

Body fluid analysis in clinical lab



- Collection
- Turn around time
- Physician/laboratory communication
- Reliable reference values



- CSF
- Synovial fluid
- Peritoneal fluid
- Pleural fluid
- Pericardial fluid



CSF Formation and Circulation



- **Formation CSF**
 - Choroid plexus
- **Circulation**
 - 500 mL/day
 - Total volume 150 mL

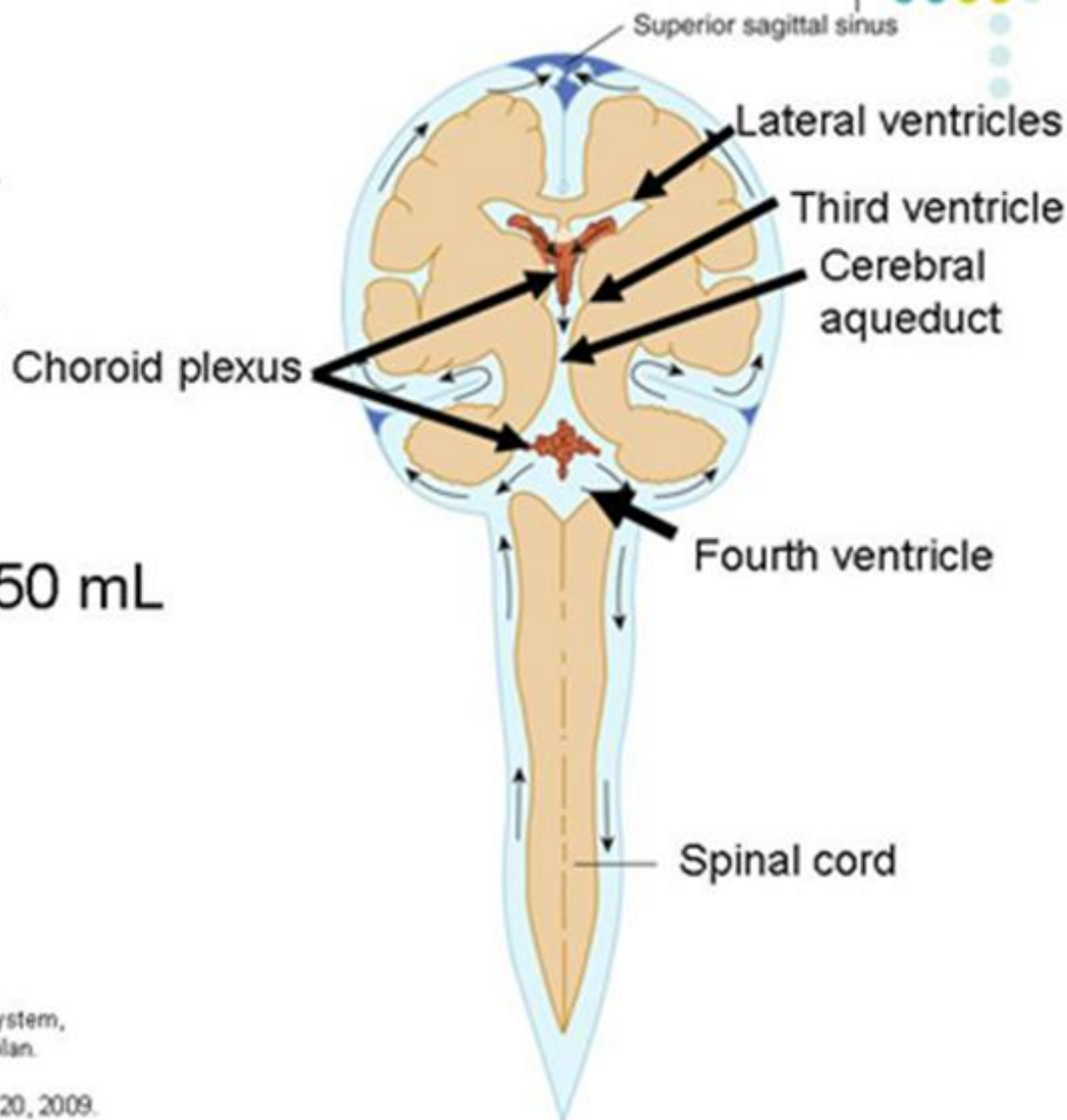


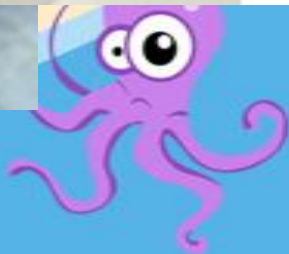
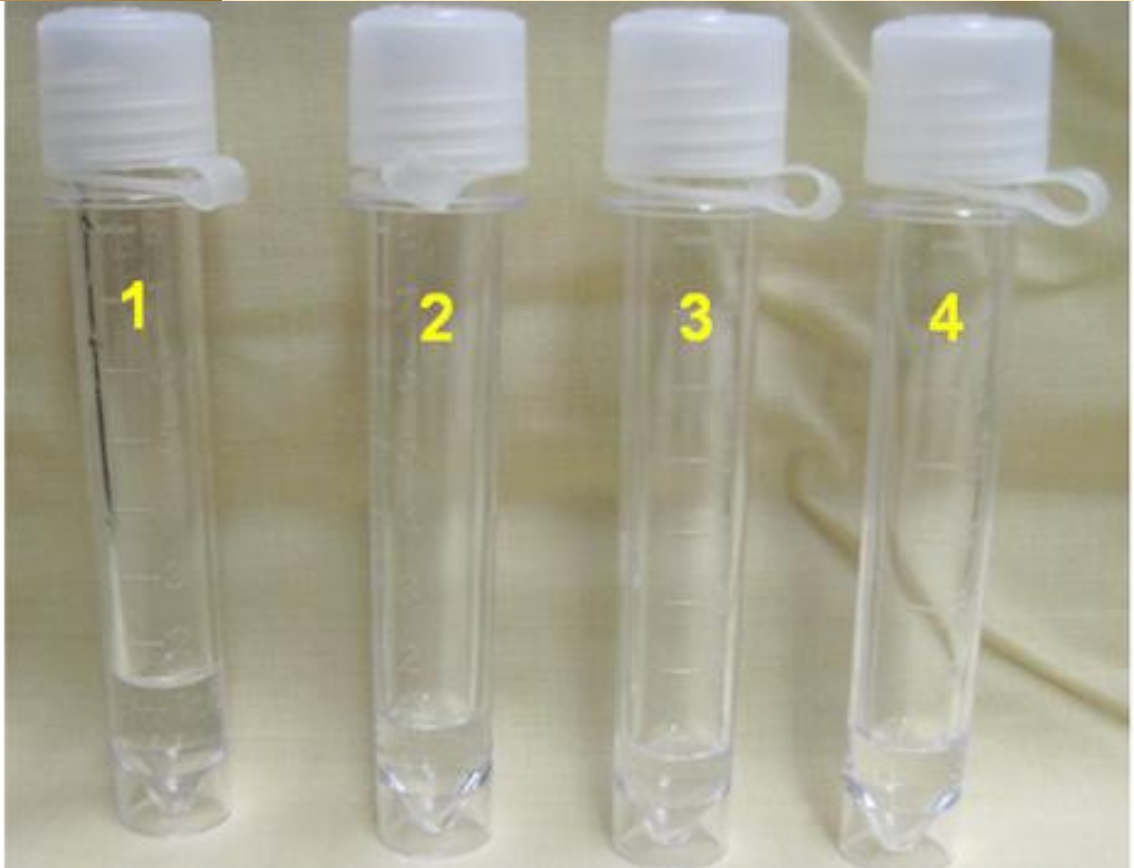
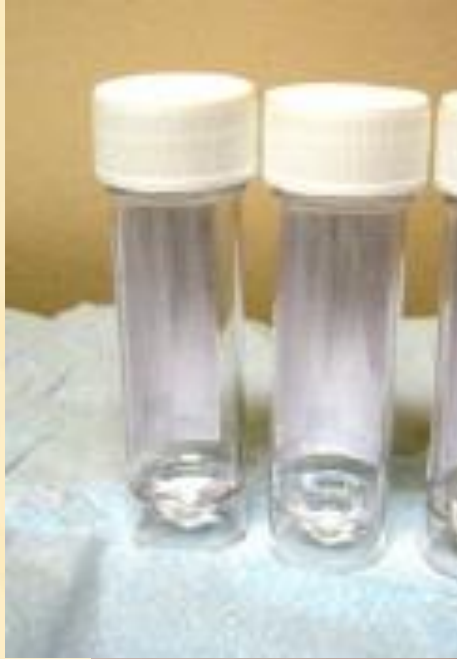
Figure 11-7. Two major divisions of the central nervous system, the brain and the spinal cord, as seen in the Midsagittal plan.
In: Waxman SG. *Clinical Neuroanatomy*, 26th ed.
<http://www.accessphysiotherapy.com>. Accessed October 20, 2009.

REASONS TO TEST

- Meningitis
- SAH
- Malignancy
- Demyelinating disease



Specimen collection



Required

Opening CSF pressure

Gross Examination

Total cell count and differential

Glucose (CSF/plasma ratio)

Protein

Optional

Cultures, gram stain, antigens, cytology

Protein electrophoresis, VDRL, D-dimers



CSF Cloudiness / Turbidity



A simple test of CSF turbidity is to see if normal print can be read easily through the sample – CSF should be crystal clear.

Cloudiness usually appears at CSF WBC counts $> 200 \times 10^6$ Wbc per L



Xanthochromia

TABLE 29-3

Xanthochromia and Associated Diseases/Disorders

CSF supernatant color	Associated diseases/disorders
Pink	RBC lysis/hemoglobin breakdown products
Yellow	RBC lysis/hemoglobin breakdown products Hyperbilirubinemia CSF protein >150 mg/dL (1.5 g/L)
Orange	RBC lysis/hemoglobin breakdown products Hypervitaminosis A (carotenoids)
Yellow-green	Hyperbilirubinemia (biliverdin)
Brown	Meningeal metastatic melanoma



Traumatic tap

- Decreasing amounts of blood (Last tube will have less)
- Clot present

SAH

- All tubes uniform
- No clot
- Hemosiderin/
hematoidin pigment



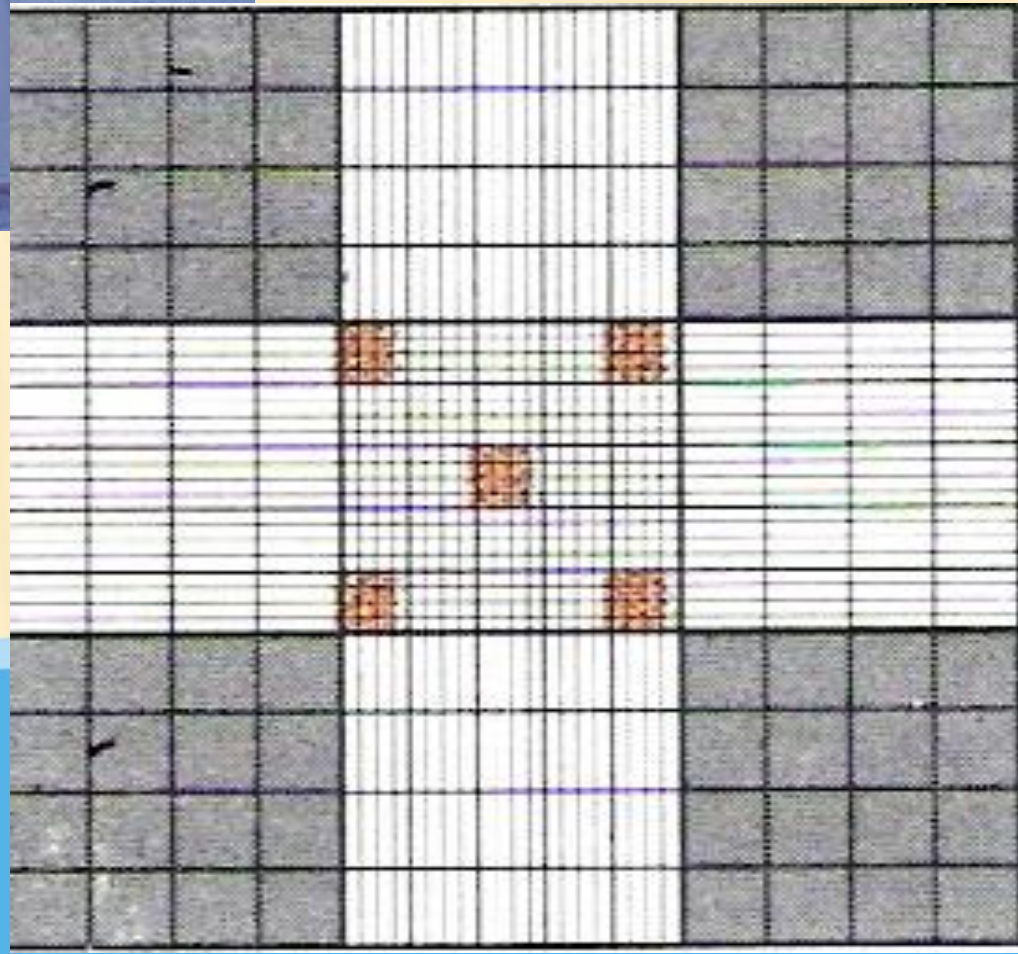
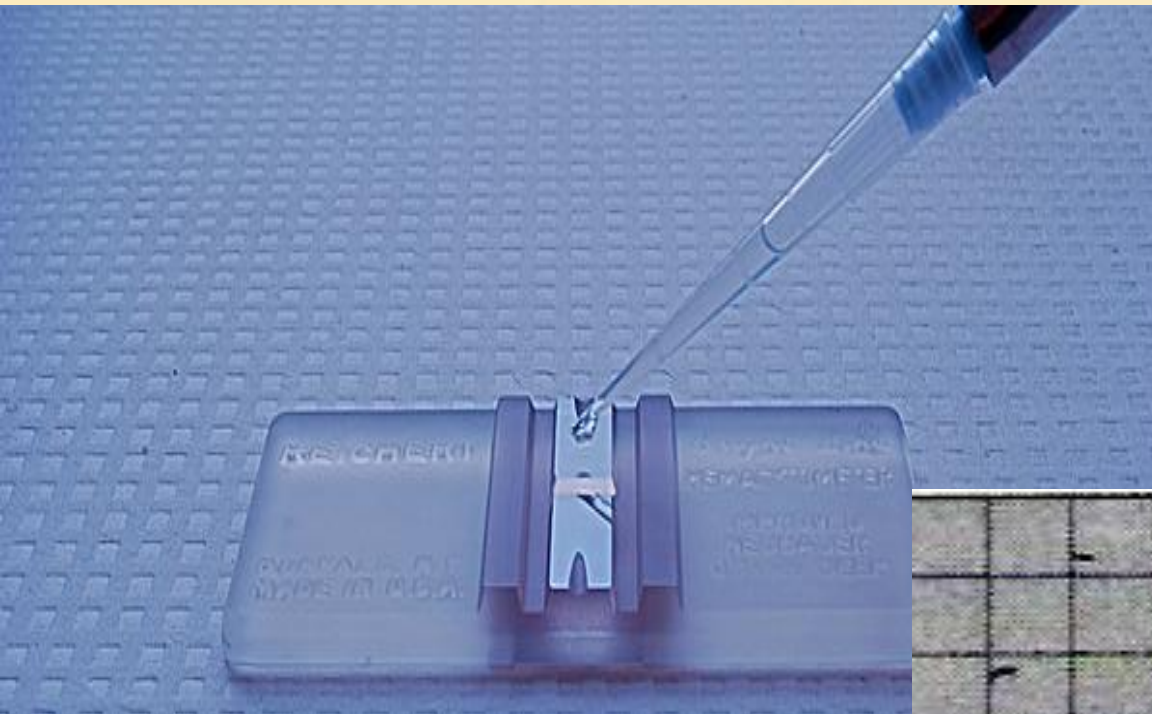
Microscopic examination

Total leucocyte count:

Adults	Neonates
0-5 cells/ μ l	0-30 cells/ μ l

- 7% neutrophils with normal WBC
- $WBC_{corr} = WBC_{obs} - WBC_{added}$
- $WBC_{added} = WBC(BLD) \times RBC(CSF) / RBC(BLD)$







Where to count

- a) < 200 cells are present in all nine squares, count all nine squares. This area counted is 9 mm^2 .
- b) > 200 cells are present in all nine squares, then count the **four corner squares**. This area counted is 4 mm^2 .
- c) > 200 cells are present in **one square**, then count five of the squares within the center square for an area of 0.2 mm^2

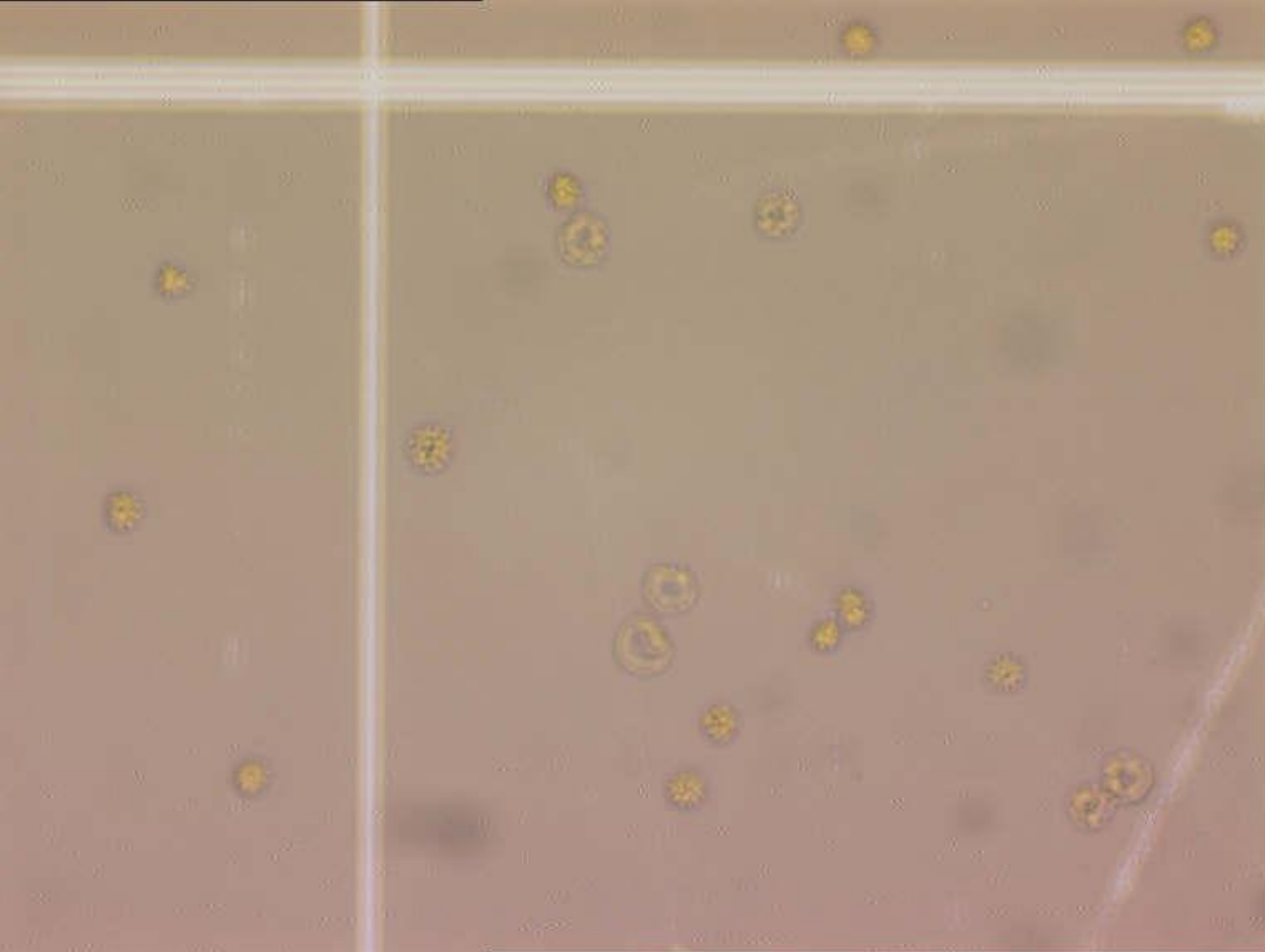


Calculation of Cell count

$$\text{Total cell count} = \frac{N \times \text{Dilution factor}}{\text{Area of total squares counted} \times \text{Depth}}$$

Correlation of cell count with cytomorphological findings is essential.

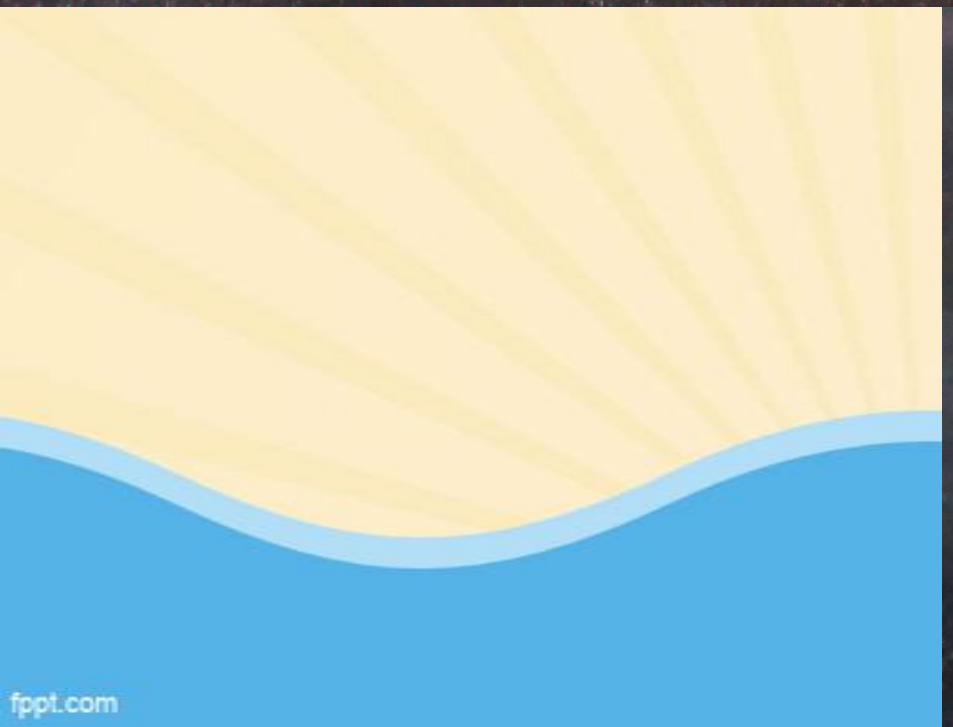
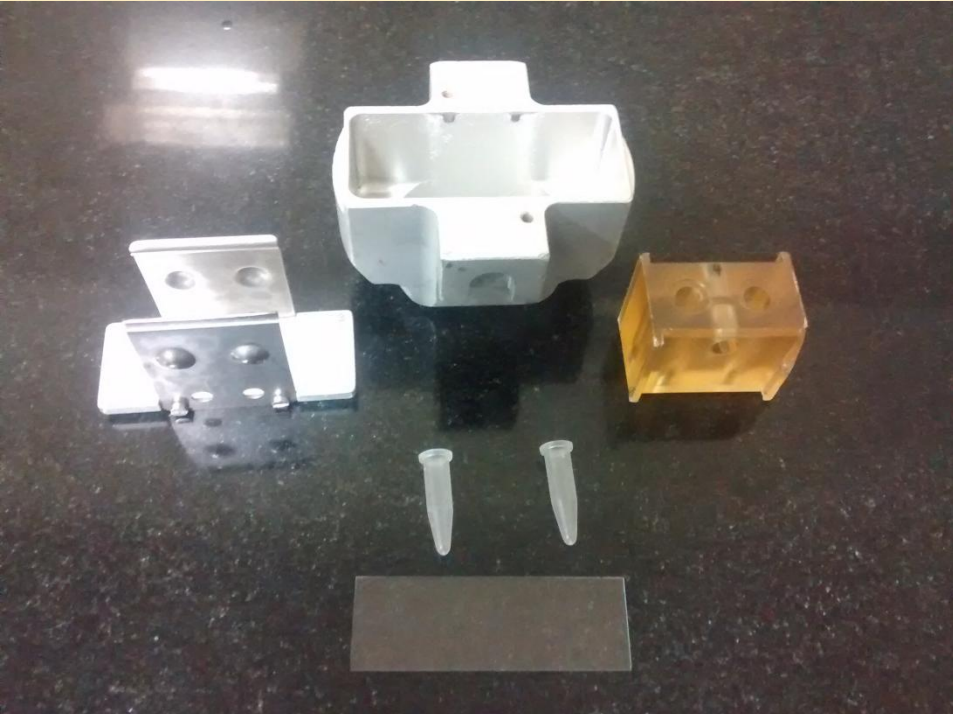




Slide for DLC

- Cytocentrifuge, 500g for 5 min
- 2 drops of 22% albumin
- Wrights stain









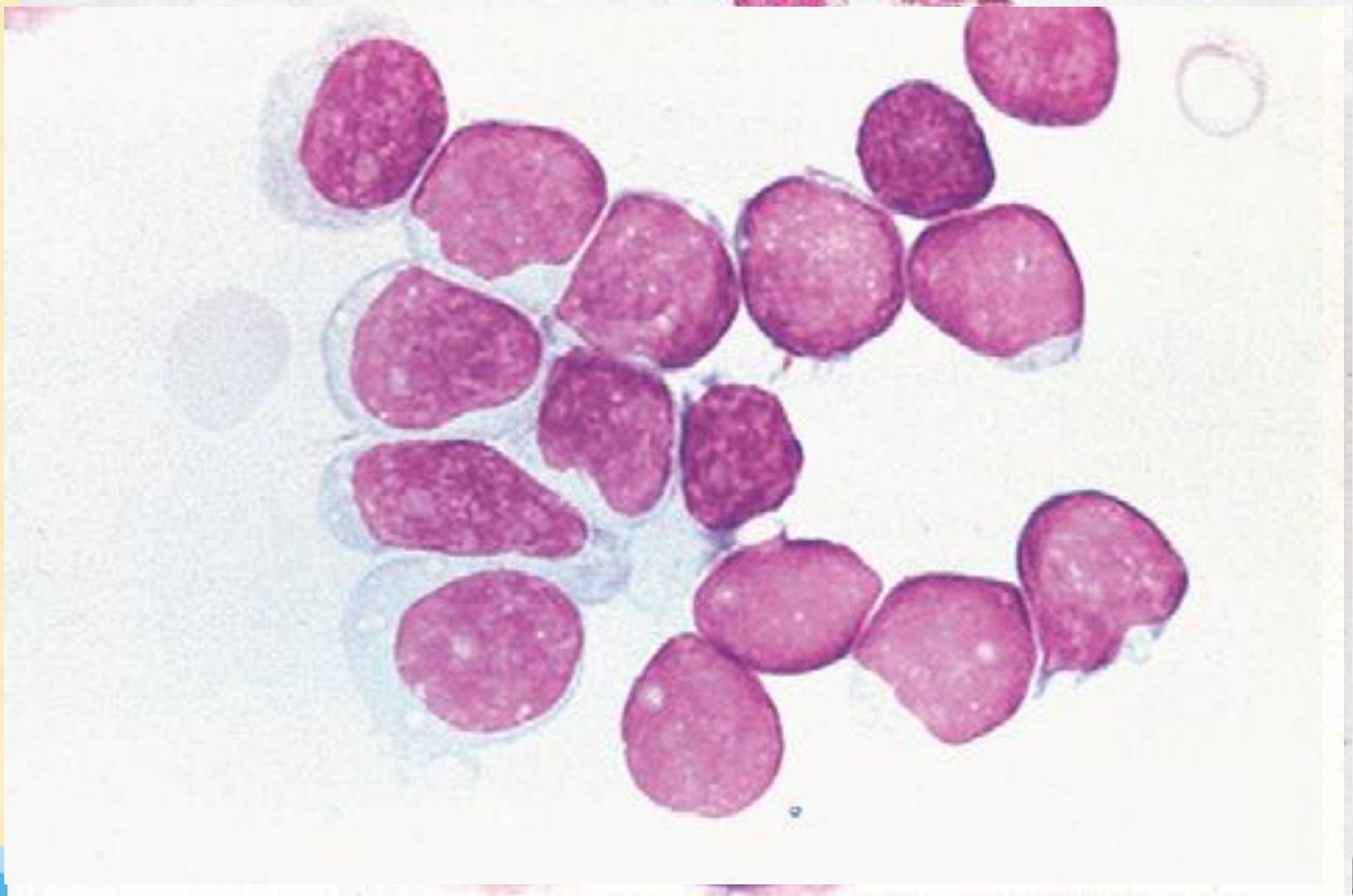
A



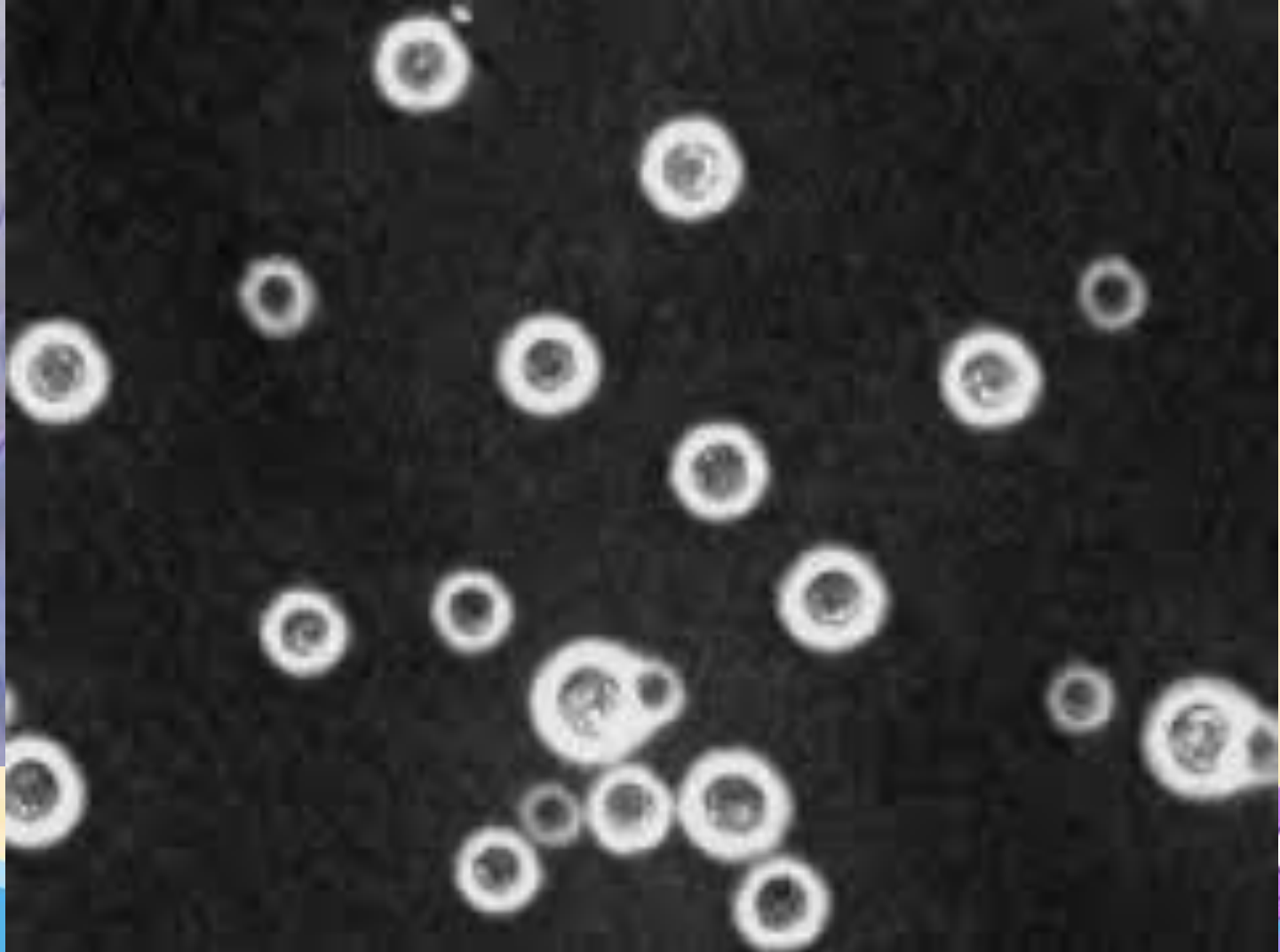
B



C



Parasites



Causes of CSF Lymphocytosis

Meningitis

- Viral meningitis

- Tuberculous meningitis

- Fungal meningitis

- Syphilitic meningoencephalitis

- Leptospiral meningitis

- Bacterial due to uncommon organisms

- Early bacterial meningitis where leukocyte counts are relatively low

- Parasitic infestations (e.g., cysticercosis, trichinosis, toxoplasmosis)

- Aseptic meningitis due to septic focus adjacent to meninges

Degenerative disorders

- Subacute sclerosing panencephalitis

- Multiple sclerosis

- Drug abuse encephalopathy

- Guillain-Barré syndrome

- Acute disseminated encephalomyelitis

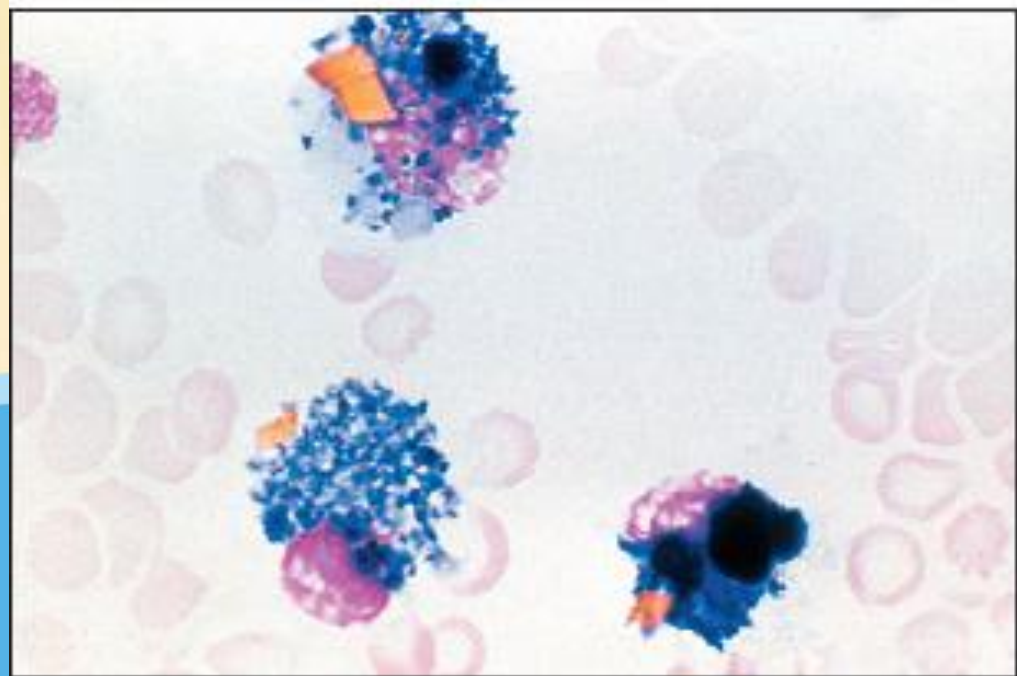
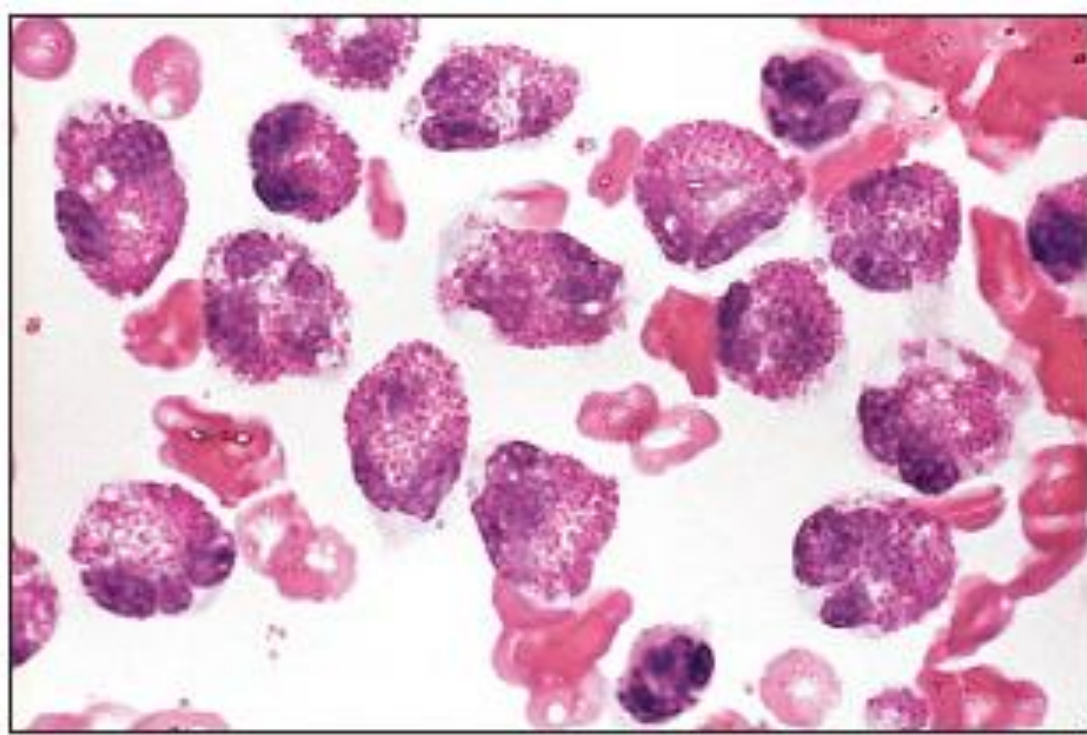
Other inflammatory disorders

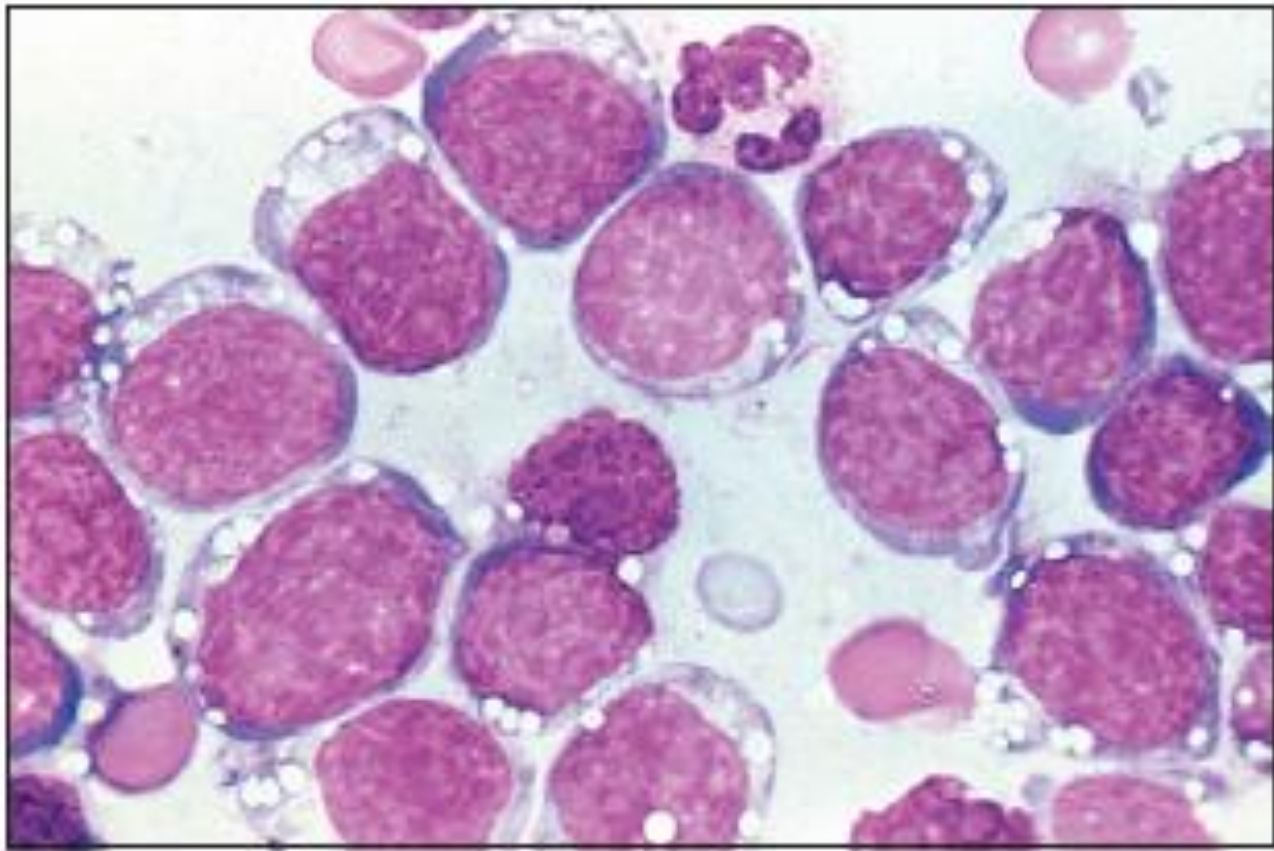
- Handl syndrome (headache with neurologic deficits and CSF lymphocytosis)

- Sarcoidosis

- Polyneuritis

- CNS vasculitis





Typical Lumbar CSF Findings in Meningitis

Test	Bacterial	Viral
Opening pressure	Elevated	Usually normal
Leukocyte count	$\geq 1000/\mu\text{L}$	$< 100/\mu\text{L}$
Cell differential	Mainly neutrophils*	Mainly lymphocytes [†]
Protein	Mild–marked increase	Normal–mild increase
Glucose	Usually ≤ 40 mg/dL	Normal
CSF/serum glucose ratio	Normal–marked decrease	Usually normal
Lactic acid	Mild–marked increase	Normal–mild increase

Data from Bodv. 1987; Tang. 1988; Arevalo. 1989; Fishman. 1992; Wubbel. 1998; Zunt. 1999.



Synovial fluid

Imperfect ultra filtrate of plasma combined with hyaluronic acid

FUNCTIONS

- Lubricate
- Provide nutrients
- Remove debris

REASONS TO TEST

- Sepsis
- Hemorrhage
- Crystal induced inflammation



Specimen collection

<u>Test</u>	<u>Anticoagulant</u>	<u>Volume (mL)</u>	<u>Comments</u>
Cell count, differential, crystals, inclusions	Heparin, EDTA	3-5	Can be done on a few drops of fluid. Mix thoroughly.
Glucose	Fluoride or none	3-5	8-hr. fast preferred
Protein	None		
CH50	None		Freeze if not tested immediately.
C3, C4	None or EDTA		Requires 1 mL
Culture	SPS, none, or anticoagulant without bactericidal or bacteriostatic effect	3-5	Sterile tube required

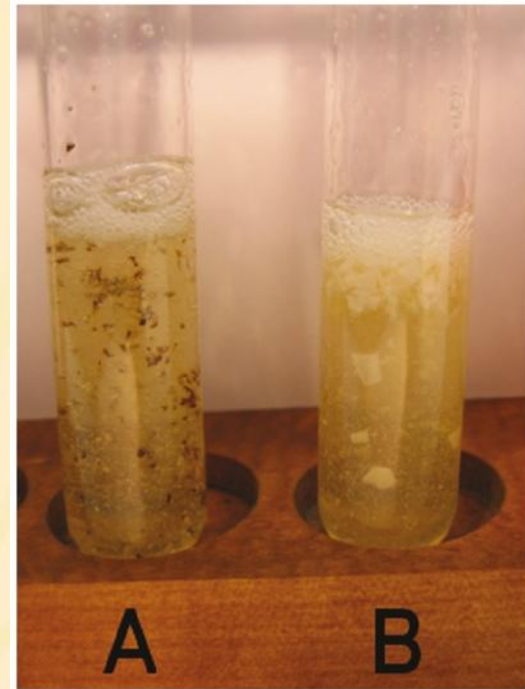


GROSS

- Color
- Volume
- Clarity

LOSS OF CLARITY

- Leucocytes
- Crystals
- Rice bodies
- Onychonosis



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Recommended Synovial Fluid Tests

Routine tests

Gross examination (color, clarity)

Total and differential leukocyte counts

Gram stain and bacterial culture (aerobic and anaerobic)

Crystal examination with polarizing microscope and compensator

Useful tests in certain circumstances

Fungal and acid-fast stains and cultures

PCR for bacterial and mycobacterial DNA

Serum–synovial fluid glucose differential

Lactate and other organic acids

Complement

Enzymes

Uric acid



Lymphocytes:

- 15% of SF cells
- RA and other collagen disorders, chronic infections.

Monocytes & macrophages

- Most common cells present in normal SF, 65% of the cell count.

Eosinophilia

- >2% of the leukocyte count,
- Rheumatoid arthritis, rheumatic fever, metastatic carcinoma, Lyme disease, parasitic infection, chronic urticaria, and angioedema & following arthrography.



Crystals

- Neutrophil predominant collection
- Intracellular crystals - pathognomic
 - ✓ Monosodium urate monohydrate
 - ✓ Calcium pyrophosphate dihydrate
 - ✓ Apatite & basic calcium phosphates
 - ✓ Calcium oxalate
 - ✓ Lipids



Type of crystal

Monosodium urate (MSU)

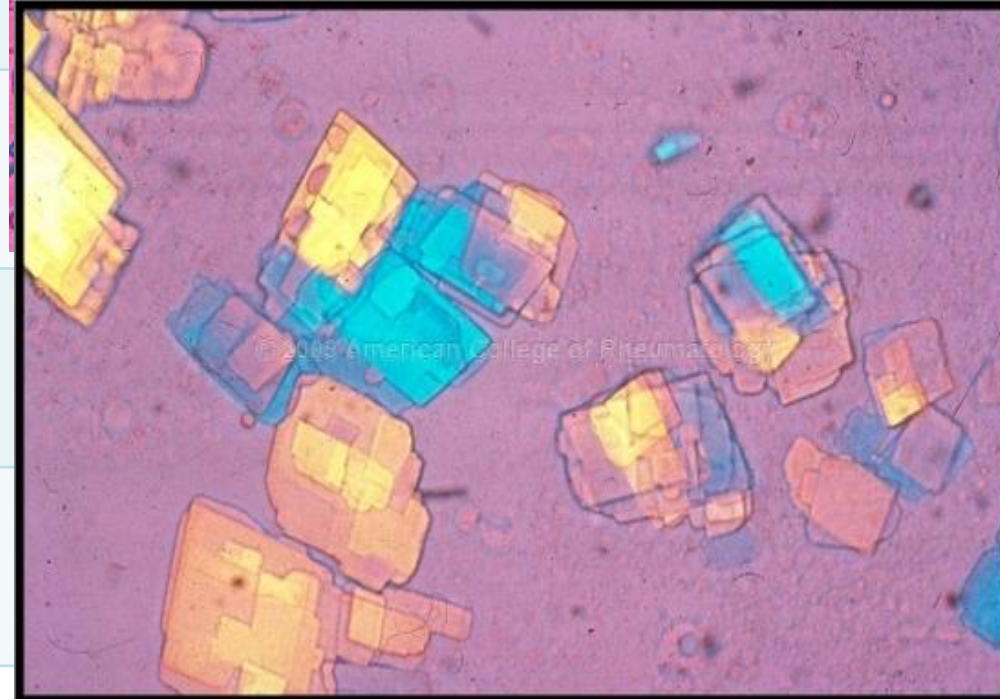
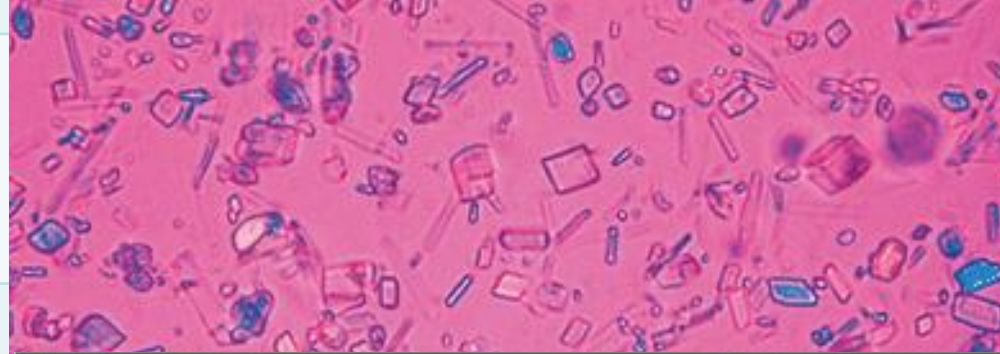
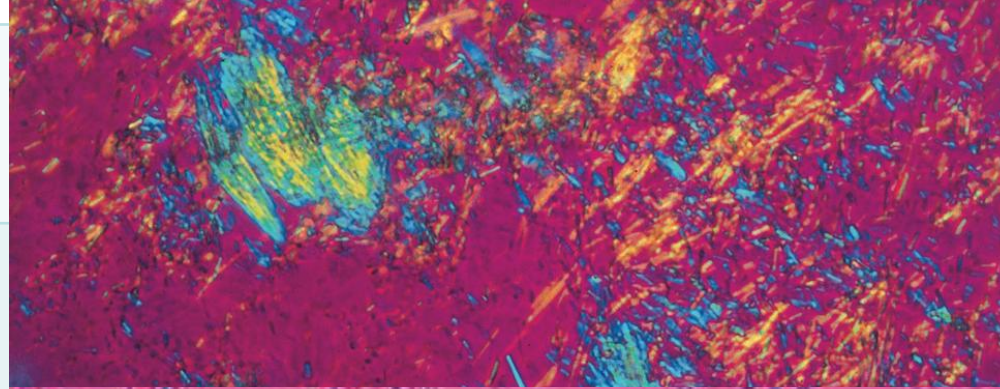
Calcium pyrophosphate dihydrate (CPPD)

BCP

Calcium oxalate dihydrate

Lipid crystals

Cholesterol crystals



Conditions

gout

inflammation, osteoarthritis, pseudogout & hyperparathyroidism

diagnosis & treatment, inborn errors of metabolism

urate arthritis

conditions (e.g., rheumatoid arthritis, SLE)

Axis of first order red compensator



polarizer



Monosodium urate monohydrate
(gout)

Calcium Pyrophosphate Dihydrate
(pseudogout)



Crystal mimics

- *Glove powder* •
- Talc
- Anticoagulants
- Prosthetic fragments
- Dust particles
- corticosteroids



Serous fluids

- Pleural
- Peritoneal
- Pericardial



Reasons to test

- Detect sepsis
- Malignancy
- Systemic disease



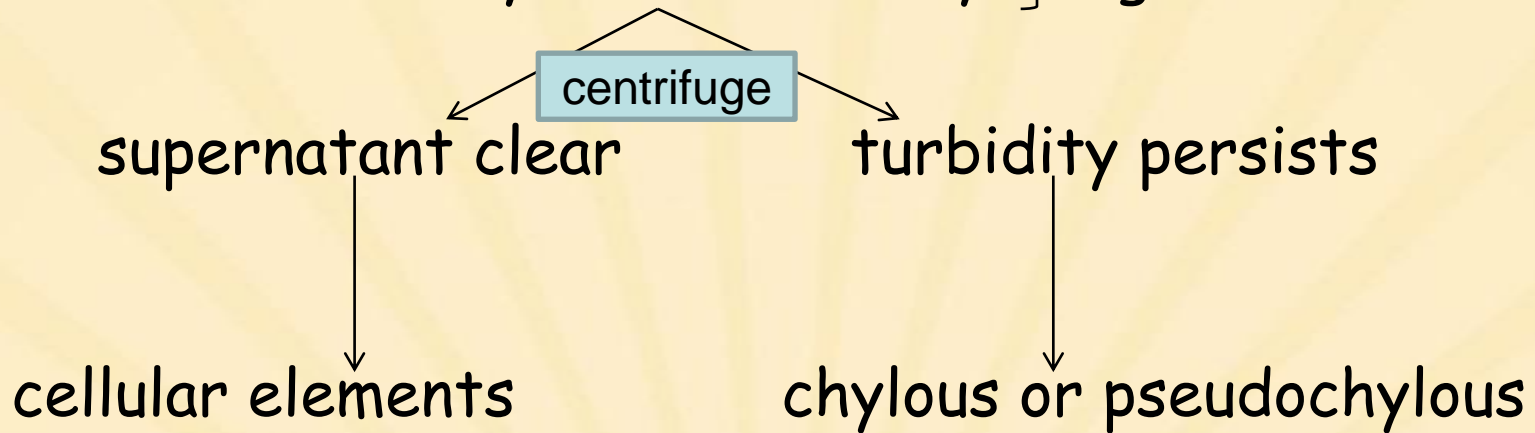
Specimen collection

<u>Tests</u>	<u>Anticoagulant</u>	<u>Volume (mL)</u>
RBC, WBC, differential	EDTA	5-8
Total protein, LD, glucose amylase	Heparin, none	8-10
Gram stain, bacterial culture	SPS*, none, or anticoagulant without bactericidal or bacteriostatic effect	8-10
AFB culture	SPS, none, or anticoagulant without bactericidal or bacteriostatic effect	15-50
PAP stain, cell block	None, heparin, EDTA	5-50



Gross

- Transudates- usually clear
 - Exudates - milky, turbid, bloody
- } lights criteria



Serous fluids

- Leucocytes
- RBC
- Mesothelial cells
- Macrophages(mononuclear phagocytes)
- Vacuolated histiocytes(can be confused with signet ring cells)
- Bacteria
- Yeast



Mesothelial cells

- Distinguishable cell borders
- Flat sheets
- Individual cells
- uniform

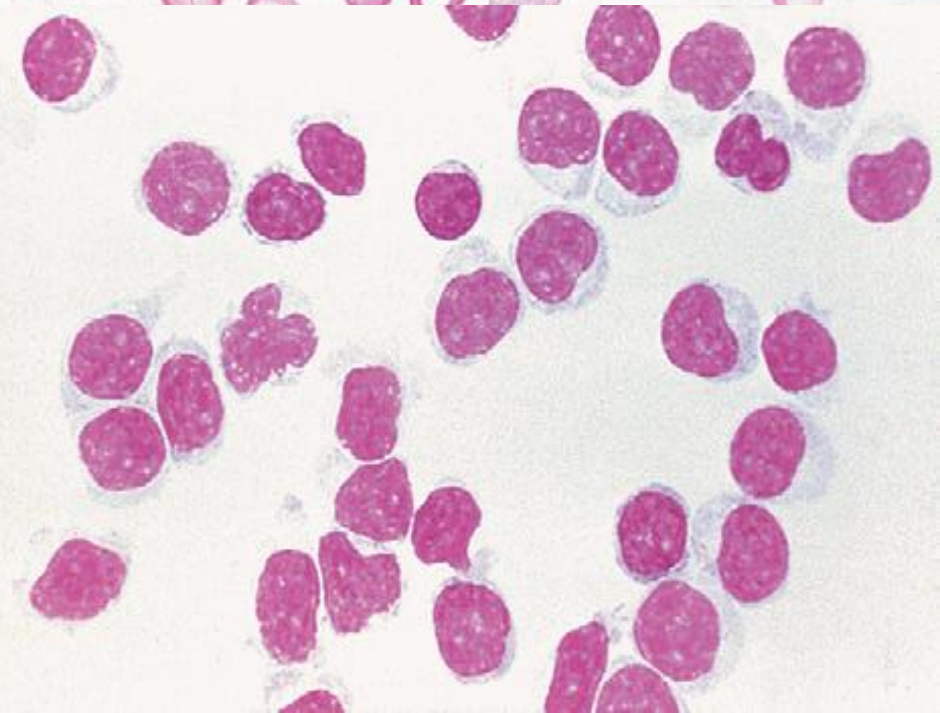
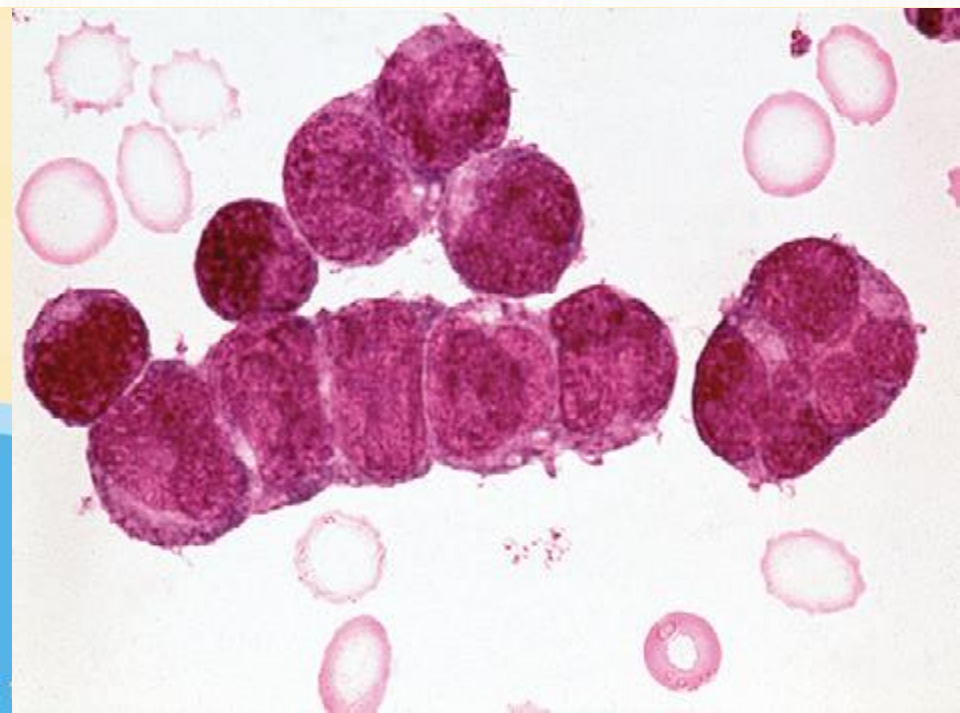
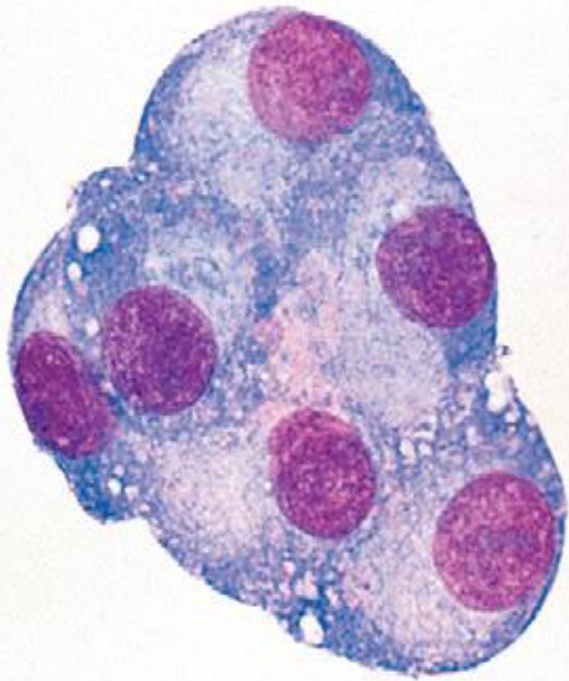
Malignant cells

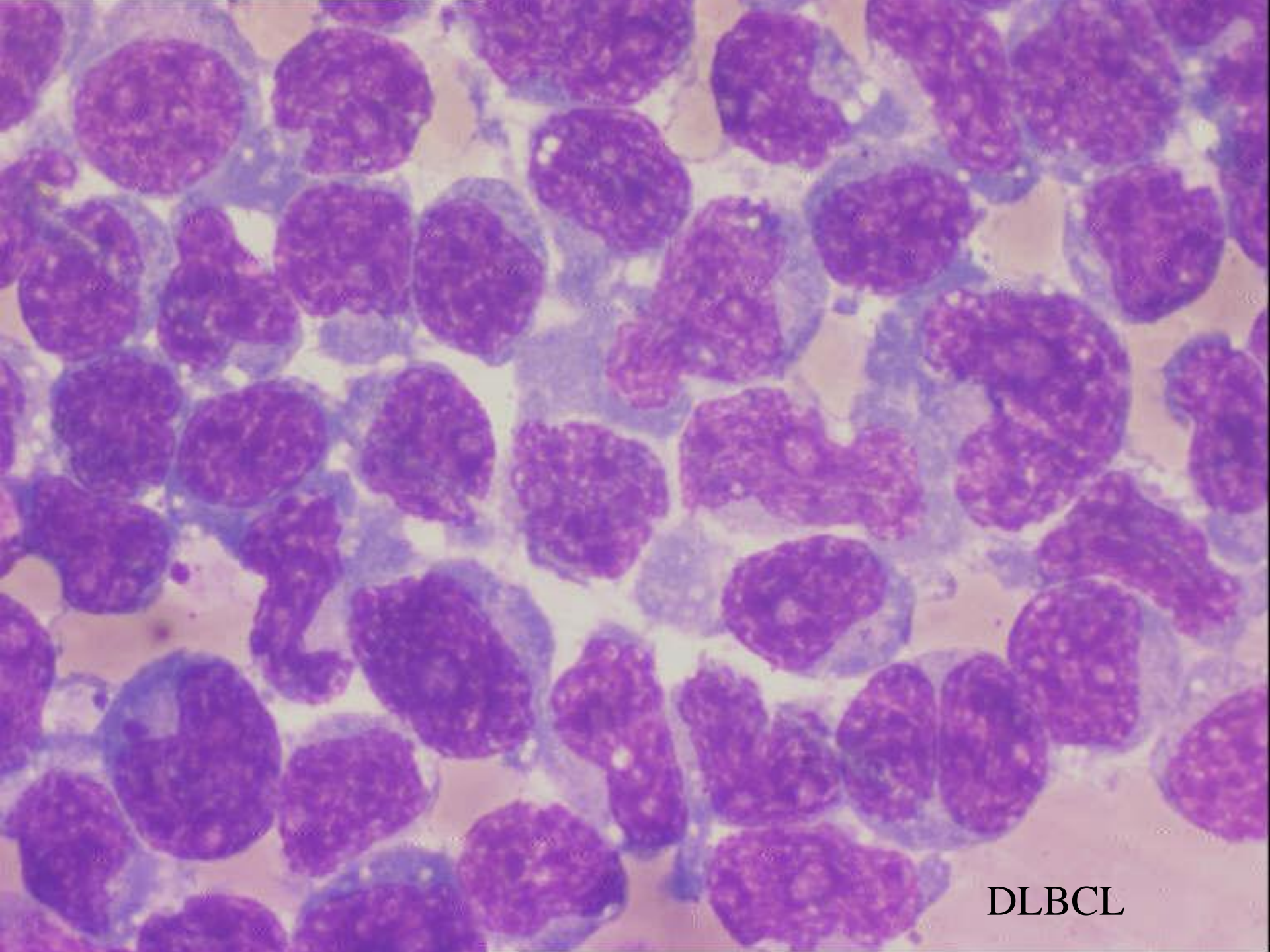
- Poorly defined cell borders
- Ball like clusters
- Cannibalism
- Non uniform



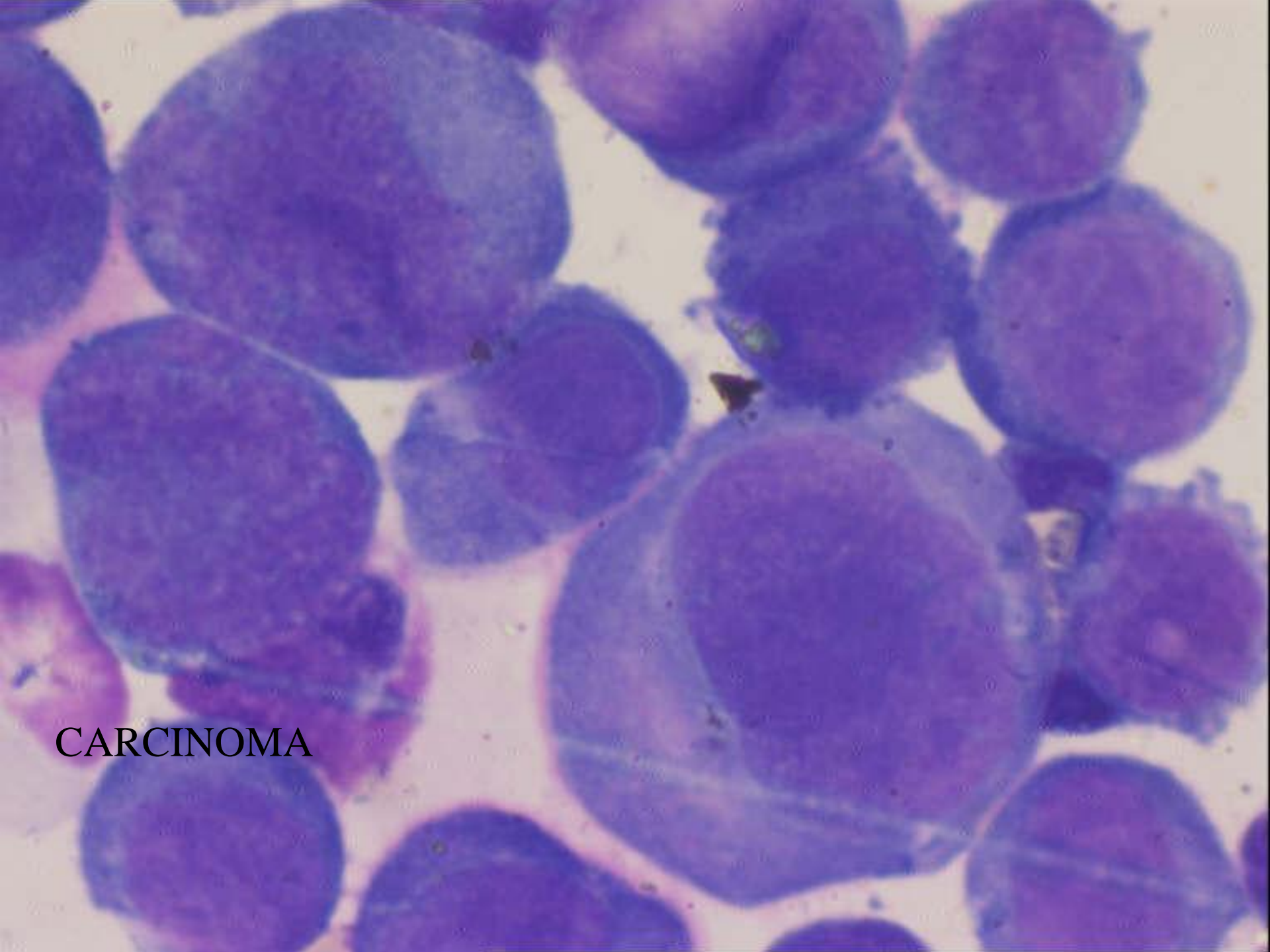
- Smooth nuclear membrane
- No clefts
- Vacoules are limited to cytoplasm
- Irregular nuclear membrane
- Clefts and moulding
- Vacoules are all over including over nuclei



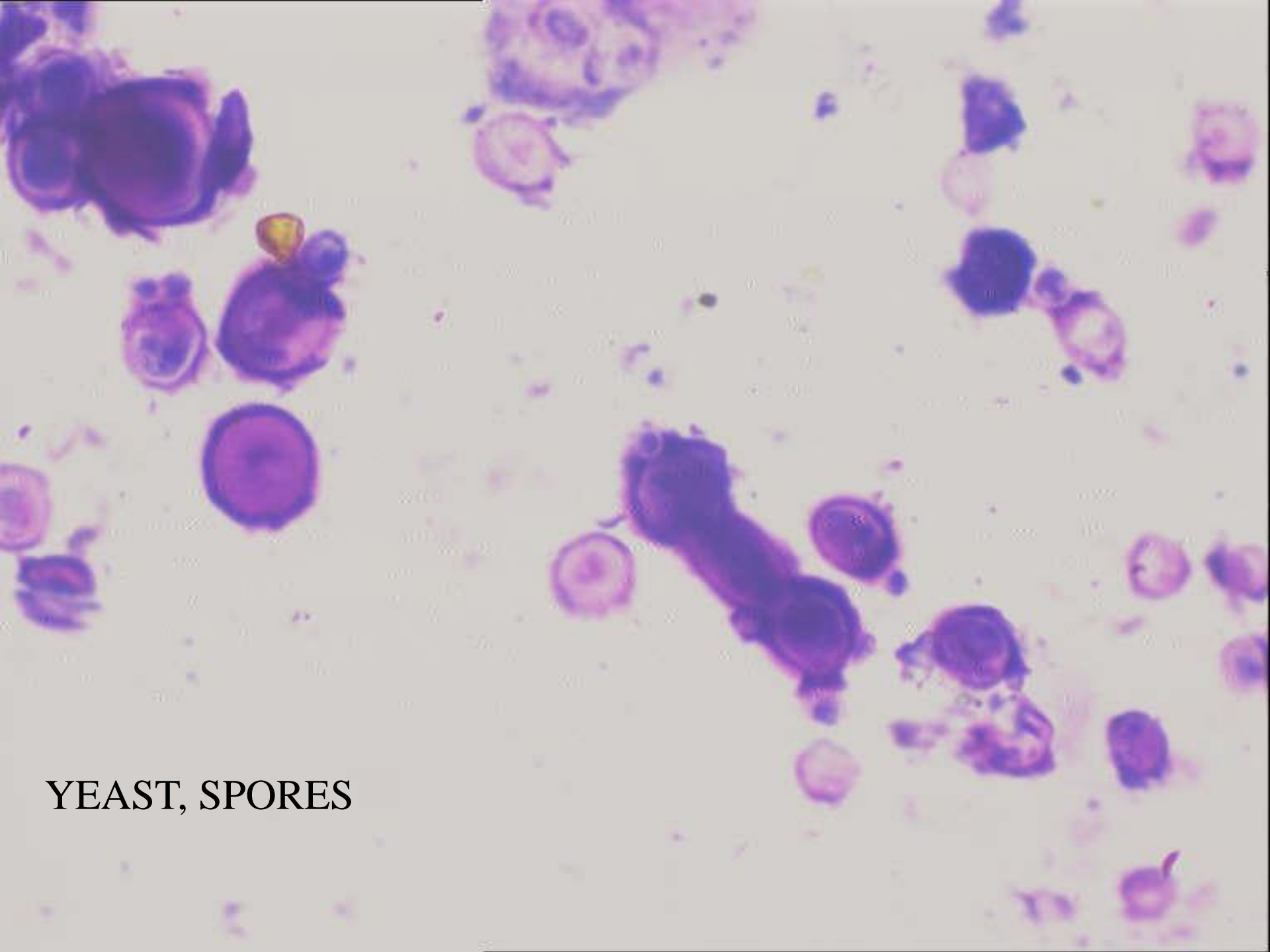




DLBCL



CARCINOMA



YEAST, SPORES

Automation

Why automation

- ✓ Labour intensive
- ✓ Time consuming
- ✓ Skilled person(24x7)
- ✓ Interobserver variability
- ✓ Biohazard



Problems with automation

- Carryover
- Background counting
- Flaging of abnormal cells
- Clogging of machine

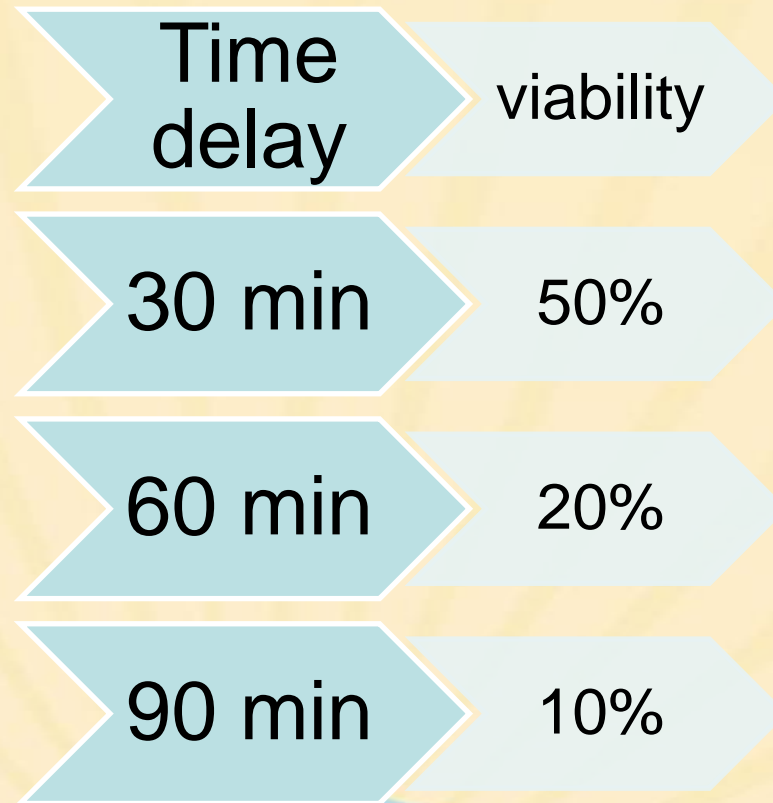


Ancillary Techniques

- **Cytochemistry**
- **Immunocytochemistry**
- **Flow Cytometry**
- **Molecular studies**



CSF for flow cytometry



Dux et al, J Neural Sci, 1994;121:74-78



References

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