-brain barrier



Pellicle formation in refrigerated specimen associated with tubercular meningitis (left tube)

Methods of protein assay in CSF

- **Sulfosalicylic acid (3%)** → turbidimetry
- **Biuret method**
- **Nonne-Apelt** (globulin precipitation in saturated ammonium sulfate). Normal range: **negative**

Chemical Analysis

⌘ protein

☒ ↑ **Albumin** in increased BBB permeability found in inflammation or tumor and increased intracranial pressure and bleeding

Increased permeability is determined by
$$\frac{\text{Albumin}_{\text{CSF}}(\text{mg/dl})}{\text{Albumin}_{\text{Serum}}(\text{g/dl})} < 9$$

☒ ↑ **IgG**

- IgG synthesis by lymphocytes (in Multiple Sclerosis) or
- BBB permeability

$$\frac{\text{IgG}_{\text{CSF}}}{\text{Albumin}_{\text{CSF}}} < 0.27$$

☒ **Nonne - Apelt** (globulin precipitation in saturated ammonium sulfate). Normal range: **negative**

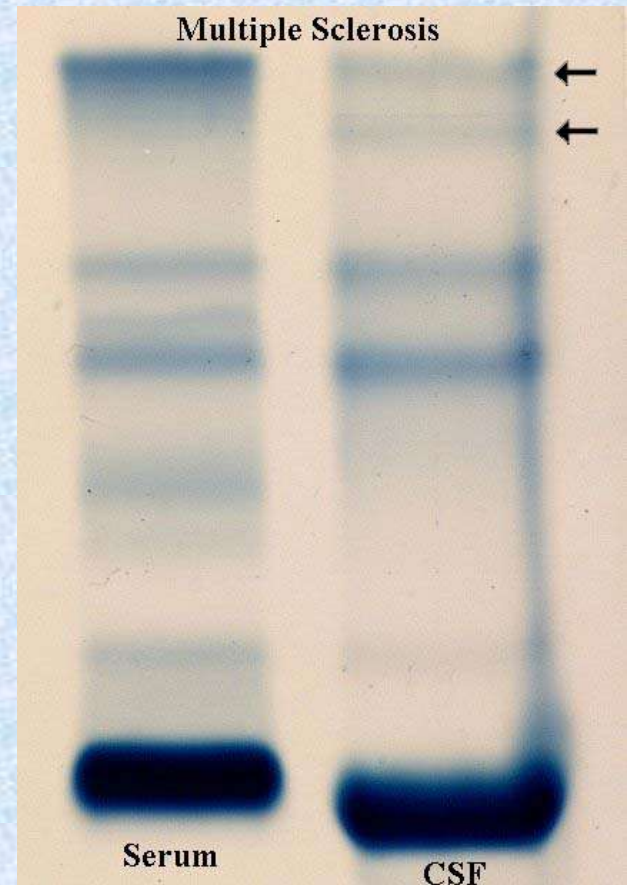
☒ **MBP** (myelin basic protein)

Multiple Sclerosis panel

- CSF Protein electrophoresis (oligoclonal bands)
- Myelin Basic Protein
- IgG/Albumin index:

$$\frac{\text{CSF}_{\text{IgG}}/\text{plasma}_{\text{IgG}}}{\text{CSF}_{\text{Albumin}}/\text{plasma}_{\text{Albumin}}}$$

- Normal reference: 0.65-0.81



Tumor markers

- CEA (breast, lung, intestine and stomach)
- β_2 -microglobulin (primary brain tumor, leukemia, lymphoma)
- AFP (metastatic germ cell carcinoma)
- Lactate dehydrogenase

Chemical Analysis

☒ Glucose

☒ lactate (to differentiate between bacterial and viral meningitis)

☒ CK-BB

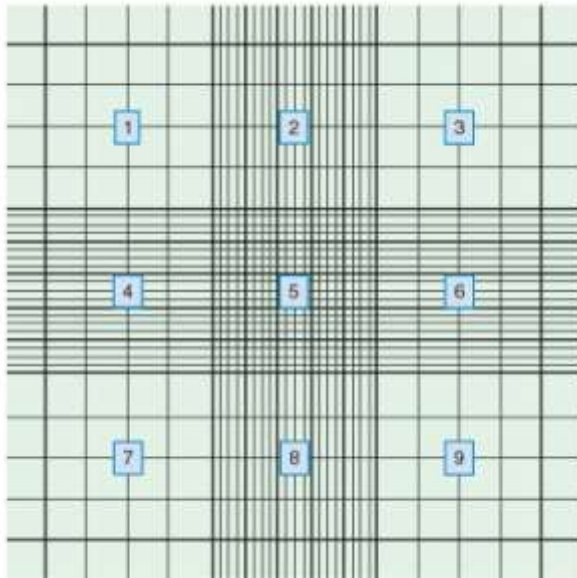
☒ lactate dehydrogenase (LD1&LD2 are in brain tissue) glutamine and acid-base parameters (ammonia intoxication)

Cellular examination

- ⌘ Centrifuge the sample and stain with Wright method
- ⌘ **Manual count using** Neubauer hemocytometer (Electronic counters generally unusable)
- ⌘ **Methylene blue staining** will improve visualization
- ⌘ **correction of blood contaminated samples:**
For every 10,000 RBC, reduce the number of leukocytes as many as 14 and 8 mg/dl for albumin

Neubauer hemacytometer / counting chamber

- **Formula for calculations** - results in # cells / μL
 - 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 below have volume of 0.1
 - Small squares - in center # 5 have volume of 0.004



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

Test	Appearance	Pressure	WBC/ μ L	Protein mg/dL	Glucose mg/dL	Chloride
Normal CSF	Clear	90 – 180 mm	0-8 lymph.	15-45	50-80	115-130 mEq/L
Acute bacterial meningitis	Turbid	Increased	1000 -10000	100 – 500	< 40	Decreased
Viral meningitis	Clear	Normal to moderate increase	5-300, rarely >1000	Normal to mild increased	Normal	Normal
Tubercular meningitis	Slightly opaque cobweb formation	Increased/decreased, spinal block	100-600 mixed or lymph.	50-300 due to spinal block	Decreased	Decreased
Fungal meningitis	Clear	Increased	40-400 mixed	50-300	Decreased	Decreased
Acute syphilitic	Clear	Increased	About 500 lymph	Increased but <100	Normal	normal

Microbiological studies

- Antigen tracing
- Rapid diagnosis:
 - I. urine strip
 - II. limulus lysate (reacts with bacterial endotoxin or lipopolysaccharide of Gram negative bacteria)
- Staining:
 - I. Gram
 - II. Acid-fast
 - III. India ink or nigrosine
- Culture (the most important test)

Bacterial meningitis

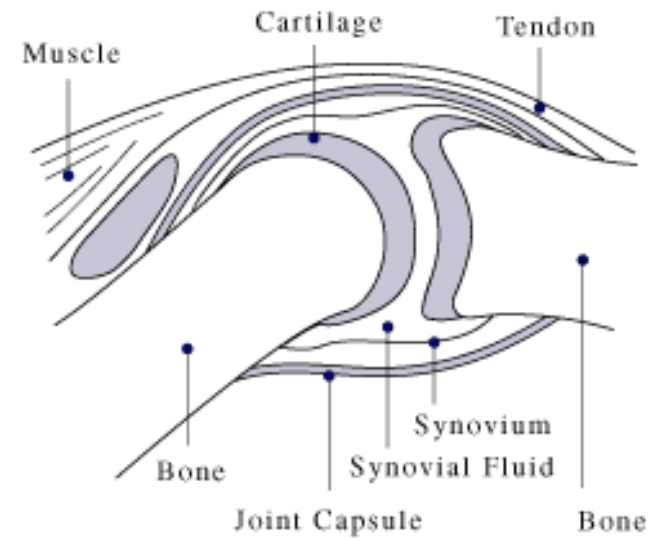
- Newborns
 - E. coli
- Children
 - Streptococcus pneumoniae
 - Hemophilus influenzae
 - Neisseria meningitidis
- Adults -
 - Neisseria meningitidis
 - Streptococcus pneumoniae
- Staph. aureus (if a shunt is present)

Bacterial	Viral	Tubercular	Fungal
Increased WBC count	Increased WBC count	Increased WBC count	Increased WBC count
Neutrophils	Lymphs	Lympts & 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>

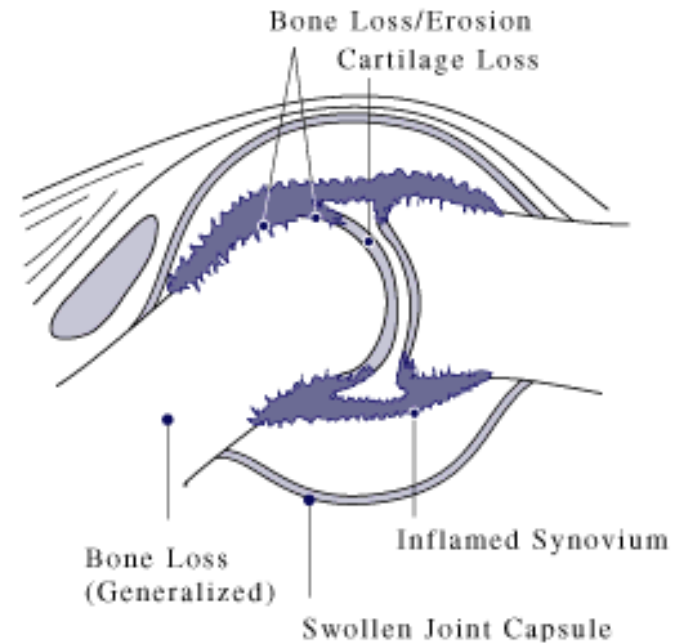
Synovial Fluid

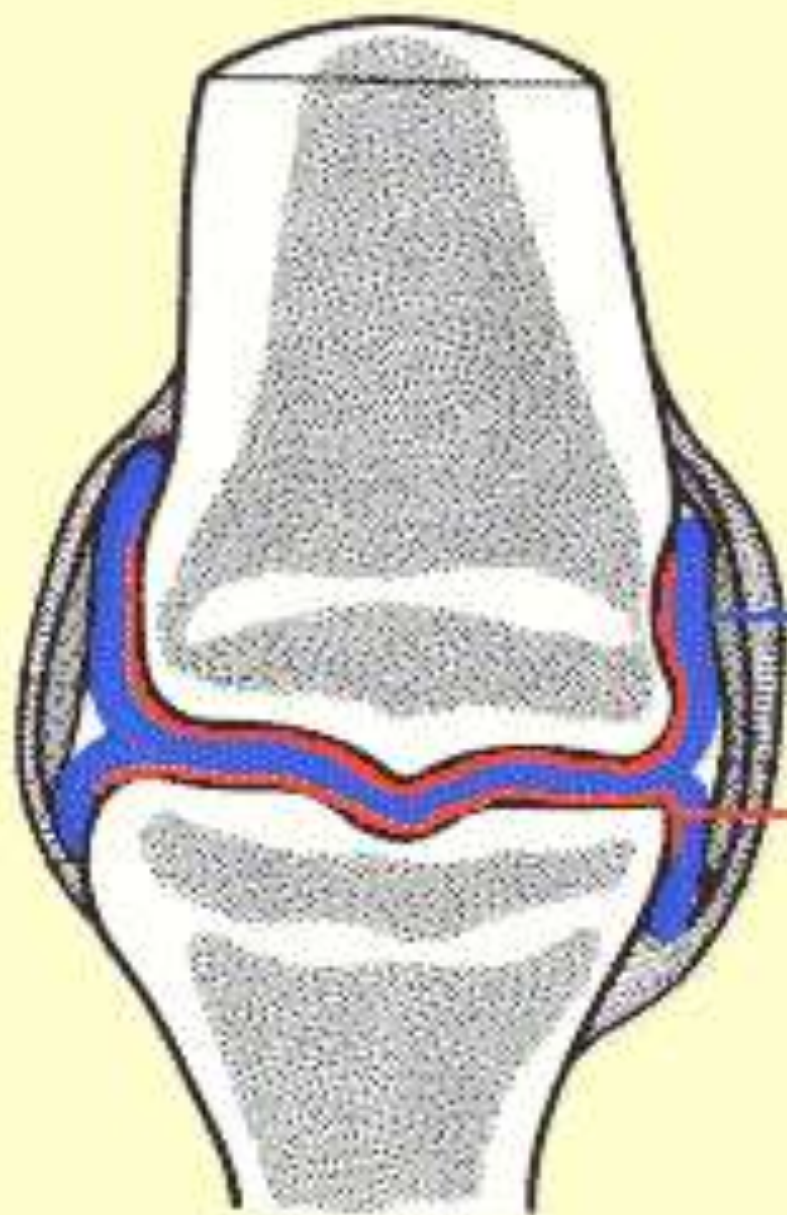


Normal Joint



Joint Affected by Rheumatoid Arthritis





**Synovial
Fluid**

**Articular
Cartilage**



Needle is inserted
into the joint, and
fluid is withdrawn



Arthrocentesis



```
graph TD; A[Arthrocentesis] --> B[Heparin (25U/ml)  
For microbiological and crystal studies  
(2-5 ml)]; A --> C[EDTA  
For microscopic studies  
(2-5 ml)]; A --> D[Without anticoagulant  
For clot study  
(2-5 ml)];
```

Heparin (25U/ml)

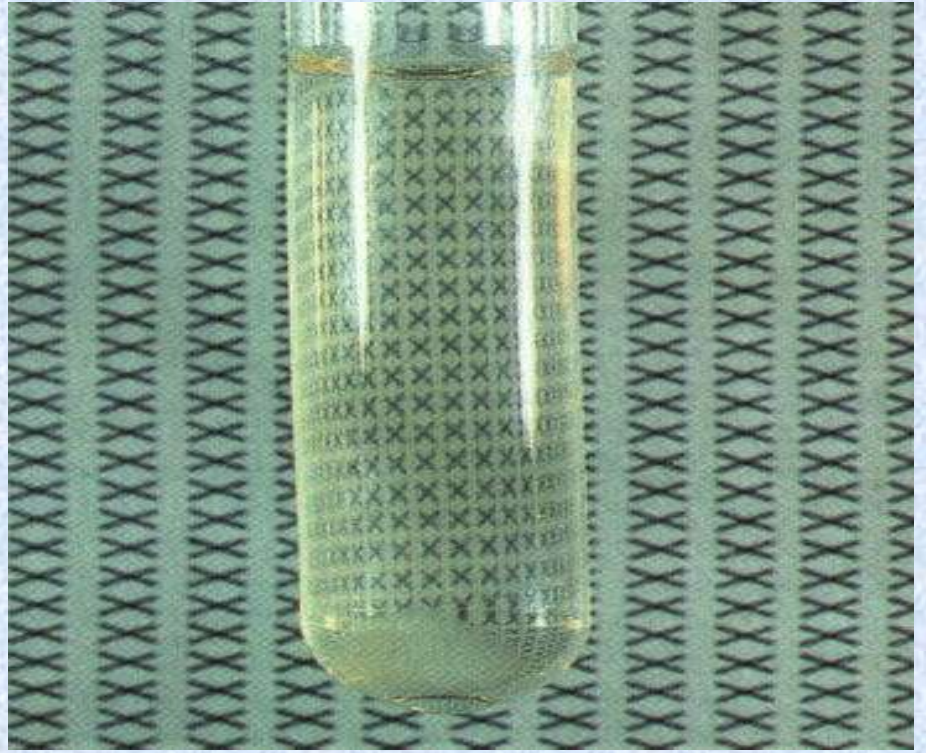
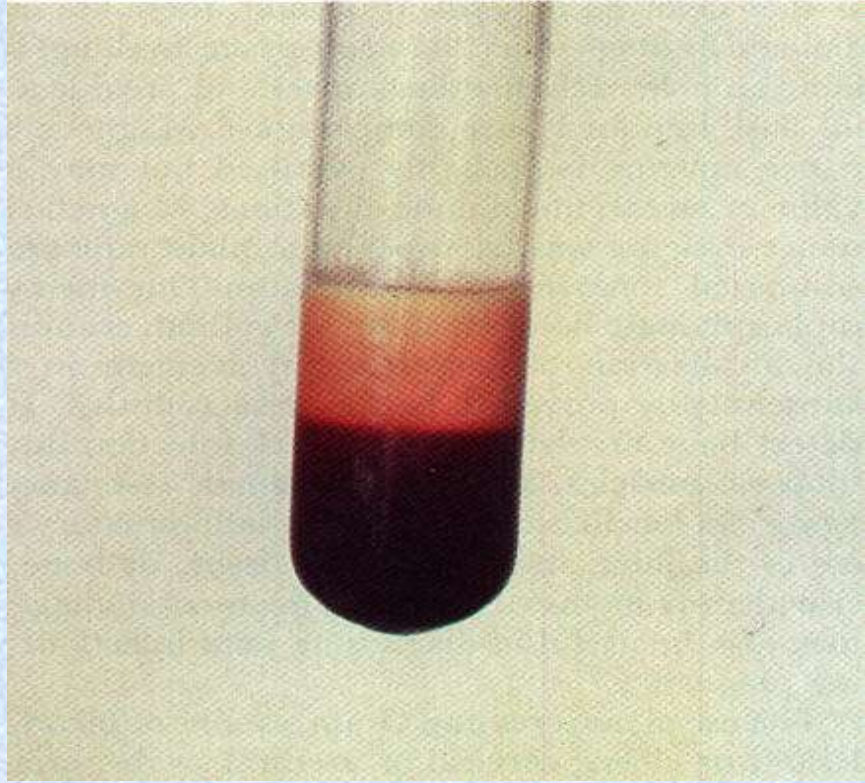
For microbiological and crystal studies
(2-5 ml)

EDTA

For microscopic studies
(2-5 ml)

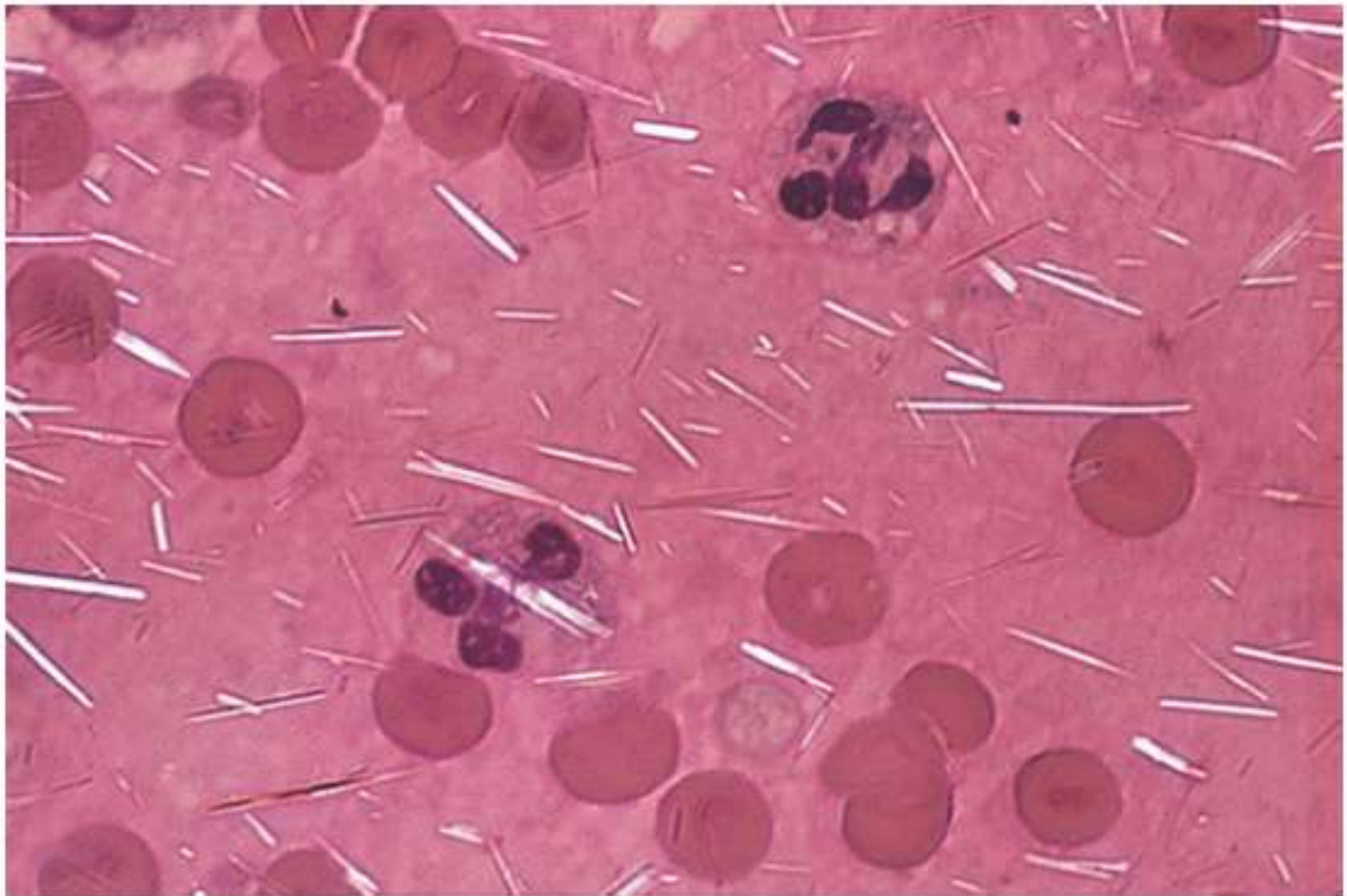
Without anticoagulant

For clot study
(2-5 ml)

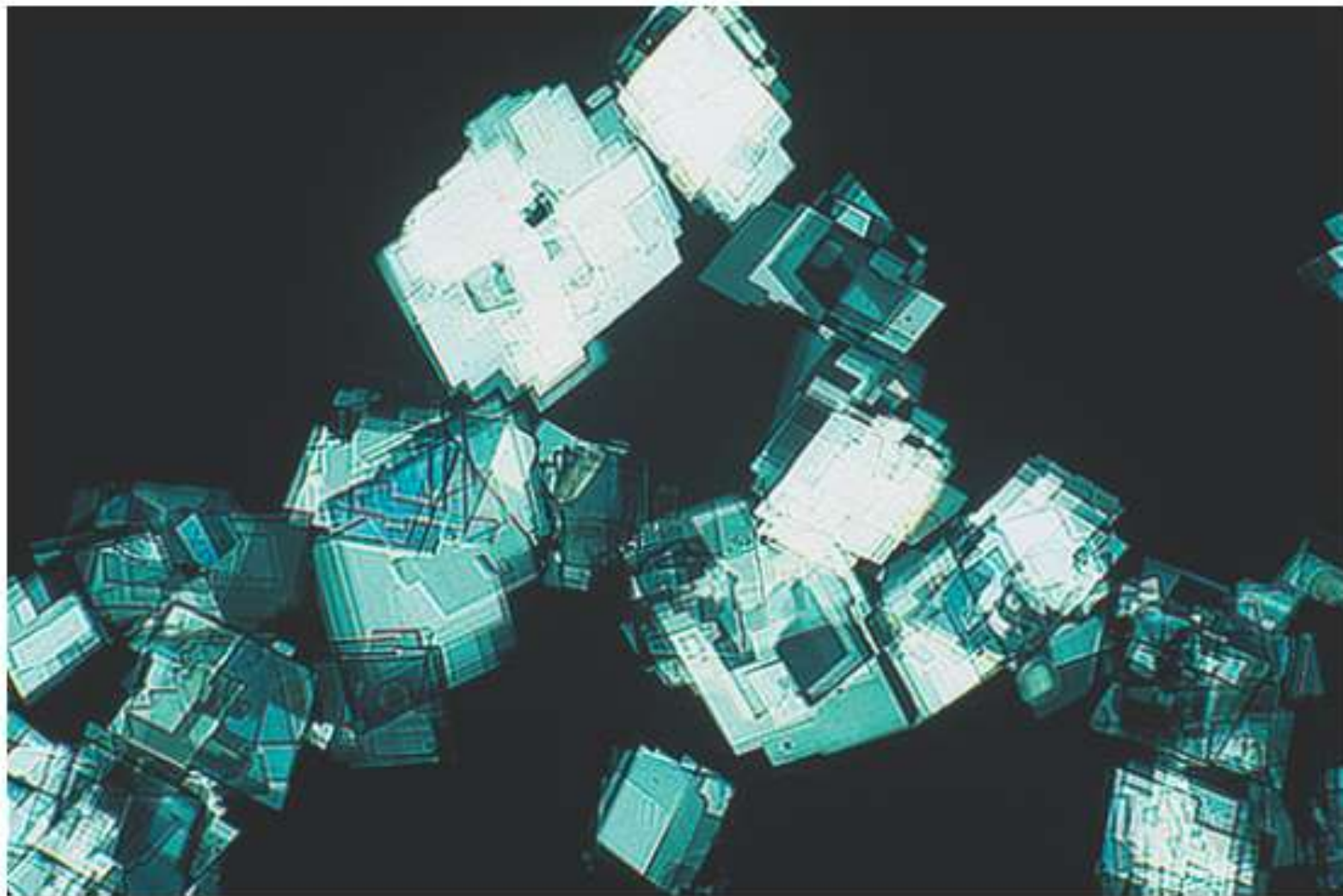


Microscopic examination

- **Leucocyte count:**
 - By hemocytometer
 - Concentrated sample is diluted by saline
 - WBC; 150-200
 - Neu; 20% increase to 70% in Gout and Rheumatoid arthritis
- **RBC count**
- **Crystal:**
 - Sodium urate (with birefringent property), seen in gout
 - calcium pyrophosphate
 - apatite (stain with alizarin S)
 - calcium oxalate seen in kidney failure
 - Cholesterol (needlelike) seen in Rheumatoid arthritis



Monosodium urate crystals under polarized light from a patient with urate gout.



Cholesterol crystals in synovial fluid. Polarized light.

arthrocentesis with synovial fluid analysis

Normal findings

Appearance	Clear, straw colored, no blood
RBC	None
WBC	0-150/mm ³
WBC differential	
Neutrophils	7%
Lymphocytes	24%
Monocytes	48%
Macrophages	10%
Glucose	Equal to fasting blood glucose
Protein	1-3 dL
LDH	<25 mg/dL
Uric acid	6-8 mg/dL
Gram stain	Negative

Microbiologic studies

- Gram staining (*staphylococcus*)
- Ziehl-Neelsen staining for Acid-Fast bacteria (*Mycobacterium tuberculosis*)
- Culture for mycoplasma

Biochemical Analysis

- **Glucose** (↓ in septic and inflammatory arthritis)
- **Protein** (↑ fibrinogen in inflammation)
- **Lipid** (↑cholesterol in chylous effusion in trauma, and systemic Lupus erythromatose)
- **Mucin Clot Test** (for testing hyaluronate in SF)
- 0.5 ml SF + 2 ml acetic acid → hyaluronate precipitates into a mucin clot
- Precipitation may be graded as good, fair, or poor. A fair to poor mucin clot test reflects dilution and depolymerization of hyaluronic acid—a nonspecific finding of several inflammatory arthritides.

Synovial Fluid Findings by Disease Category

		CATEGORY			
Finding	Normal	Group I NonInflammatory	Group II Inflammatory	Group III Infectious	Group IV Hemorrhagic
Clarity	Transparent	Transparent	Transparent/opaque	Opaque	Opaque
Color	Clear to pale yellow	Xanthochromic	Xanthochromic to white/bloody	White	Red-brown or xanthochromic
WBCs/mL	0-150	<3000	3000-75,000	50,000-200,000	50-10,000
PMNs, %	<25	<30	>50	>90	<50
RBCs	No	No	No	Yes	Yes
Glucose (blood/SF difference, mg/dL)	0-10 (0-0.56 mmol/L)	0-10 (0-0.56 mmol/L)	0-40 (0-2.2 mmol/L)	20-100 (1.11-5.5 mmol/L)	0-20 (0-1.11 mmol/L)

Reference Intervals for Synovial Fluid Constituents

Constituent	Synovial Fluid	Plasma
Total protein	1-3 g/dL	6-8 g/dL
Albumin	55%-70%	50%-65%
α_1 -Globulin	6%-8%	3%-5%
α_2 -Globulin	5%-7%	7%-13%
β -Globulin	8%-10%	8%-14%
γ -Globulin	10%-14%	12%-22%
Hyaluronic acid	0.3-0.4 g/dL	
Glucose	70-110 mg/dL	70-110 mg/dL
Uric acid	2-8 mg/dL	2-8 mg/dL
Lactate	9-29 mg/dL	9-29 mg/dL

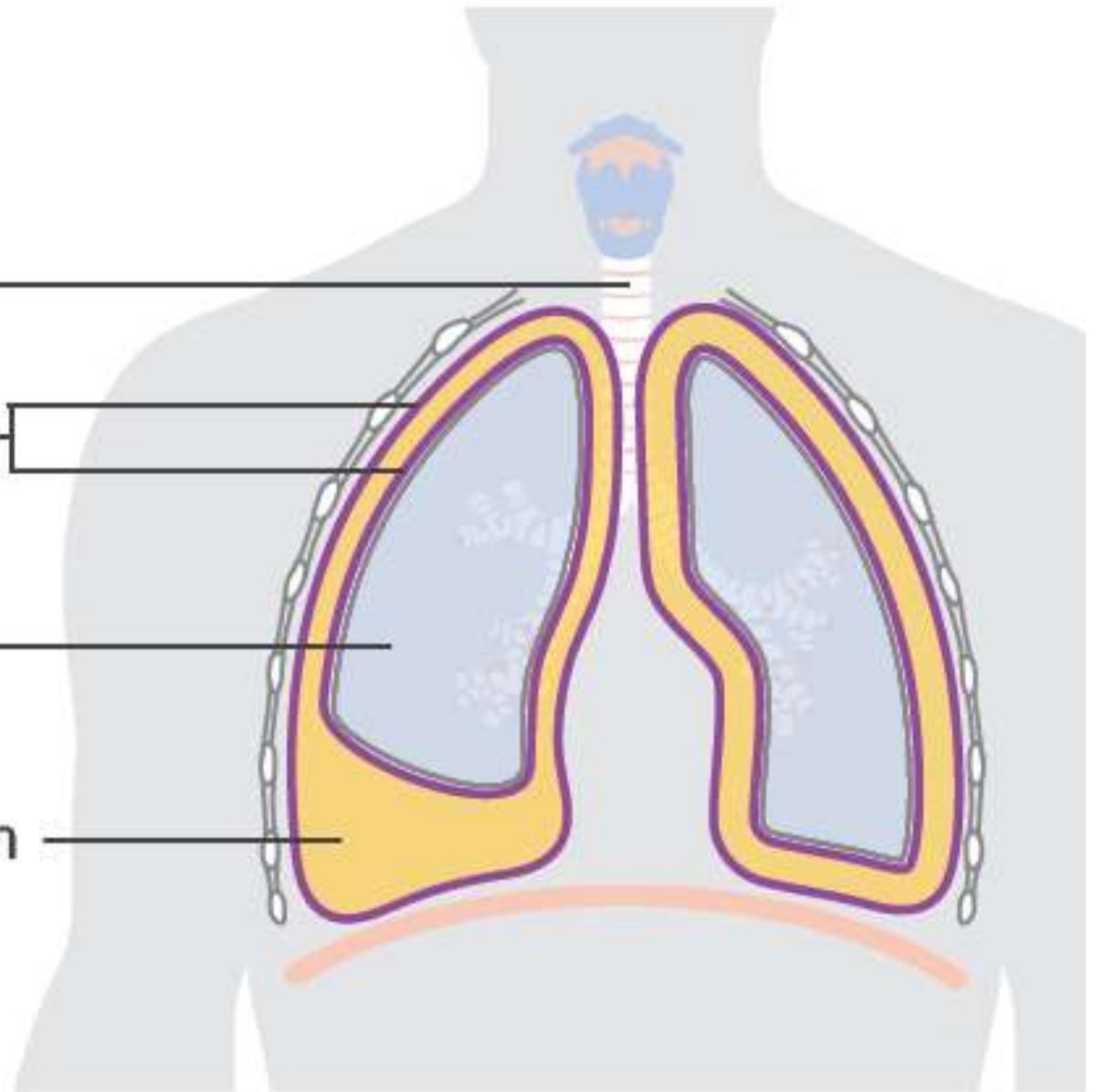
Pleural fluid

Trachea
(windpipe)

Pleura
(lung lining)

Lung

Pleural effusion
(fluid between
pleural space)



Schematic diagram of pleural anatomy

- The lymphatic vessels in the parietal pleura are in direct communication with the pleural space by means of stomas. These stomas are the only route through which cells and large particles can leave the pleural space.
- Although there are abundant lymphatics in the visceral pleura, these lymphatics do not appear to participate in the removal of particulate matter from the pleural space.

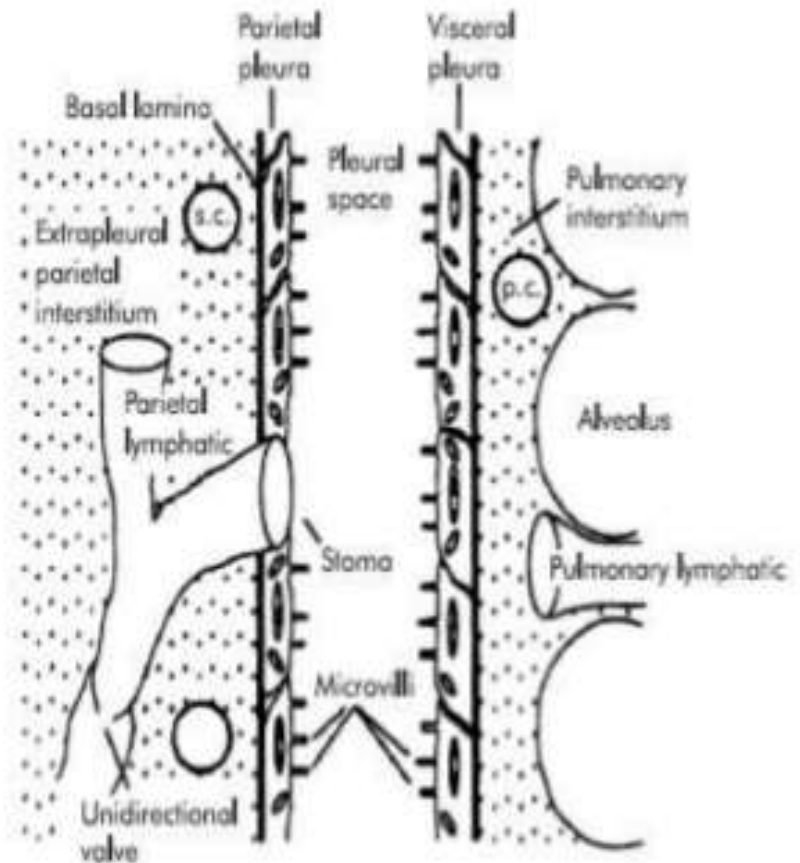
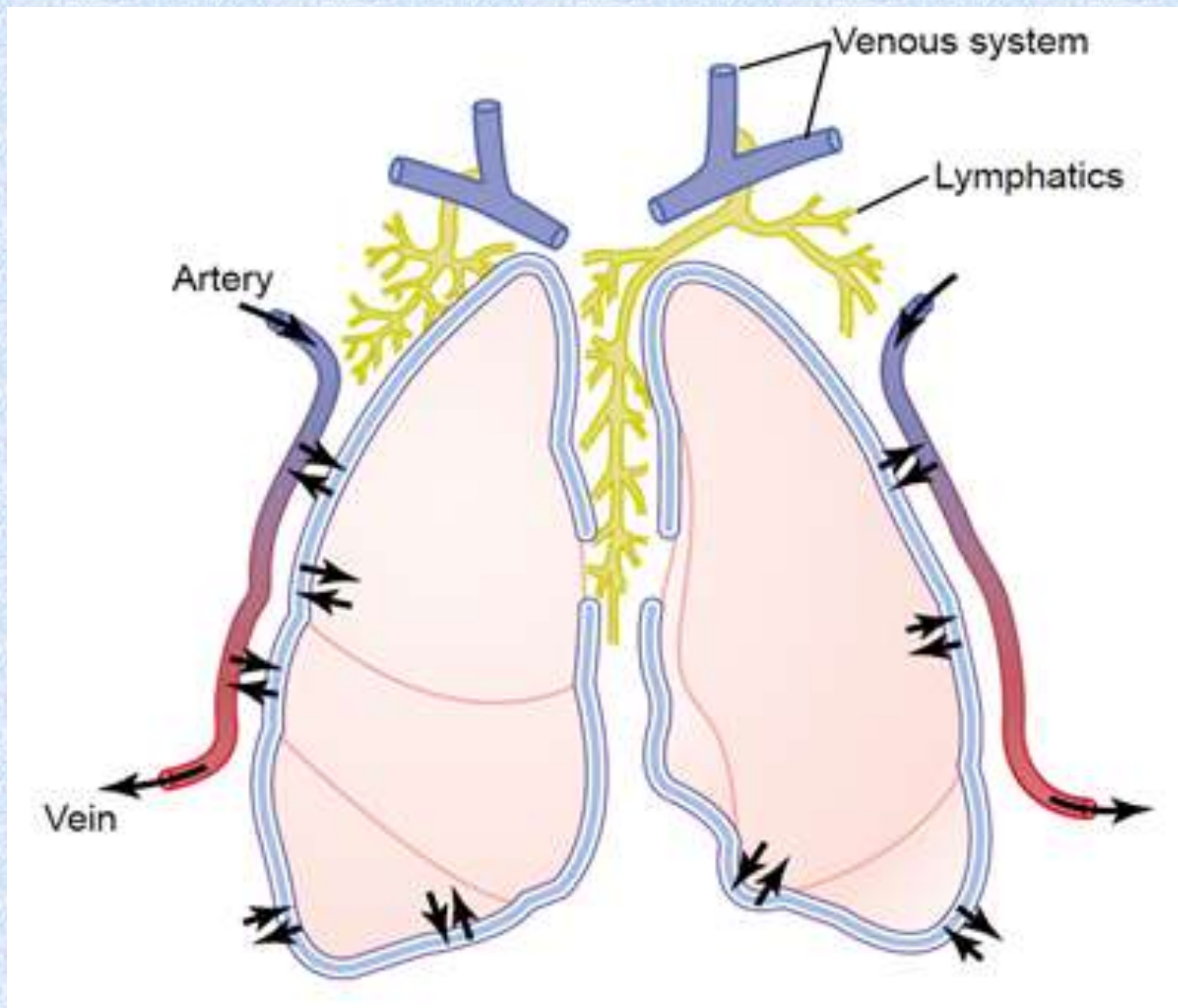


Figure 2 Schematic diagram of pleural anatomy; s.c.=systemic capillary; p.c.=pulmonary capillary. Modified from Miserocchi¹⁰ with permission.



Transudate

■ Congestive heart failure (CHF)

- Hepatic cirrhosis
- Hypoproteinemia
- Nephrotic syndrome
- Pulmonary atelectasis
- Myxedema
- Peritoneal dialysis
- Pulmonary embolism

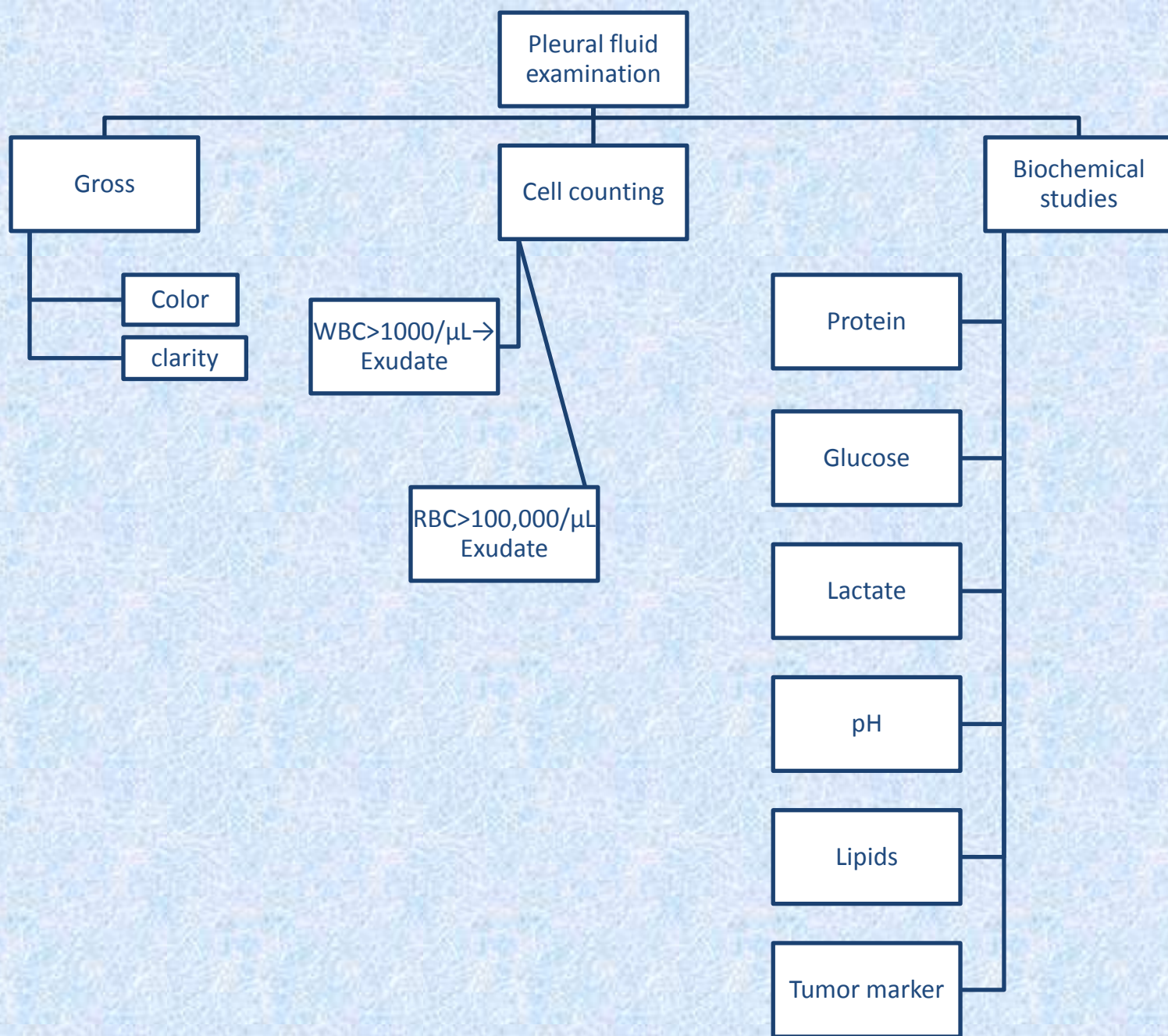
Exudative causes

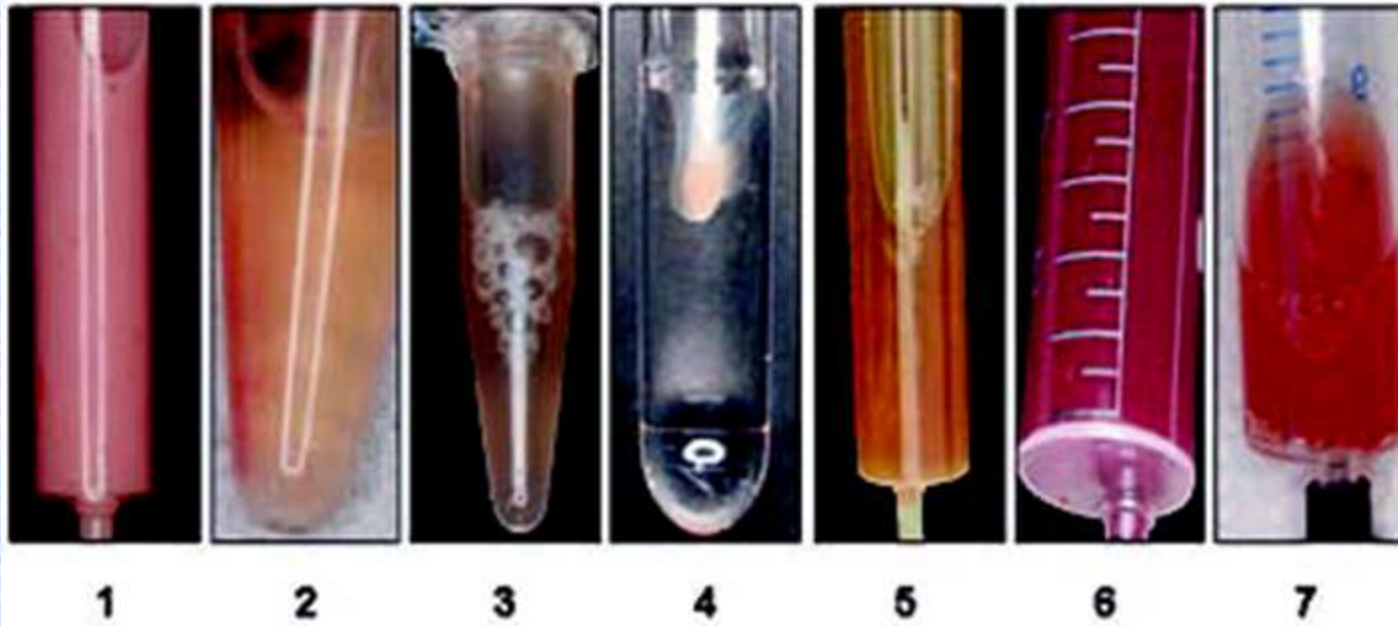
■ Malignancy

- Infection
- Trauma
- Pulmonary infarction
- Pulmonary embolism
- Autoimmune disorders
- Pancreatitis
- Ruptured esophagus

Thoracentesis







different pleural effusions:

- 1) Chylothorax,
- 2) Traumatic effusion,
- 3) Lymphoma,
- 4) Positive Rivalta test,
- 5) Pulmonary carcinoma,
- 6) Pulmonary carcinoma,
- 7) Traumatic effusion



Biochemical studies

- **Protein**
 - $\text{Alb}_{\text{ser}} - \text{Alb}_{\text{PF}} \leq 1.2 \rightarrow \text{Exudate}$
 - Rivalta test (one drop PF + acetic acid \rightarrow turbidity \rightarrow positive)
 - Ligh't's: Transudate $\leftarrow 3 \text{ g/dL} > \text{protein} > 3 \text{ g/dL} \rightarrow \text{Exudate}$
- **Glucose** (in septic exudate and rheumatoid pleuritis)
- **Lactate** (evaluation of infectious pleuritis)
- **pH** (in pneumonia effusion < 7.2)
- **Lipids**
 - Chylous $\leftarrow 110 \text{ mg/dL} < \text{TG} < 50 \rightarrow$ chylous is negative
 - Cholesterol $> 45 \text{ mg/dL} \rightarrow \text{exudate}$

Rivalta test

7-8 ml distilled water + 1 drop of glacial acetic acid (mix thoroughly) + 1 drop of the effusion fluid is carefully layered.

1- If the drop disappears and the solution remains clear → Rivalta's test is negative.

2- If the drop retains its shape, stays attached to the surface or slowly floats down to the bottom of the tube (dropper jellyfish like) → the Rivalta's test is positive.



Characteristic Features of Chylous and Pseudochylous Effusions

Feature	Chylous	Pseudochylous
Onset	Sudden	Gradual
Appearance	Milky-white, or yellow to bloody	Milky or greenish, metallic sheen
Microscopic examination	Lymphocytosis	Mixed cellular reaction, cholesterol crystals
Triglycerides	≥ 110 mg/dL (≥ 1.24 mmol/L)	< 50 mg/dL (< 0.56 mol/L)
Lipoprotein electrophoresis	Chylomicrons present	Chylomicrons absent

thoracentesis and pleural fluid analysis (Pleural tap)

Type of test Fluid analysis

Normal findings

Gross appearance: Clear, serous, light yellow, 50 mL

Red blood cells (RBCs): None

White blood cells (WBCs): <300/mL

Protein: <4.1 g/dL

Glucose: 70-100 mg/dL

Amylase: 138-404 units/L

Alkaline phosphatase

Adult male: 90-240 units/L

Female: <45 years: 76-196 units/L

Female: >45 years: 87-250 units/L

Lactic dehydrogenase (LDH): Similar to serum LDH

Cytology: No malignant cells

Bacteria: None

Fungi: None

Carcinoembryonic antigen (CEA): <5 ng/mL

Peritoneal fluid

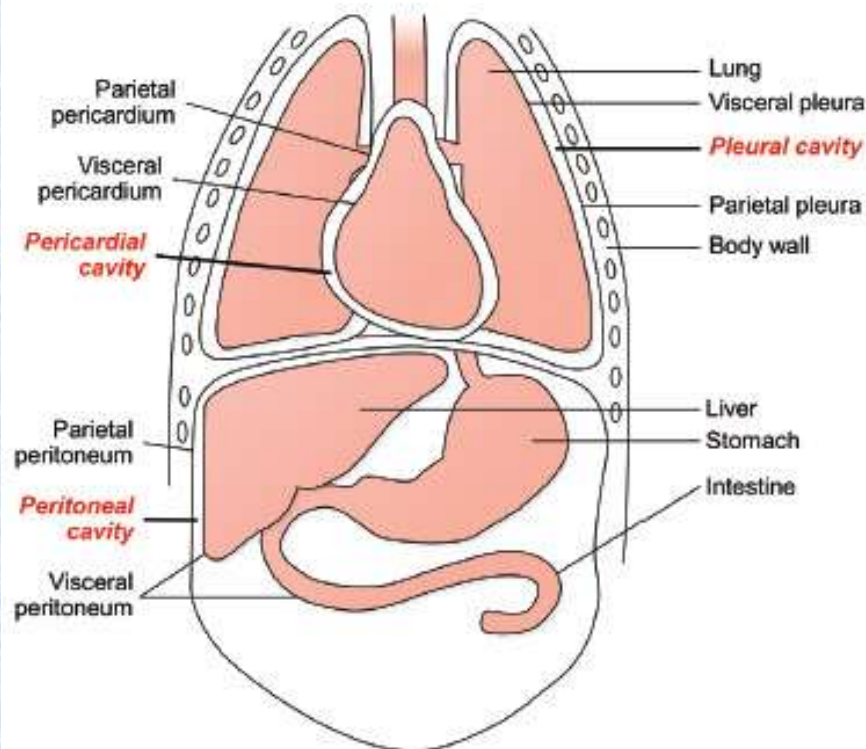
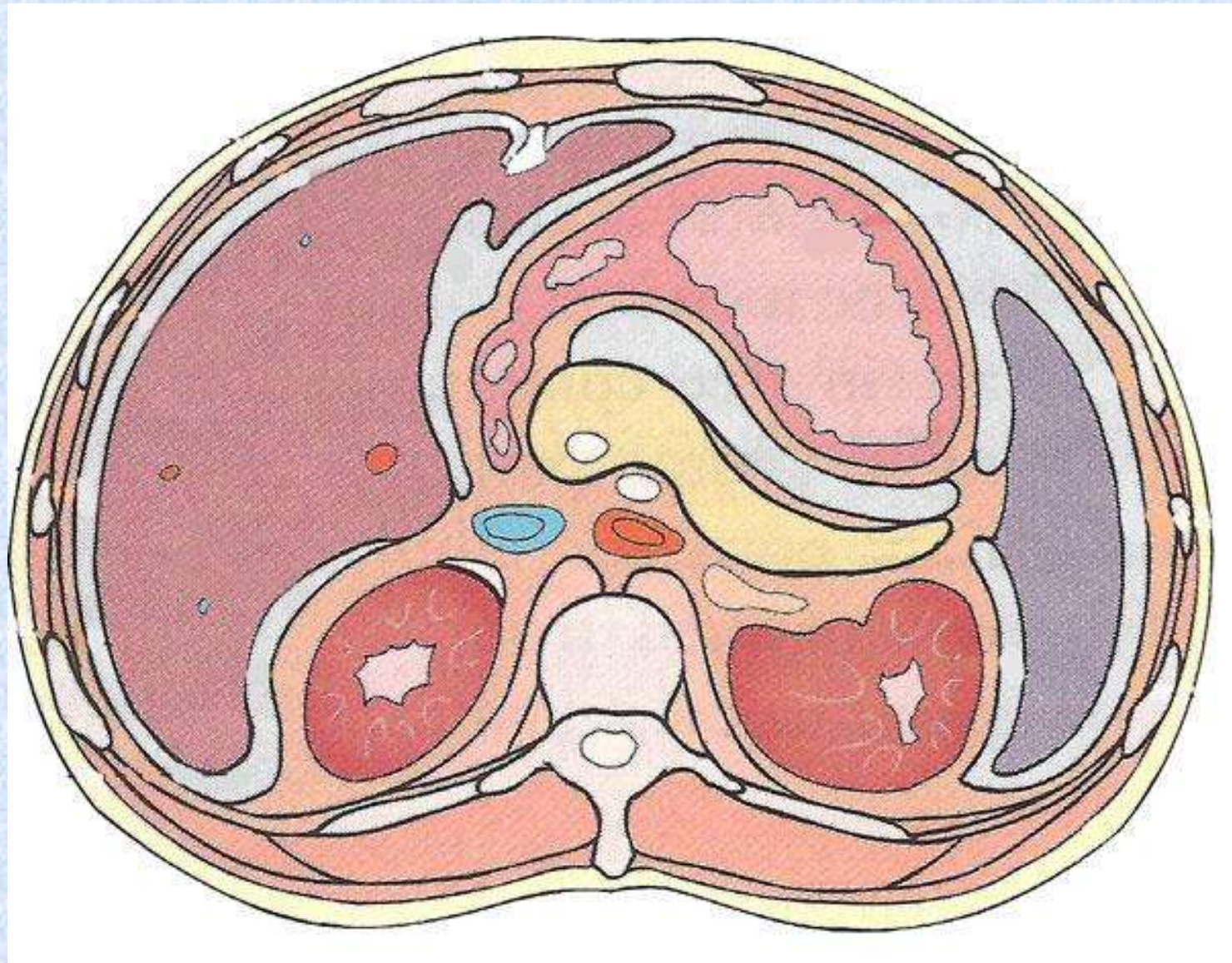


Figure 1. Anatomic location of commonly collected body fluid specimens including pleural, pericardial, and peritoneal fluids. Reprinted with permission from Glasser L: *Extravascular Biological Fluids*. In *Clinical Chemistry: Theory, Analysis, Correlation*. Fifth edition. Edited by LA Kaplan, AJ Pesce, SC Kazmierczak. Philadelphia, Mosby Company. pp 895-903. Copyright Elsevier (2009)



Paracentesis

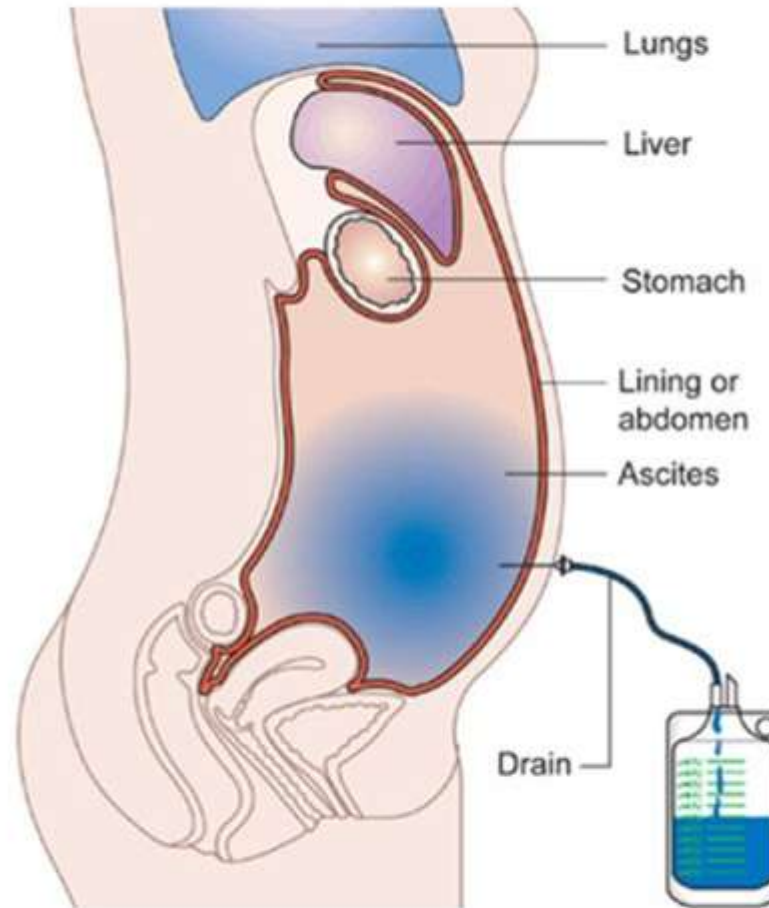
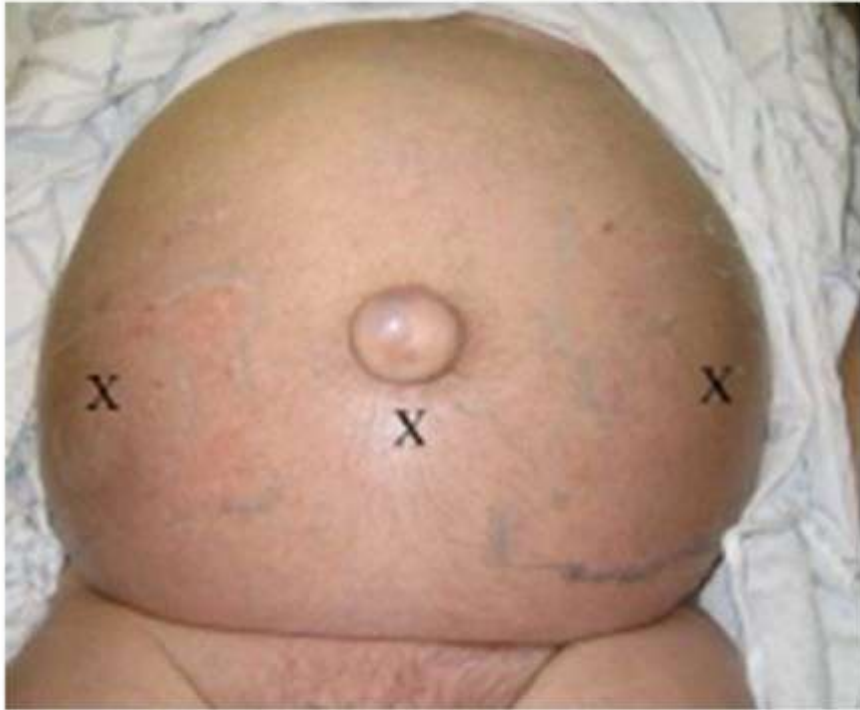
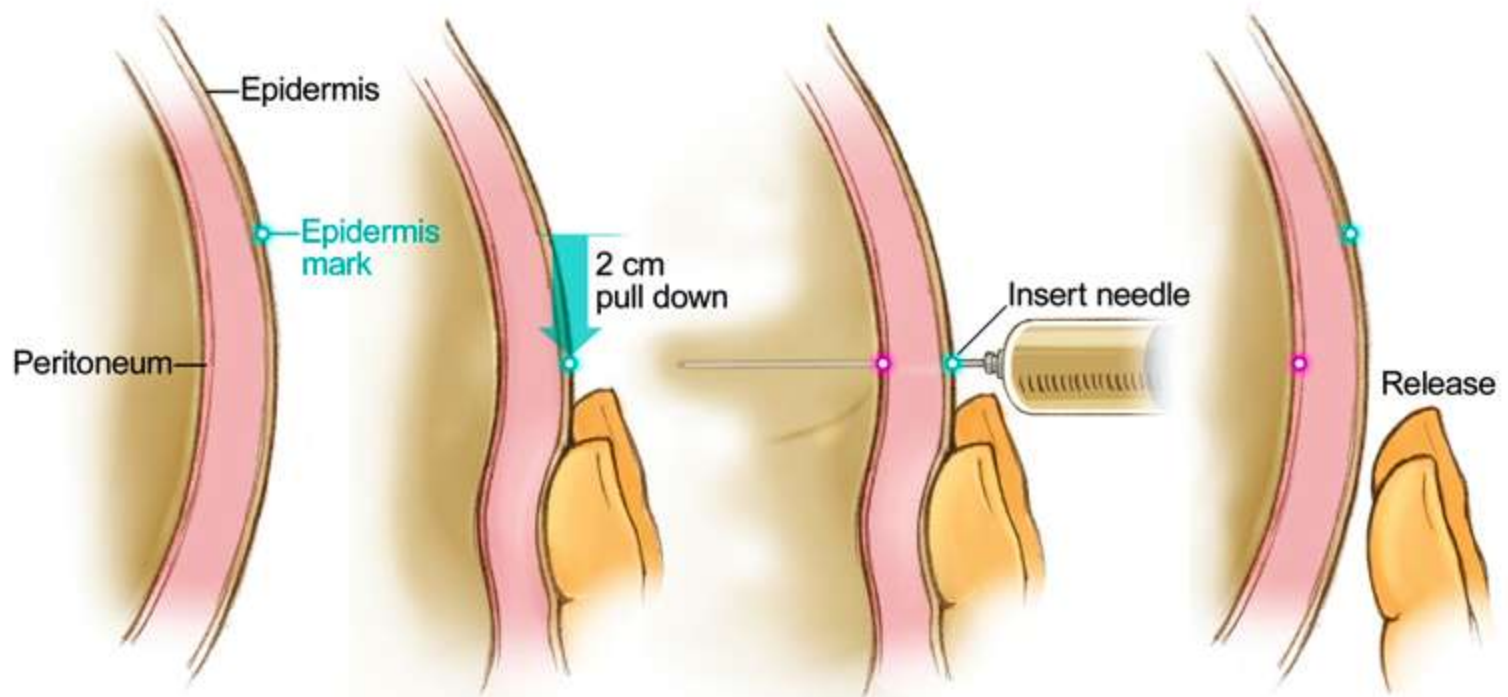


Diagram showing fluid (ascites) being drained from the abdomen
Copyright © CancerHelp UK



Let's
Copyright © 2010, Cognition Studio, Inc. All rights reserved.



Paracentesis

```
graph TD; A[Paracentesis] --> B["EDTA  
For microscopic studies  
(100 ml)"]; A --> C["For biochemical studies  
(30 ml)"];
```

EDTA

For microscopic
studies
(100 ml)

For biochemical
studies
(30 ml)

To diagnose

- Ascites with unknown origin
- Tenderness and abdominal pain
- Intestinal perforation
- Any suspected intra-abdominal malignancies

Distinguishing criteria are not clear

- **Transudate**

- Viral hepatitis and liver necrosis and cirrhosis
- Obstruction of inferior vena cava
- Congestive heart failure
- Nephrotic syndrom

- **Exudate**

- Tuberculous peritonitis
- Neoplasm
- Trauma
- Pancreatitis
- Lymphatic obstruction (as a result of lymphoma)

Causes of Peritoneal Effusions

Transudates: Increased Hydrostatic Pressure or Decreased Plasma Oncotic Pressure

Congestive heart failure

Hepatic cirrhosis

Hypoproteinemia (e.g., nephrotic syndrome)

Exudates: Increased Capillary Permeability or Decreased Lymphatic Resorption

Infections

Primary bacterial peritonitis

Secondary bacterial peritonitis (e.g., appendicitis, bowel rupture)

Tuberculosis

Neoplasms

Hepatoma

Lymphoma

Mesothelioma

Metastatic carcinoma

Ovarian carcinoma

Prostate cancer

Trauma

Pancreatitis

Bile peritonitis (e.g., ruptured gallbladder)

Chylous Effusion

Damage to or obstruction of thoracic duct (e.g., trauma, lymphoma, carcinoma, tuberculosis and other granulomas [e.g., sarcoidosis, histoplasmosis], parasitic infestation)

Gross Appearance

- **Normal: transparent, pale yellow and without turbidity**
- The transudate fluid can be straw colored and clear,
- the color may appear milky in blocked lymphatic vessels (carcinoma, tuberculosis and lymphoma)
- The color may becomes opaque or turbid in any inflammatory conditions such as (appendicitis, pancreatitis and peritonitis).
- Abdominal bleeding or trauma, hemorrhagic pancreatitis or tumor infiltrate may result in hemorrhagic color.
- In case of intestinal perforation, pancreatitis or ruptured gall bladder may cause bile stained or green colored fluid.





Cellular examination

- ⌘ Centrifuge the sample and stain with Wright method
- ⌘ **Manual count using** Neubauer hemocytometer (Electronic counters generally unusable)
- ⌘ **WBC > 500 → bacterial peritonitis**
 - For the tuberculosis and peritoneal carcinomatosis, lymphocytes are predominant.
 - Neutrophils are increased in primary bacterial peritonitis
- ⌘ **Consider malignant cells**

Biochemical examinations

- **Protein** (The diagnostic value of protein is low)
 - in exudate $> 3\text{g/dl}$ but less than 3g/dl in transudate
 - ascetic fluid protein/serum protein $> 0.5 \rightarrow$ exudate
 - serum-ascites albumin $\geq 1.1\text{ g/dl} \rightarrow$ liver disease
- **Glucose** (\downarrow in tuberculosis peritonitis, carcinoma)
- **AMS_{fluid}/AMS_{ser} > 3** pancreatitis and trauma
- **ALP $> 10\text{U/L}$** (\uparrow in Intestinal damage)
- **LD_{fluid}/LD_{ser} $> 0.6 \rightarrow$** malignancy
- **Urea and creatinine** (bladder rupture)
- **Bilirubin** (gall bladder rupture)

	TRANSUDATE	EXUDATE
COLOR	Clear, water-like, or pale yellow of plasma	Cloudy, white, yellow or red
CONSISTENCY	Thin and watery, no tissue fragments	Thick and creamy, contains tissue fragments
ODOR	None	May have an odor
pH	Alkaline	Acid
SPECIFIC GRAVITY	1.015 or lower	1.018 or higher
PROTEIN CONTENT	Low, less than 3%	High, more than 4%
CELL COUNT	Low; none or few WBC and RBC	High; many WBC and RBC
ENZYME CONTENT	Low	High
BACTERIA	None	May be present
INFLAMMATION	None present	Associated with Inflammation

paracentesis (Peritoneal fluid analysis, Ascitic fluid cytology, Peritoneal tap)

Type of test Fluid analysis

Normal findings

Gross appearance: clear, serous, light yellow, <50 mL

Red blood cells (RBCs): none

White blood cells (WBCs): <300/ μ L

Protein: <4.1 g/dL

Glucose: 70-100 mg/dL

Amylase: 138-404 units/L

Ammonia: <50 mcg/dL

Alkaline phosphatase

Adult male: 90-240 units/L

Female <45 years: 76-196 units/L

Female >45 years: 87-250 units/L

Lactic dehydrogenase (LDH): similar to serum LDH

Cytology: no malignant cells

Bacteria: none

Fungi: none

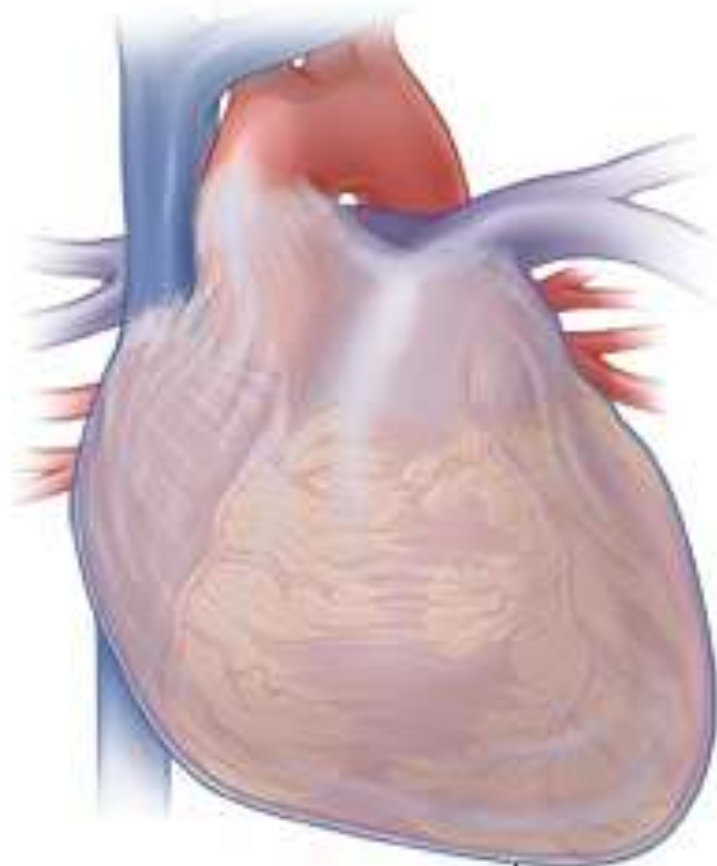
Carcinoembryonic antigen (CEA): negative

Comparison of Exudates and Transudates Based on Laboratory Profile

Laboratory Value	Exudate	Transudate
Clarity	Cloudy	Clear
Color	Yellow-green	Yellow
Common cell type	Segmented neutrophils	Mononuclear cells
White blood cell count (μL)	>1,000 (pleural) >500 (peritoneal)	<1,000 (pleural) <300 (peritoneal)
Clottable fibrinogen	Yes	No
Glucose	\leq plasma	Equal to plasma
Total protein	>50% of plasma value	<50% of plasma value
Fluid-to-plasma total protein ratio	>0.5	<0.5
Lactate dehydrogenase	>60% of plasma value	<60% of plasma value

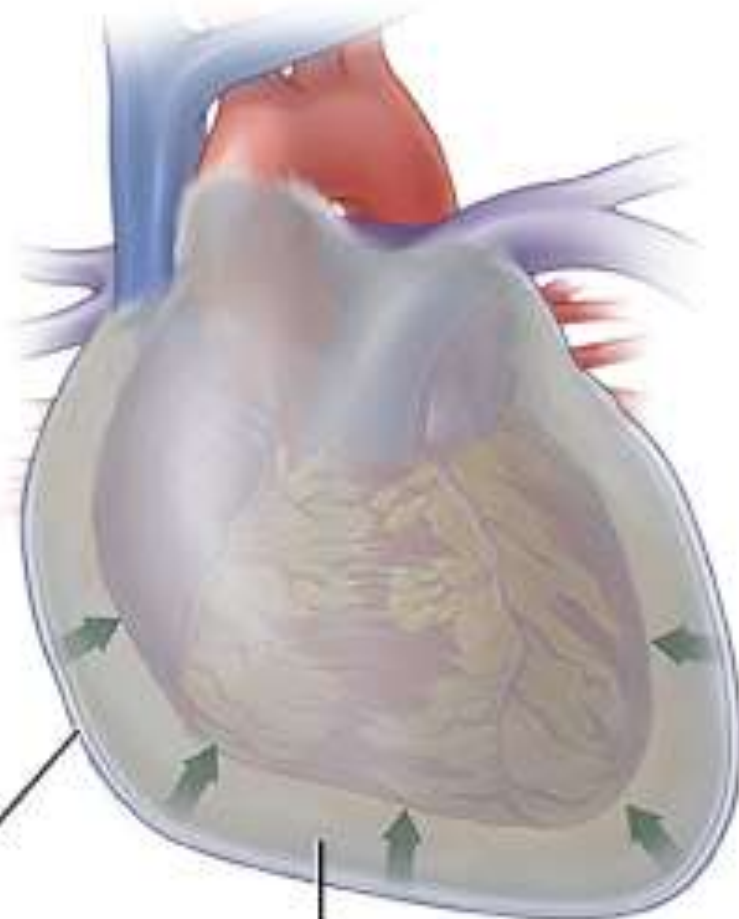
Pericardial fluid

Normal heart

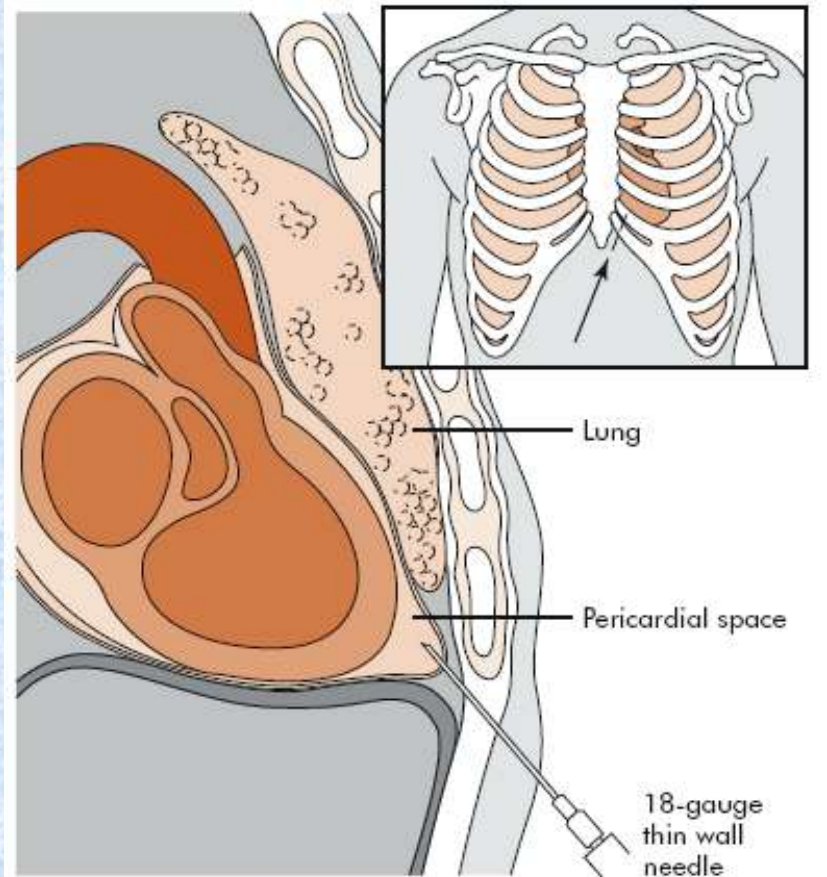
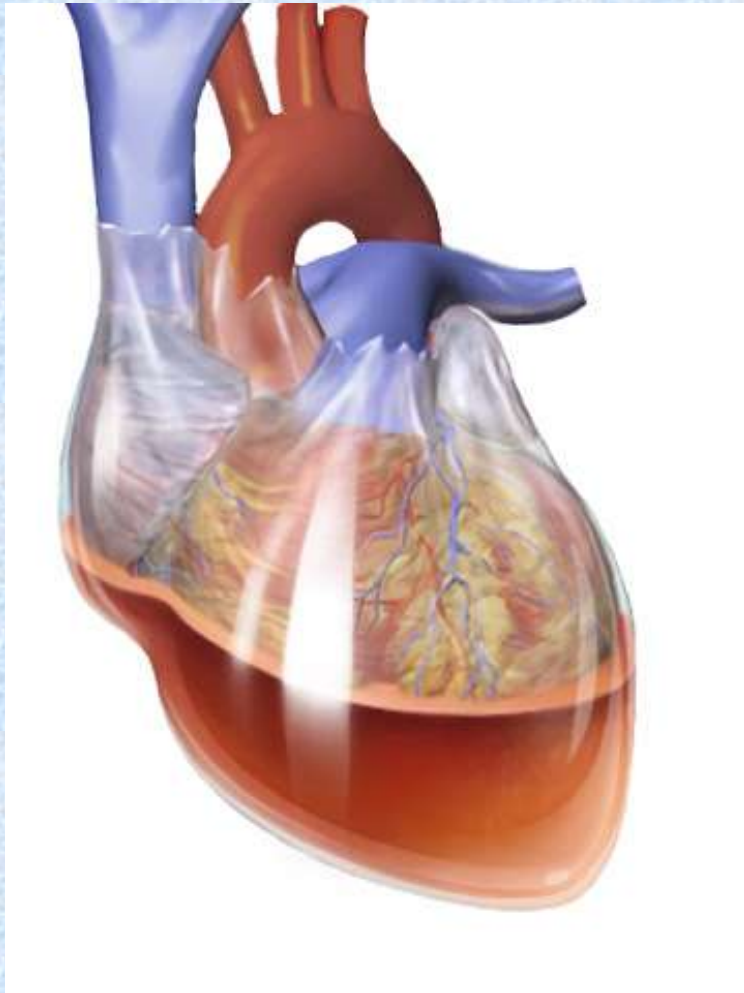


Pericardium

Pericardial effusion



Buildup of fluid



Pericardiocentesis using subxyphoid route for aspiration of pericardial fluid. The 18-gauge needle is introduced at a 30- to 40-degree angle.

Cause of effusion

- Bacteria
- AIDS
- Metastatic cancer
- Lymphoma
- Anti-coagulant therapy
- trauma
- SLE

Exudate criteria

- **WBC** > 10,000 in malignancy and bacterial infection
- **Protein** > 3 g/dl
- **Glucose** < 60 mg/dl
- **Glucose** < 40 mg/dl (bacteria and cancer)
- **LD_{fluid}/LD_{ser}** > 0.6
- **ADA** > 40 U/L → Tuberculosis

ADA is produced by lymphocytes in the areas where *Mycobacterium tuberculosis* are present.

- **ANA** in SLE

Amniotic fluid

AF collection

- Sample should be covered by aluminum foil to protect from direct light.
- Refrigerated and delivered to the Lab as soon as possible
- Polyhydramnios and oligohydramnios affect the results
- Contamination with maternal blood:
Correction: $0.05 \times \Delta A_{410} - \Delta A_{450}$

Why analysis of amniotic fluid?

1. Chromosomal abnormality and inherited metabolic diseases
2. Congenital anomalies (NTD)
3. Rh sensitization
4. Assessment of fetal lung maturity (FLM)

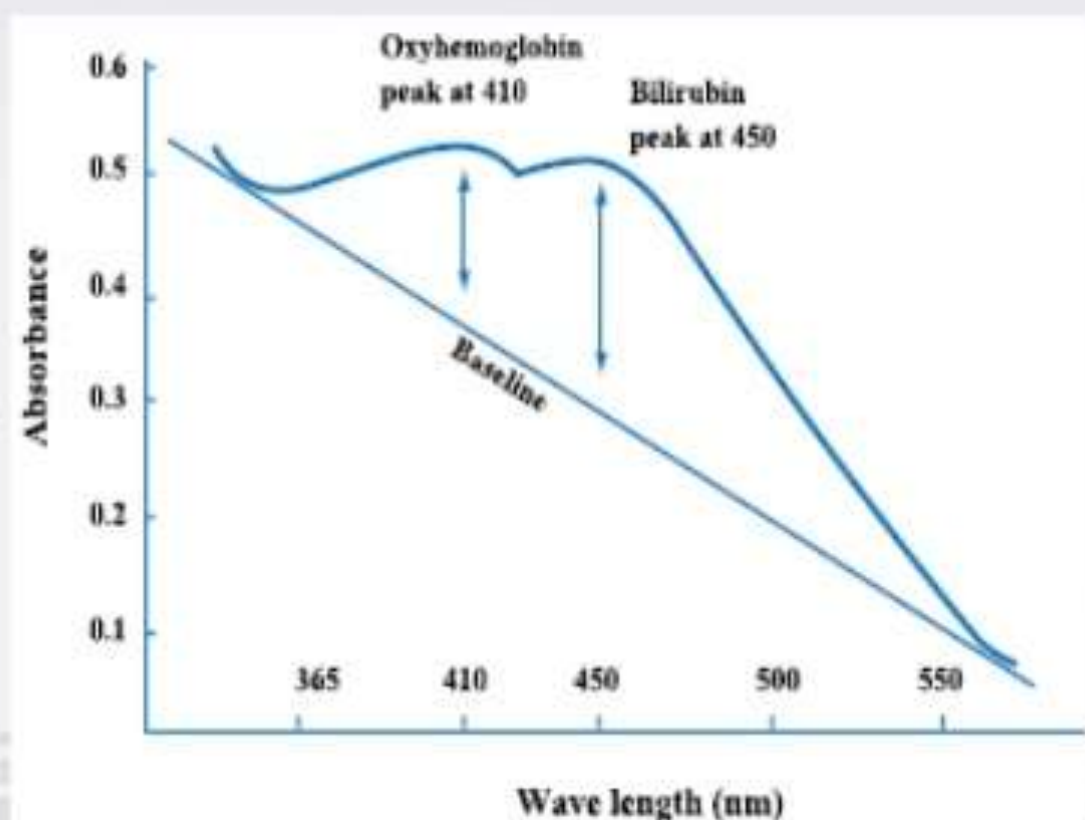
Rh sensitization

- Amniotic fluid bilirubin is measured
- AF is scanned spectrophotometrically over a spectrum of wavelength and bilirubin level is reflected by the absorbance change at 450 nm (ΔA_{450}).
- The ΔA_{450} is then used to predict the severity of hemolytic disease by plotting the ΔA_{450} on the Liley graph.

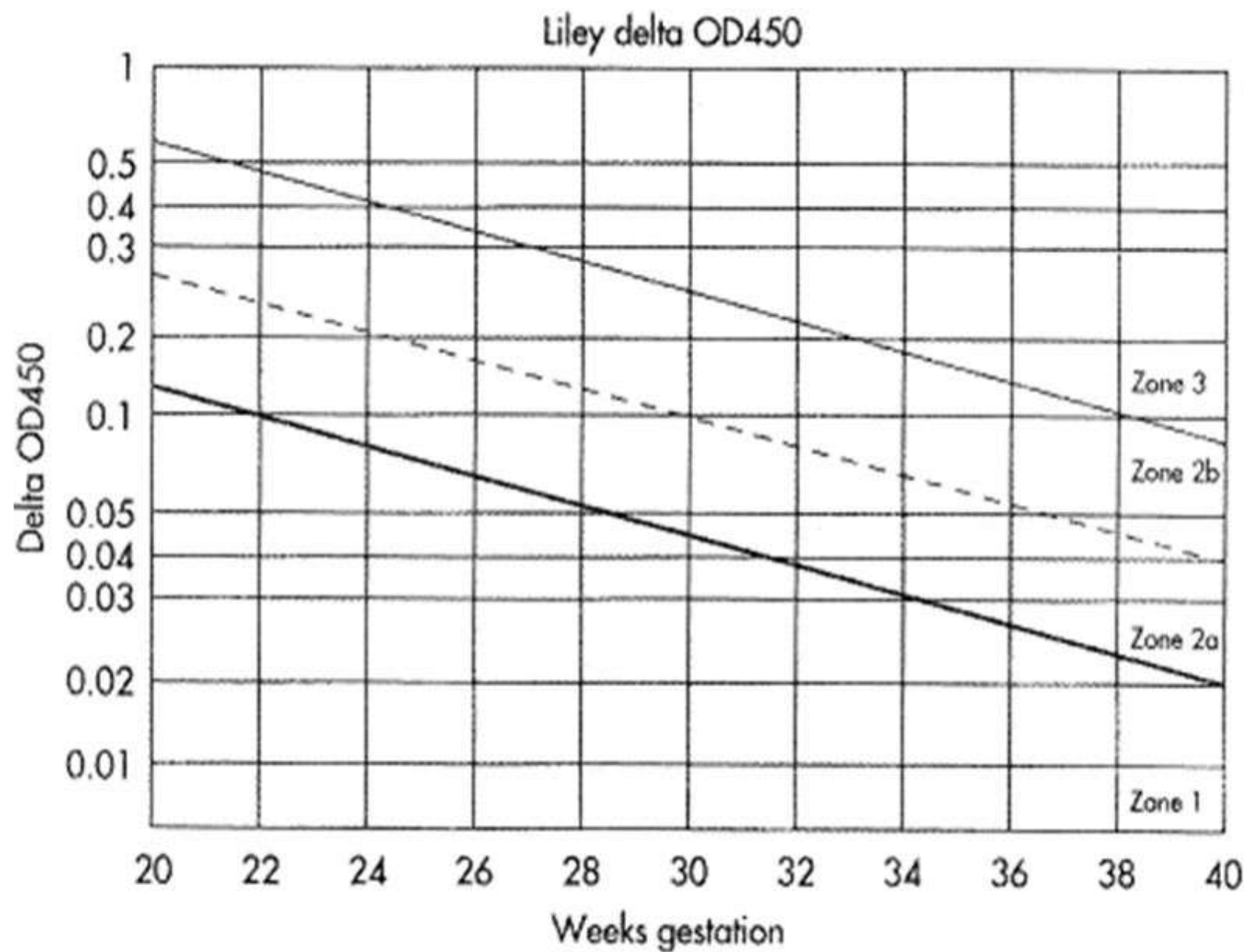
Liley Chart

- a chart that uses the spectrographic measurement of amniotic fluid bilirubin levels plotted against gestational age to estimate the severity of fetal hemolysis resulting from Rh isoimmunization. The chart is divided into three zones; a measurement falling in zone 1 indicates no disease or mild disease, while one falling in zone 3 indicates severe disease with impending fetal death.

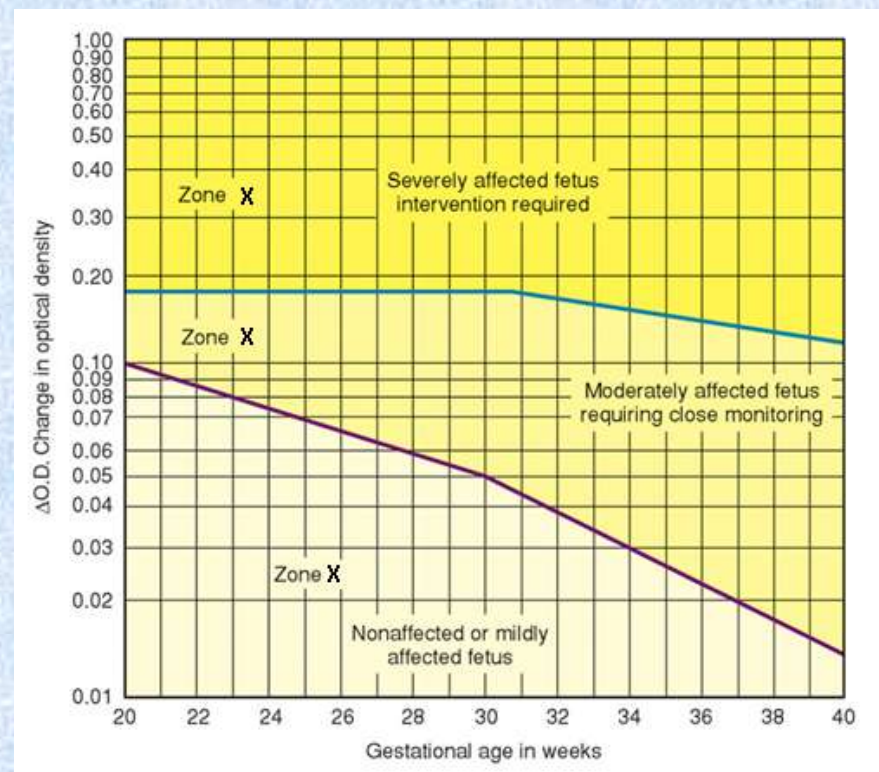
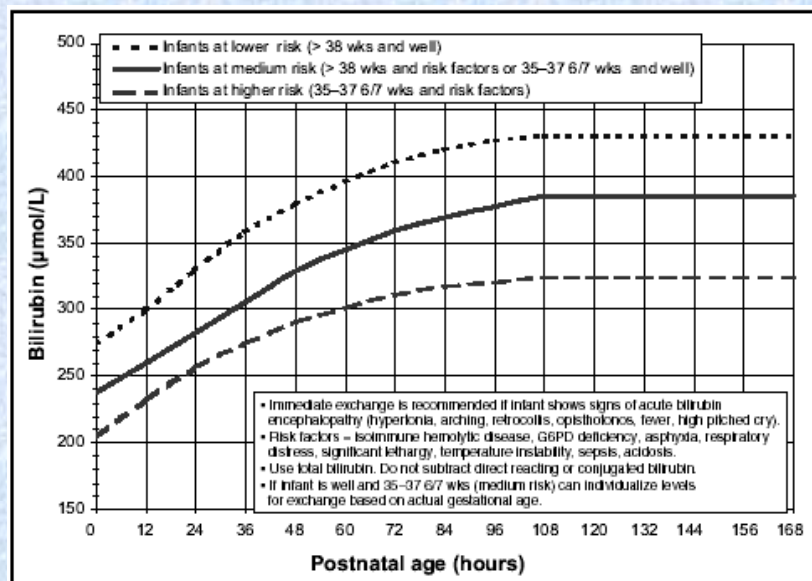
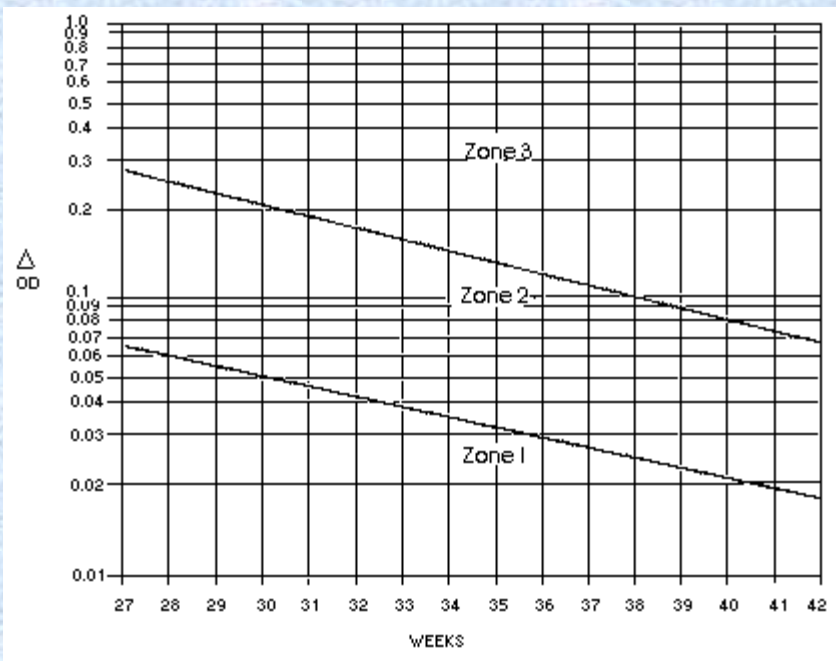
- When bilirubin is present, a rise in OD will be seen at 450 nm because this is the wavelength of maximum bilirubin absorption.
- The amount that the curve deviates from a straight line at 450 nm (the ΔA_{450}) is *directly proportional to the* amount of bilirubin in the amniotic fluid.



Spectrophotometric
bilirubin scan showing
bilirubin and
oxyhemoglobin peaks.

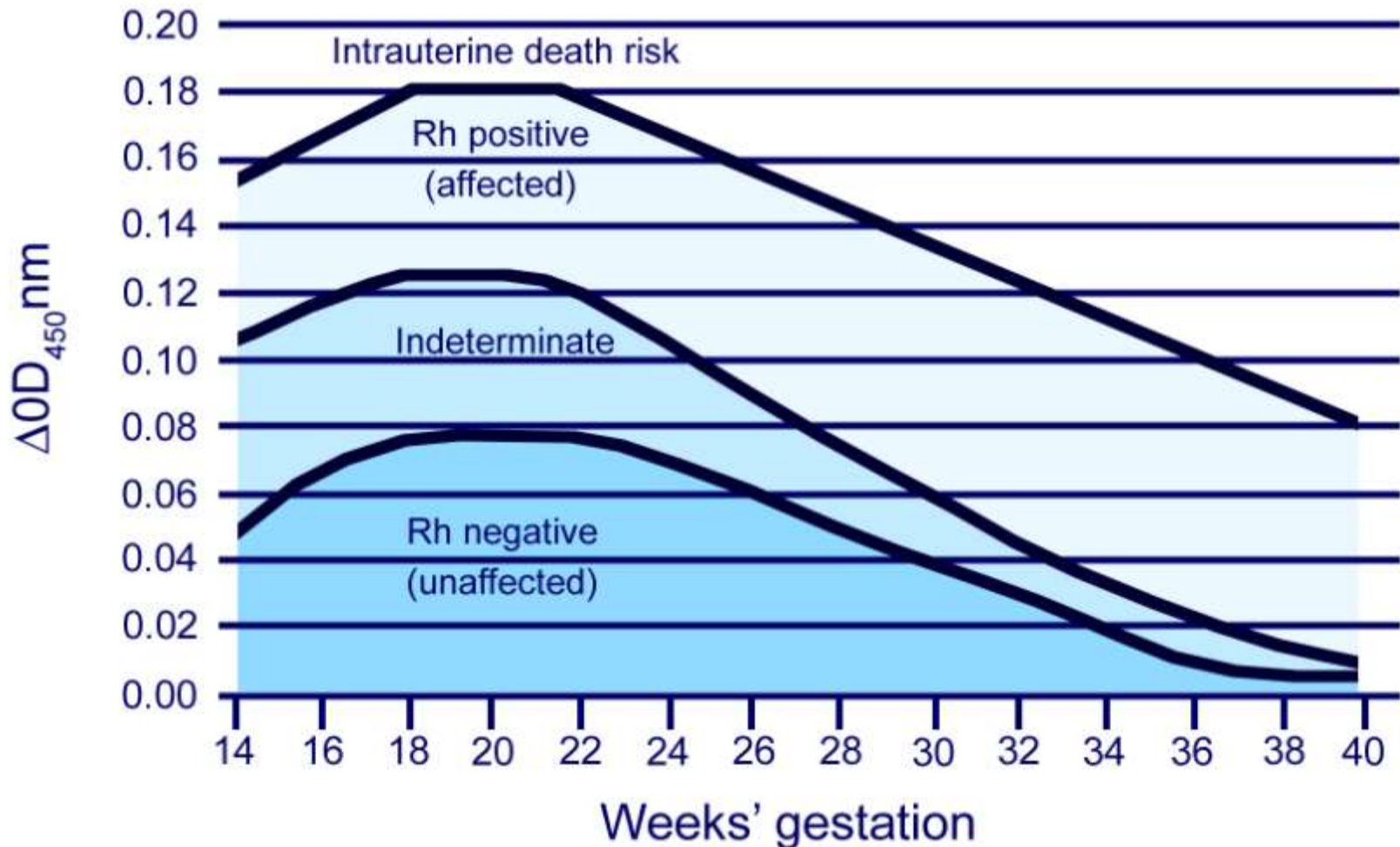


Extended Liley graph.



Guidelines for exchange transfusion in infants

Queenan curve for ΔOD_{450} values



NTD risk assessment

- AFP
- Choline esterase:
- Acetyl choline esterase (not found in normal embryo)
- Non-specific choline esterase

Assessment of fetal lung maturity (FLM)

- Respiratory distress syndrome
- **Lecithin/Sphingomyelin** (L/S) ratio (gold standard)
- **Normal lung:** $L/S \geq 2$
- Assay method: precipitation by cold acetone and then TLC
- **Phosphatidyl glycerol** (PG)
- **Foam-shake test:** amniotic fluid+ E-OH (1/1) → shake for 15'' → foam layer that is stable for 30''

L:S ratio	(Some laboratories use these values)	Lung Maturity
<1	<2.0	Very immature lungs (up to 30th wk of gestation); severe RDS is expected; lung maturity may require many wks; do not resample before 2 wks.
1.0– 1.49		Immature lungs; moderate to severe RDS is expected; lung maturity may occur in 2 wks; resample in 1 wk.
1.5– 1.9	2.0–3.0	Lungs on threshold of maturity (within 14 days); mild to moderate RDS may occur. Test should be repeated in 1 wk.
≥2	>3.0	Mature lungs (35th wk of gestation); low incidence of RDS even if PG is absent.
Abundant lecithin with trace or no sphingomyelin		Postmature lungs

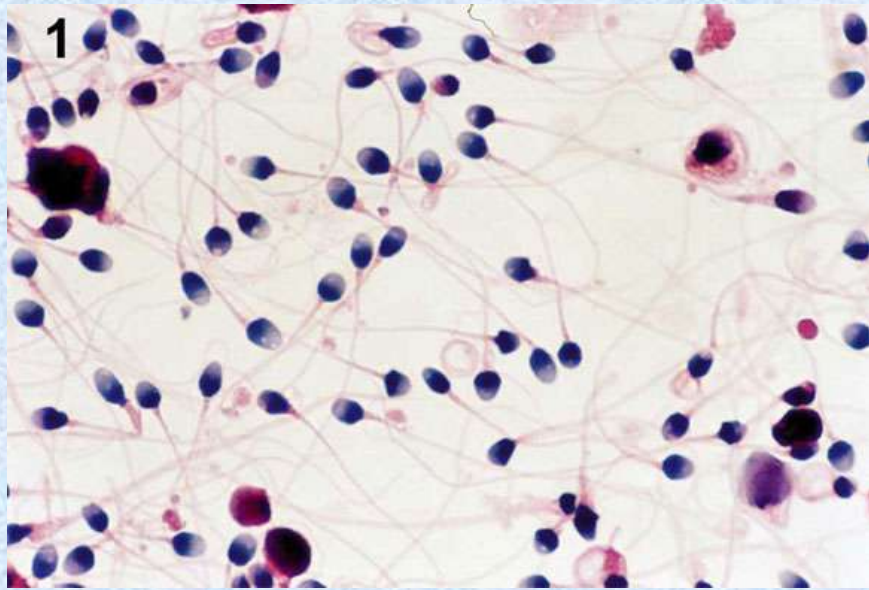
Semen

Sample Collection

- after 2 to 5 days of sexual abstinence
- The bladder should be evacuated before ejaculation occurs.
- Collect the specimen in sterile polypropylene
- Sexual intercourse with condoms or masturbation
- Be sent to the laboratory within **one hour**
- Start examination after liquefaction (less than 20 minutes)

Semen Analysis

- Volume, sperm count, motility, morphology, agglutination
- 4-8 μ l semen is required for microscopic study
- **Sperm count**: dilute semen with diluent
- Diluent: NaHCO_3 (5 gr)+ formalin (1ml) in 100 ml distilled water
- For morphologic study:
- Smear preparation \rightarrow fixation (EOH 95%) \rightarrow staining with papanicolaou



Spermatozoa stained with H-E



Papanicolaou staining

Sperm Analysis

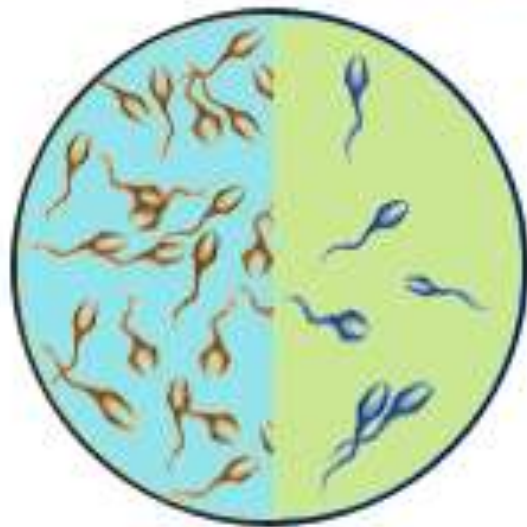
Semen Analysis	Sperm			
Volume (ml)	1.00	ml	2 - 5	7.2-7.8
PH	8.00		7.2 - 8	
Color	Milky			
Viscosity	Normal			
Liquifaction time	30	20'		
Sperm Count	5.63	20mil/ml		
A:Fast progressive	0.00			
B:Slow progressive	0.00			
C:Non progressive (%)	0.00	50%		
D:Non otile(%)	100.00			
Live Ratio (A+B+C)	0.00			
Normal Spermatozoa	61.54			
Defective Spermatozoa (%)	H 38.46		2- 3	
-Sperm With head defect (%)	38.46		After 20 Min	
-Sperm With neck defect (%)	0.00		>60:Good,20-60,Average,0-20:poor	
-Sperm With tail defect (%)	7.69	%	>50:Good,30-50,Average,0-30:poor	
Teratozospemic index	1.20		>30:Good,20-30,Average,0-20:poor	
WBC/hpf	0.08			

Fructose assay (in the case of low semen volume and low sperm count)



normal
sperm count

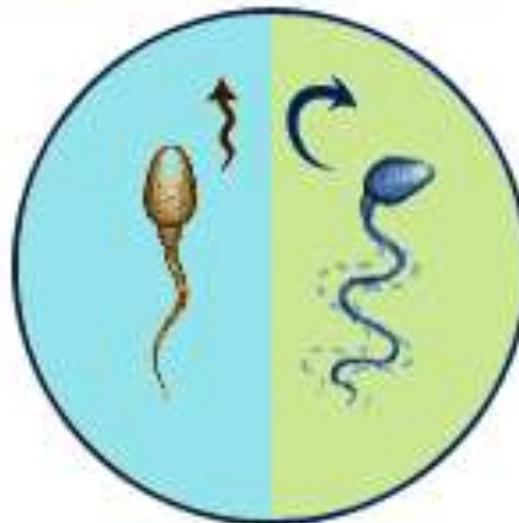
low sperm
count



sperm count

normal
progression

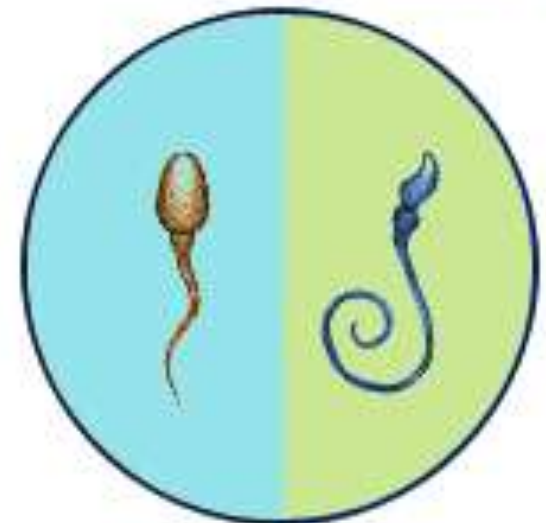
poor
motility



sperm motility

normal
shape

abnormal
shape



sperm morphology



forensic medicine

- Sperm watching
- Acid phosphatase
- Blood group
- Florence test: sample + KI → choline-periodate crystal (Dark brown and needle shaped)
- Florence reagent: (8%) W/V solution of Iodine in water containing 5% W/V of Potassium Iodide)

Saliva

- Easy to collect
- Because it is a filtrate of plasma, it contains some small molecules that are in equilibrium with the free (unbound) active forms of those substances in plasma.
- especially useful for measuring free cortisol
- Unfortunately, measurement of other steroid hormones are not reliable because of rapid fluctuations in their salivary concentrations
- Antibodies against HIV
- drugs testing (such as amphetamines, cocaine, and opioids)
- Genetic testing using buccal cells in saliva

Meconium

- for the detection of illicit drugs (cocaine and amphetamines) that the mother might have abused while pregnant; the drugs goes to the fetus, which excretes the drug in bile that remains in the meconium until birth
- measurement of ethyl glucuronide in meconium was found to be an objective measure of maternal alcohol use.



thank
you

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