Don’t Be Afraid

It’s just a Body Fluid!

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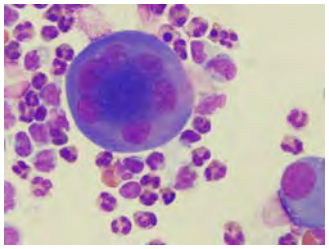
Objectives

- Discuss the advantages of the automated cell count over a manual count
- Review the meaning of analytical reference range and clinical reportable range
- Identify features of benign and malignant cells

Why Do We Fear Body Fluids?

Icky Specimens

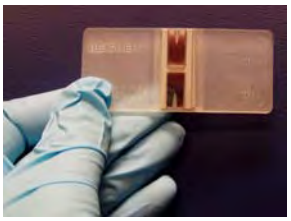


Scary Cells

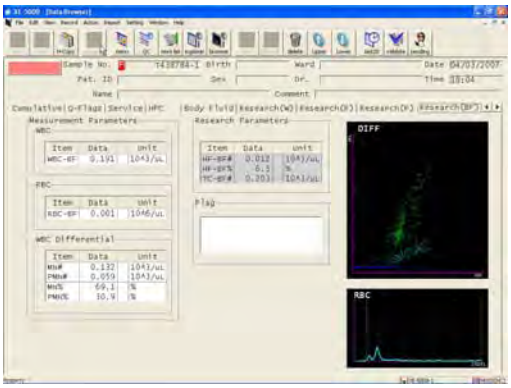
Manual Cell Counting

The Formula

$$\text{cells/}\mu\text{L} = \frac{\text{cells counted}}{\text{area x depth x dilution}}$$



Automated Cell Counting



Automated Counts Are Better

- Better Precision
- Easier to Perform
- Safer!!!

Rules to Follow

- Treat Synovial Fluids with Hyaluronidase
- Original Reference – Add 5mg to a 0.5 – 1ml aliquot.

SIGMA #H-3757
TYPE VIII BOVINE
STERILE
FILTERED

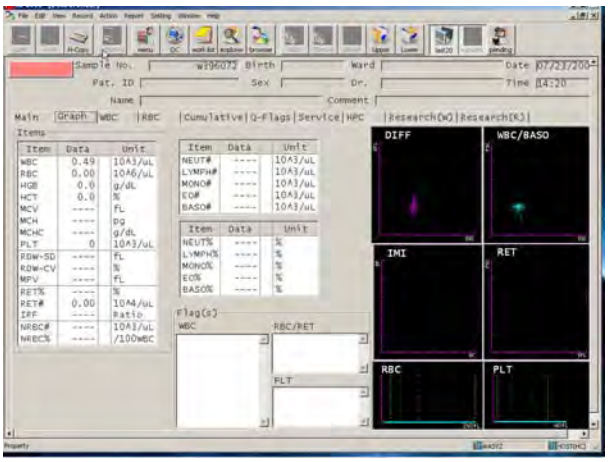
Rules to Follow

- It's a Total Nucleated Count – NOT just a WBC count
  - Mesothial Cells, Macrophages
- If there are clots or fibrin……. Take them out and report as approximate
  - You can still make a smear

Automated Cell Counts

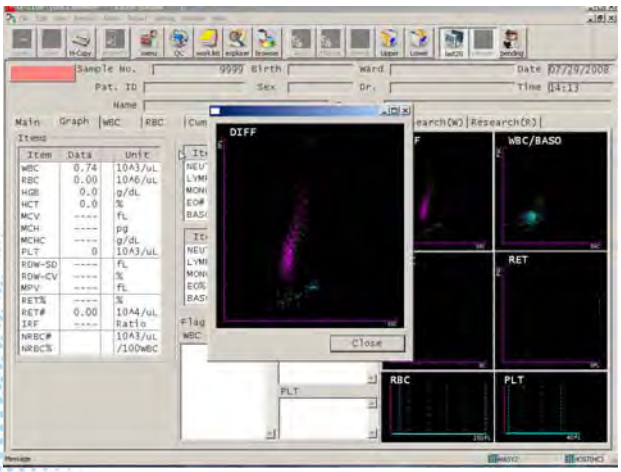
- What's Important?
  - Background counts
  - Scattergrams
  - AMR – Analytical Measurement Range
  - CCR – Clinical Reportable Range

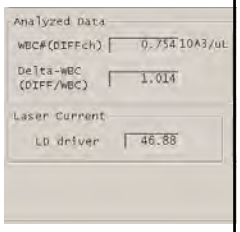
Scattergrams



SYSMEX XE2100

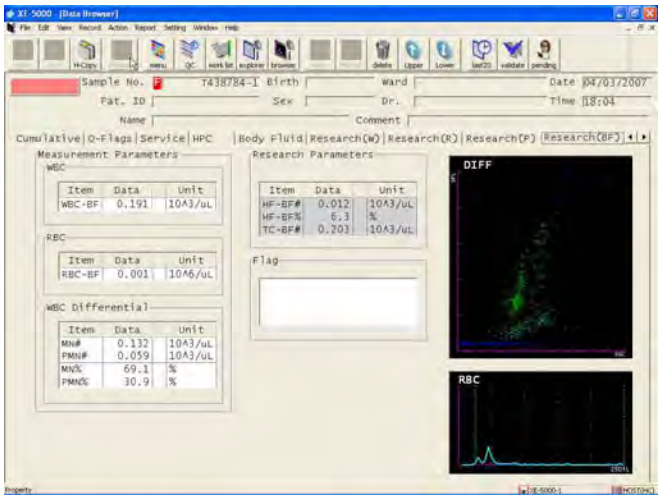
Scattergrams





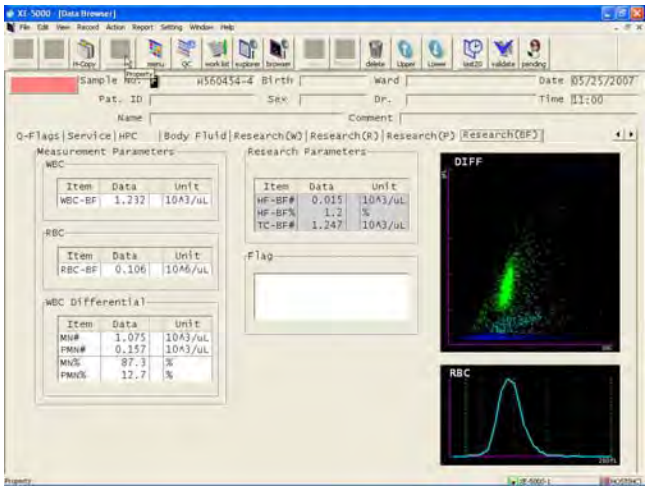
SYSMEX XE2100

Scattergrams

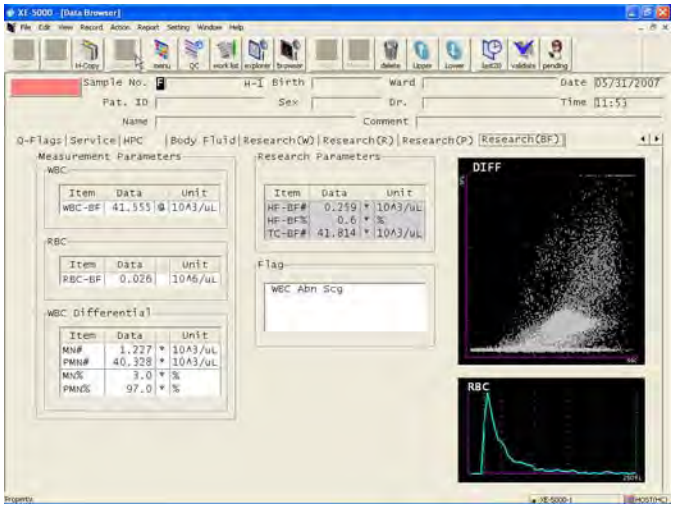


SYSMEX XE5000

Know When it is Good



And When it is Not



Things to Consider

- AMR
  - How low or high can the instrument measure
- CRR
  - How low or high can you report results

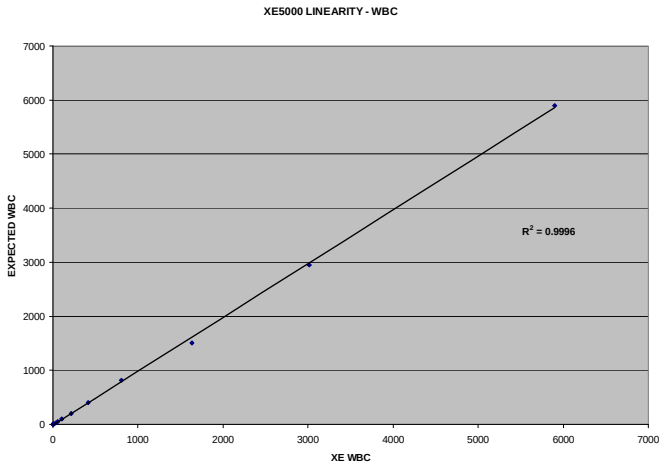
Terminology

- Linearity = Analytical Measurement Range (AMR)
  - Range of analyte values that a method can directly measure on the specimen without any dilution, or other pretreatment not part of the usual assay process

AMR = LINEARITY

XE5000 LINEARITY - SYNOVIAL FLUID				
	XE5000	EXPECTED	XE5000	EXPECTED
DILUTION	nucleated cells		RBC	
0	5901	5901	1445000	1445000
2	3015	2950.5	749000	722500
4	1631	1507.5	384000	374500
8	809	815.5	191000	192000
16	411	404.5	97000	95500
32	213	205.5	50000	48500
64	107	106.5	27000	25000
128	56	53.5	14000	13500
256	29	28	7000	7000
512	11	14.5	4000	3500
1024	6	5.5	2000	2000
2048	3	3	1000	1000
4096	1	1.5	1000	500
CORR	1.0		1.0	
SLOPE	1.0		1.0	

AMR - Linearity



BUT!!

There are limits …….

That **MUST** Be Taken Into Consideration

Things to Consider

- Does the method have limitations based on:
  - Linearity Limits — YES
  - Precision or Reproducibility — YES
  - Decimal Place limits — YES
  - Carryover/Background — YES
  - Clinical Decision Points — POSSIBLY

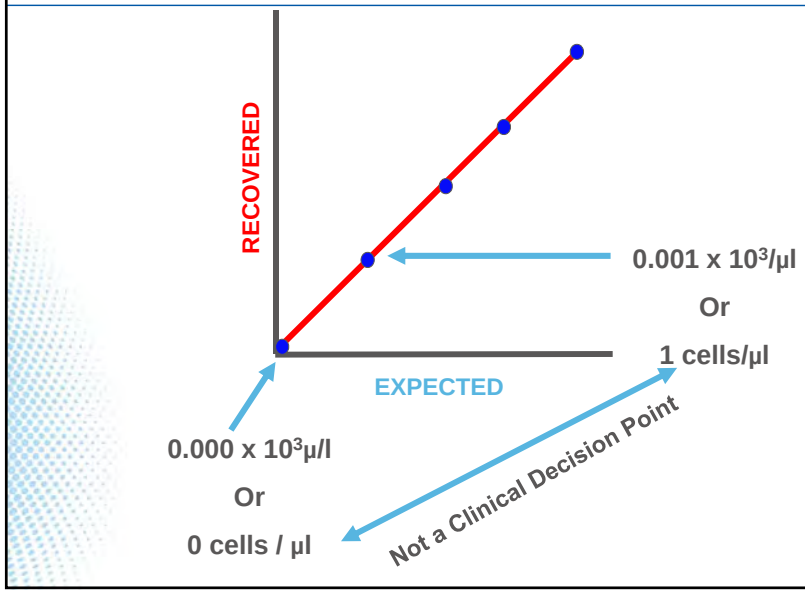
Things to Consider

- Clinical Reportable Range (CRR)
- LOD – Limits of Detection
  - Taken From Chemistry
  - Required by FDA for new analyzers

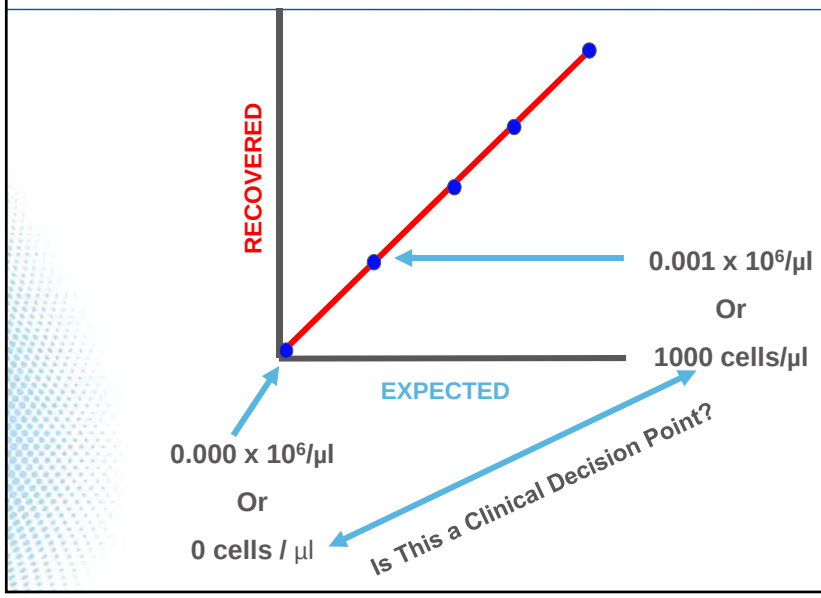
Clinical Reportable Range (CRR)

- Clinical Reportable Range (CRR) - Analyte values that a method can report as a quantitative result, allowing for specimen dilution, concentration or other pre-treatment used to extend the AMR
- Establishment of the CRR is a **medical judgment** made by the Laboratory Medical Director, and is based in part on the assay technology.
- Values outside the CRR are reported as < or > a numeric value.

WBC-BF & TC-BF are Linear to Zero



RBC-BF is Linear to Zero



Clinically Reportable Range

Example**: RBC-BF**

- **AMR is 0.000 - 5.000 x 10^6/µl (method specification).**
- **CRR is 0.001 - 8.000 x 10^6/µl (clinical usefulness).**
- **Results of 0.000 are reported as "< 0.001 x 10^6/µl ".**
- **Results >5.000 are diluted and rerun to obtain quantitative values up to 8.000 x 10^6/µl .**
- **Results >8.000 are reported as ">8.000 x 10^6/µl ".**

****NOTE: CRR limits are the Judgment of the Lab Director and may be different for different labs**

Clinical Significance of Body Fluids

Body Fluid Type	WBC	RBC
CSF	Adults: 0-5 mononuclear cells/µL Children: 0-30 mononuclear cells/µL*	Limited diagnostic value
Peritoneal fluid	>500/mL	NA
Pleural fluid	WBC have limited value	>10,000/ul
Synovial fluid	Up to 200/µL Gp 1: Noninflammatory 200-3000/µL Gp 2: Inflammatory 3000-75,000/µL Gp 3: Infectious 50,000-200,000/µL Gp 4:Crystal associated 500-200,000/µL	NA
Lavage (Peritoneal)	>500/mL	>10,000/ul

*Children have intermediate leukocyte values, less than 20/µL the first year of life and less than 10/µL until adolescence.
Source: Body Fluids Kjeldsberg, C. Third Edition, 1993.

Clinical Significance of RBCs in Body Fluids

Tips from the clinical experts
Edited by Daniel M. Baer, MD

Answering your questions

A varied range of queries challenges experts

- RBCs should be present under normal conditions
- Numerous RBC suggest a pathologic, malignancy, trauma, infarct or hemorrhage
- RBCs should be correlated with WBCs
  - Is the fluid contaminated with peripheral blood

Daniel M Baer MD. MLO March 2004

Reasons for knowing RBC values in CSF

- Subarachnoid or intracranial bleeding
  - RBC Count unchanged between first and third tubes
  - Blood does not clot
  - Xanthochromia suggests bleeding
    - See CSF Color
    - CSF supernatant xanthochromic on centrifugation
    - Requires at least 2 to 4 hours from onset of bleed
- Traumatic tap
  - RBC Count decreases between first and third tubes
  - CSF becomes clear on centrifugation
  - Consider repeating tap at higher interspace
- References
- Kooiker in Roberts (1998) Procedures in ER, p. 1067-75
- Ravel (1995) Lab Medicine, Mosby, p. 294-9
- Tunkel in Mandell (2000) Infectious Disease, p. 974-8
- Seehusen (2003) Am Fam Physician 68:1103

Sysmex Body Fluid Comparison Chart

	Sysmex XE-Series & XT-Series	Sysmex XE-5000
WBC-BF	N/A	0.000 – 10.000 x 10^3/µL*
TC-BF	> = 0.050 x 10^3/µL	0.000 – 10.000 x 10^3/µL*
RBC	> = 0.01 x 10^6/µL	0.000 – 5.000 x 10^6/µL**

*+/- 10 µl (0.000 – 0.050 x 10^3µl ; +/- 20% (0.050 – 10.000x 10^3µl
** +/- 2% (0.000 – 5.000 x 10^6µl

What's Really Important

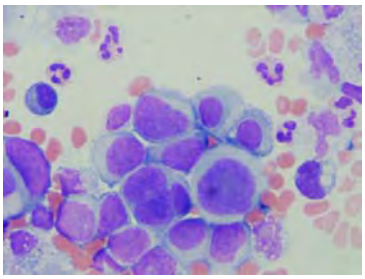
The Type of Cell Present

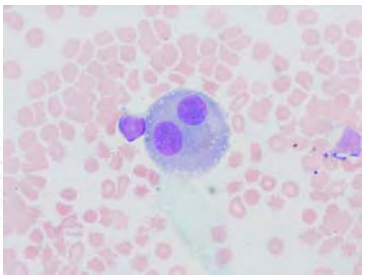
Necessary Tools

- CytoCentrifuge

Body Fluid Fears

- Problem Cells
  - Mesothelial Cells
  - Malignant Cells





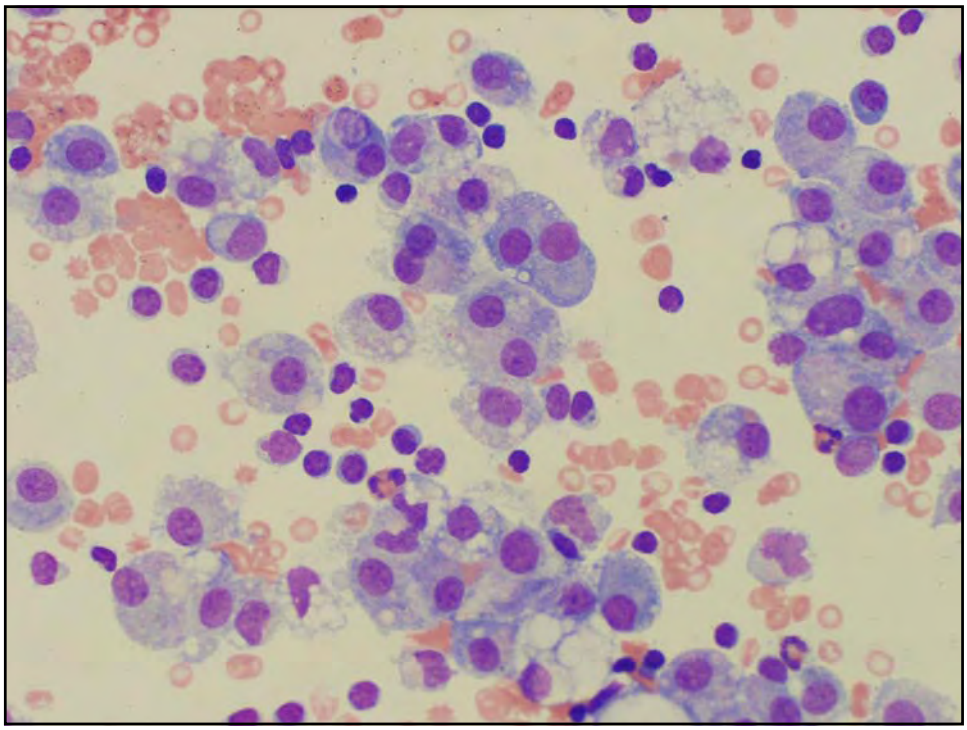
How To Tell Them Apart

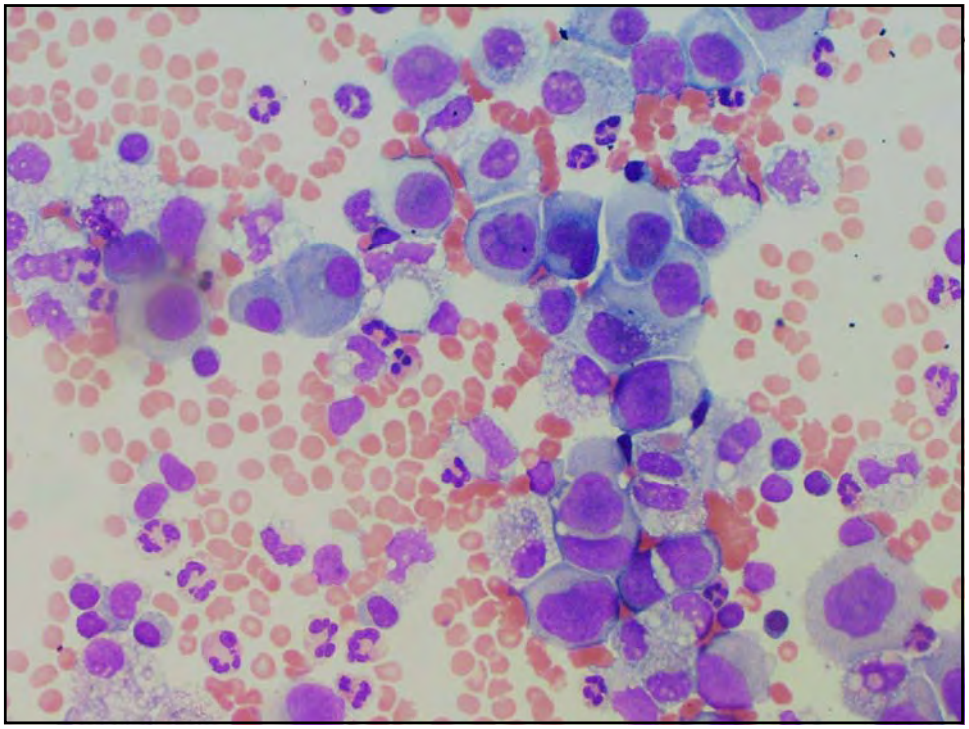
Mesothelial

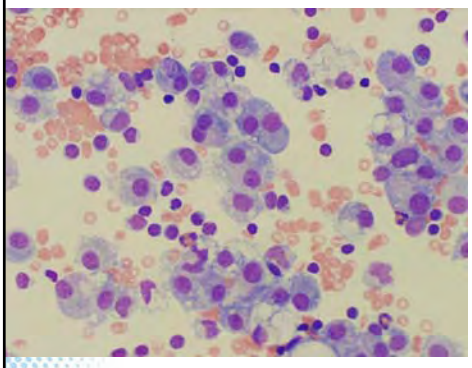
- Individual
- Uniform
- Flat clusters
- N/C ratio low

Malignant

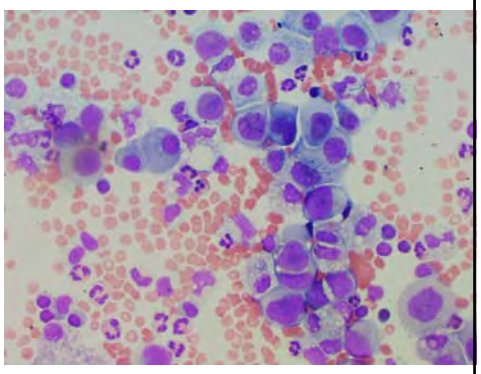
- Cannibalism
- Bizarre
- Ball-like clusters
- N/C ratio high



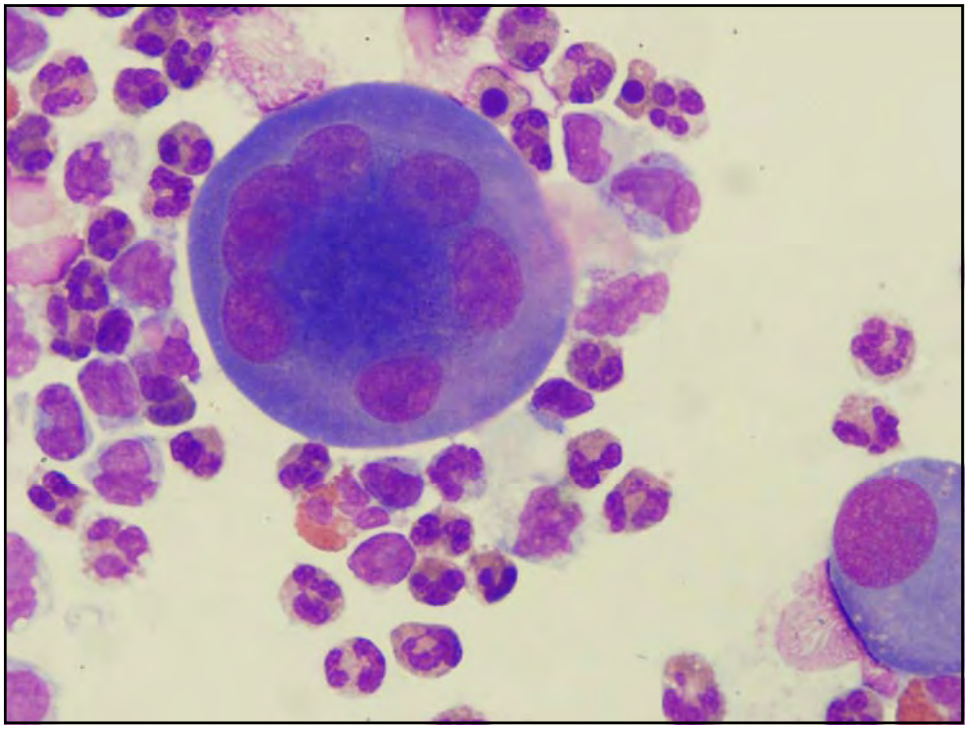


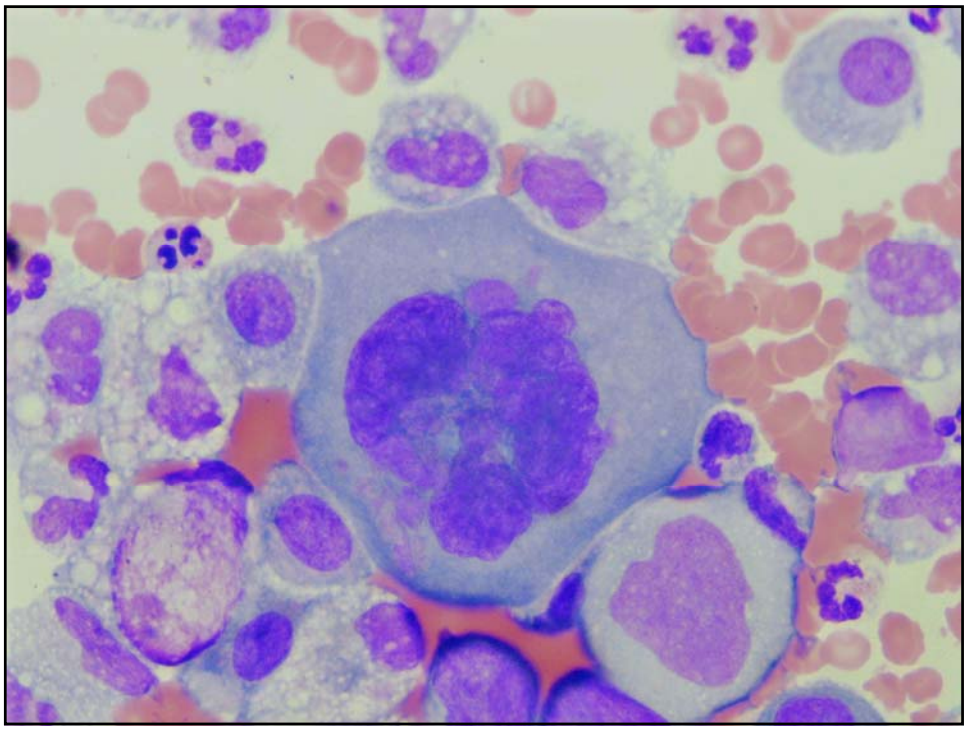


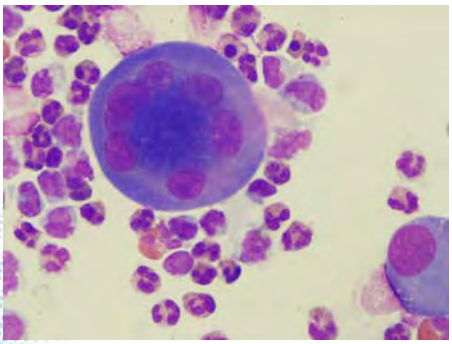
Mesothelial Cells



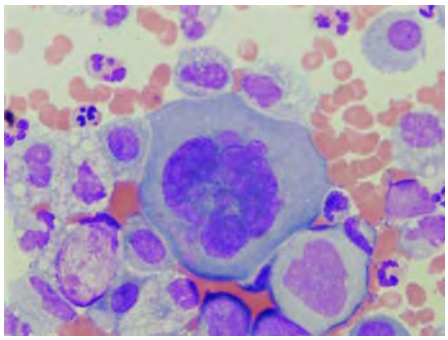
Malignant Cells



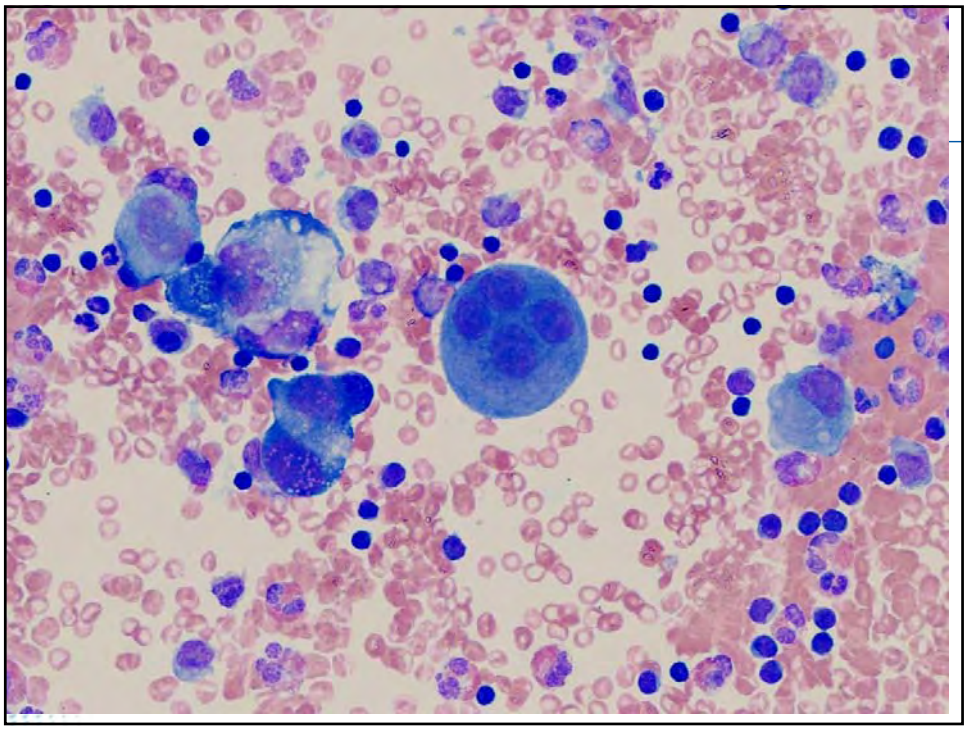




Mesothelial Cells



Malignant Cells



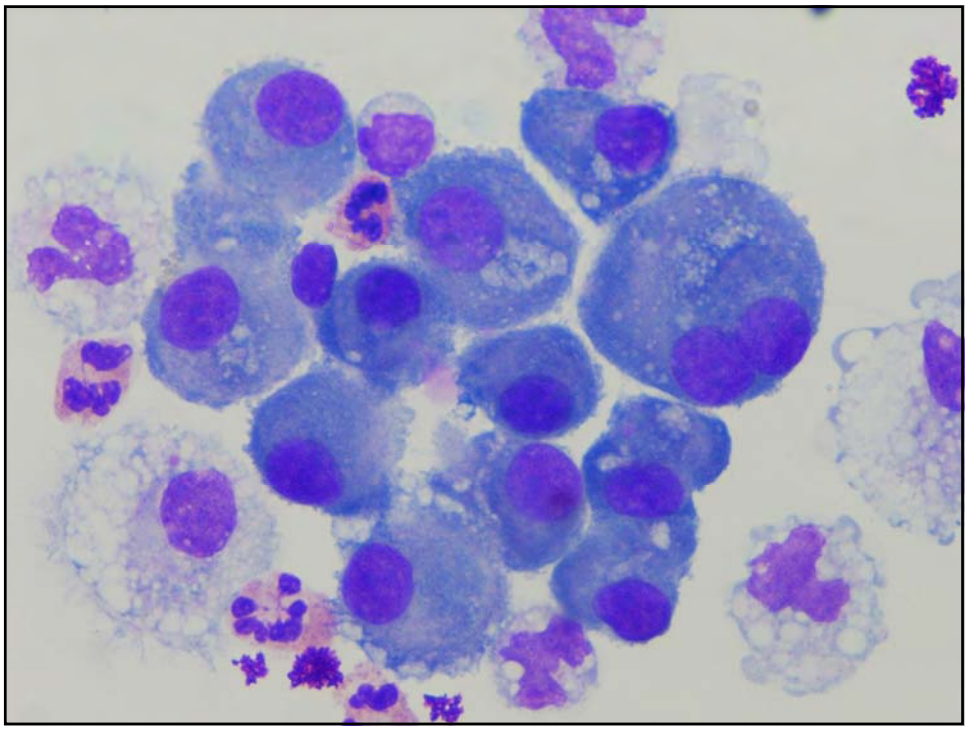
How To Tell Them Apart

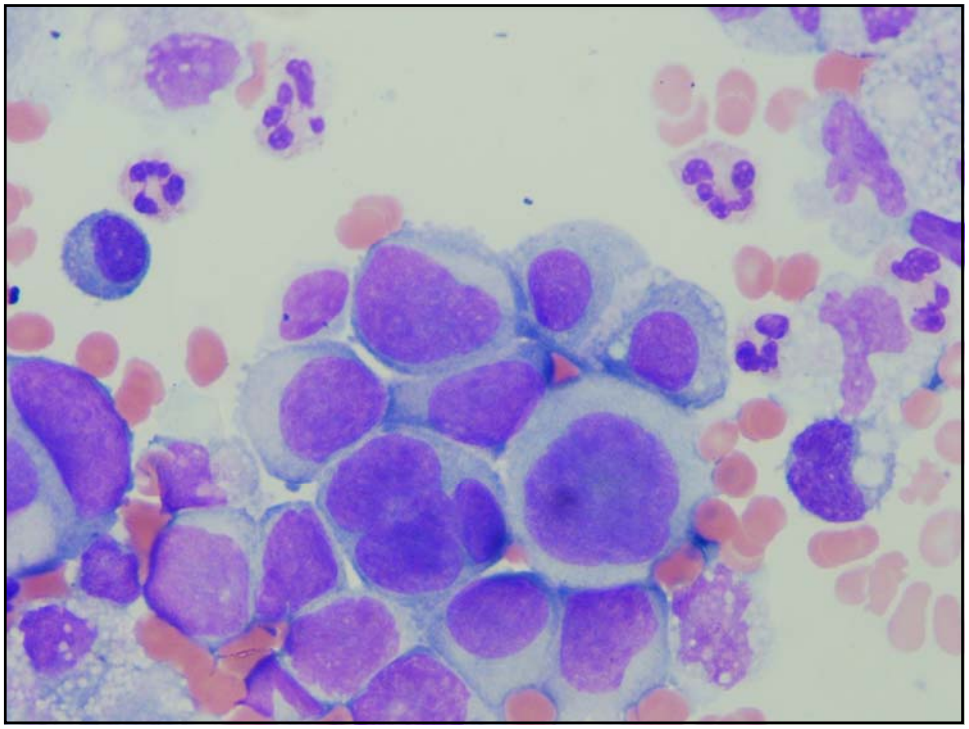
Mesothelial

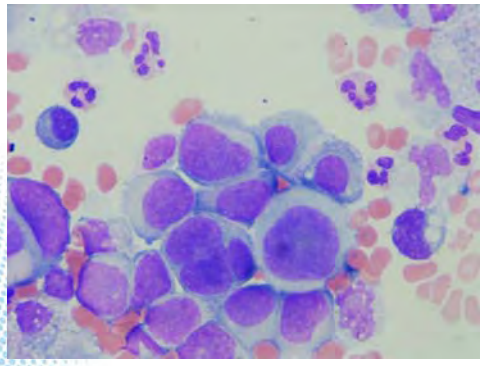
- Smooth chromatin
- Smooth nuclear membrane
- Round/oval nuclei
- Multinucleated/uniform
- Minimal vacuolization

Malignant

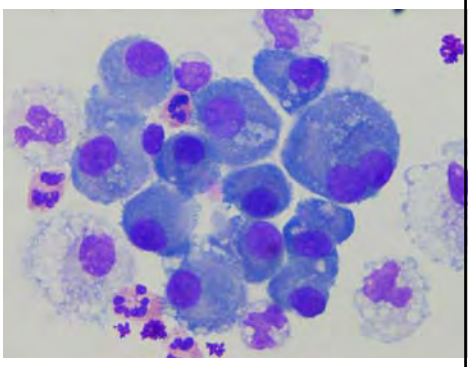
- Uneven chromatin
- Irregular nuclear membrane
- Nuclear clefting/molding
- Multinucleated /non-uniform
- Dramatic vacuolization



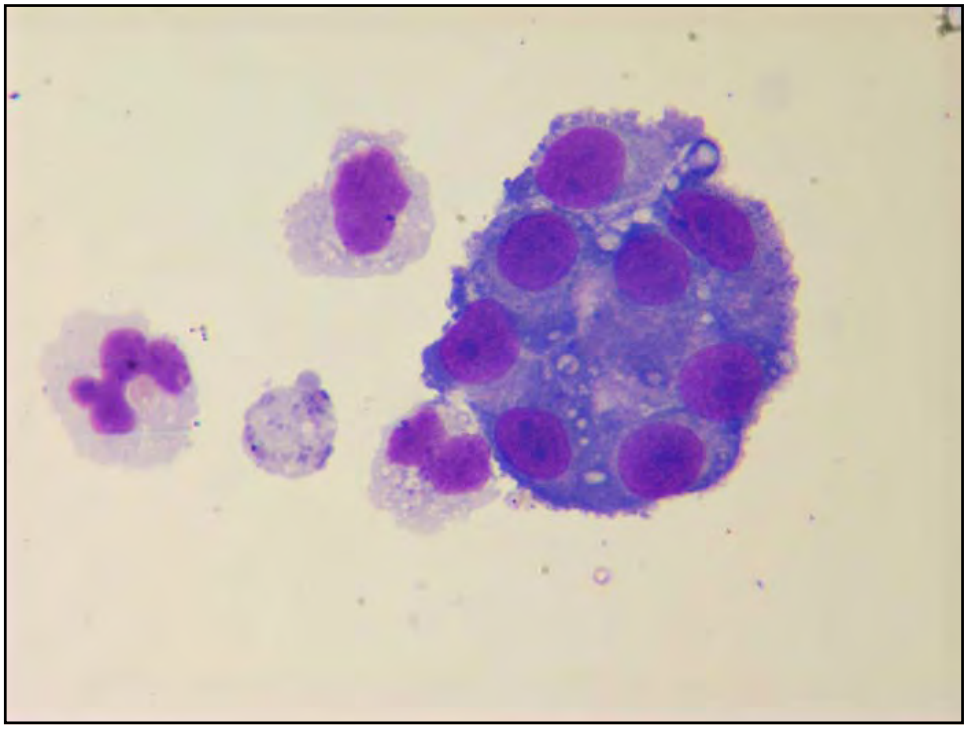


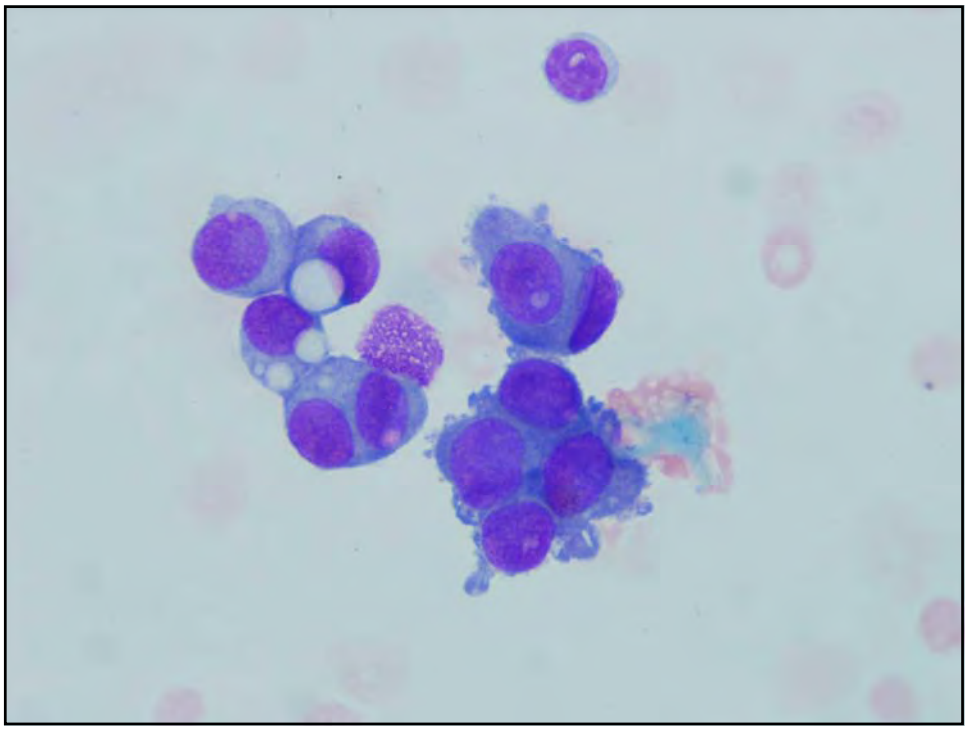


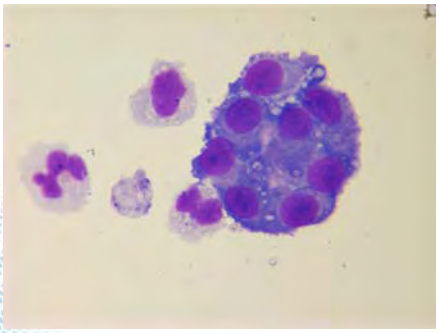
Malignant Cells



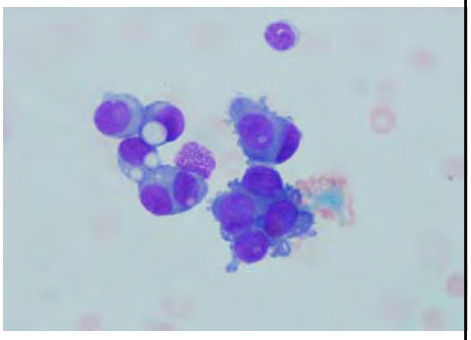
Mesothelial Cells



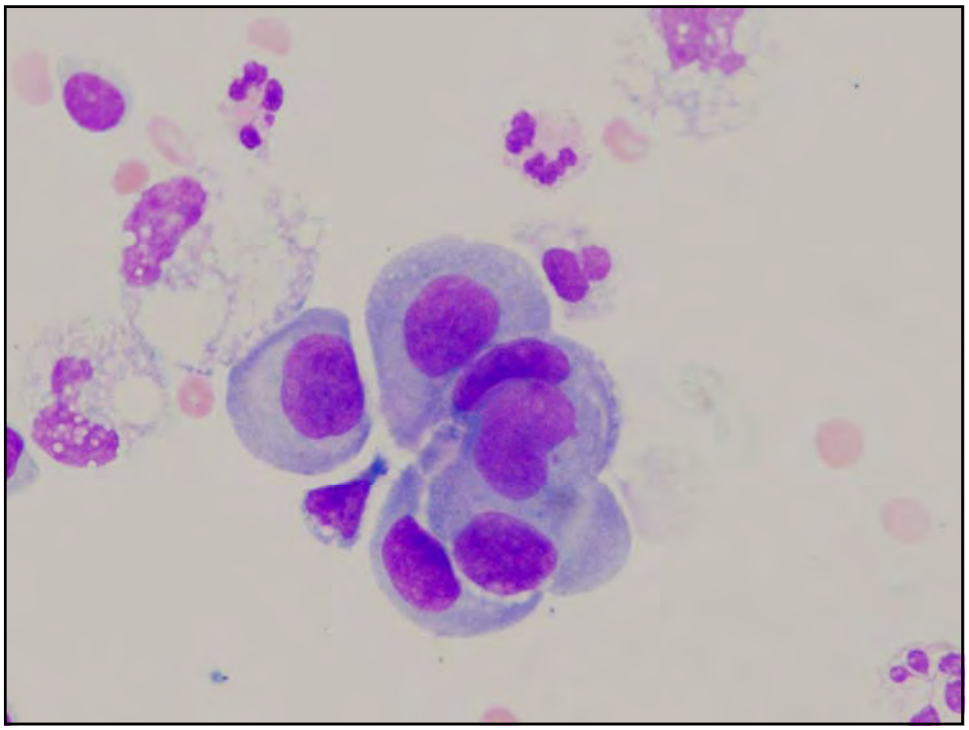




Mesothelial Cells



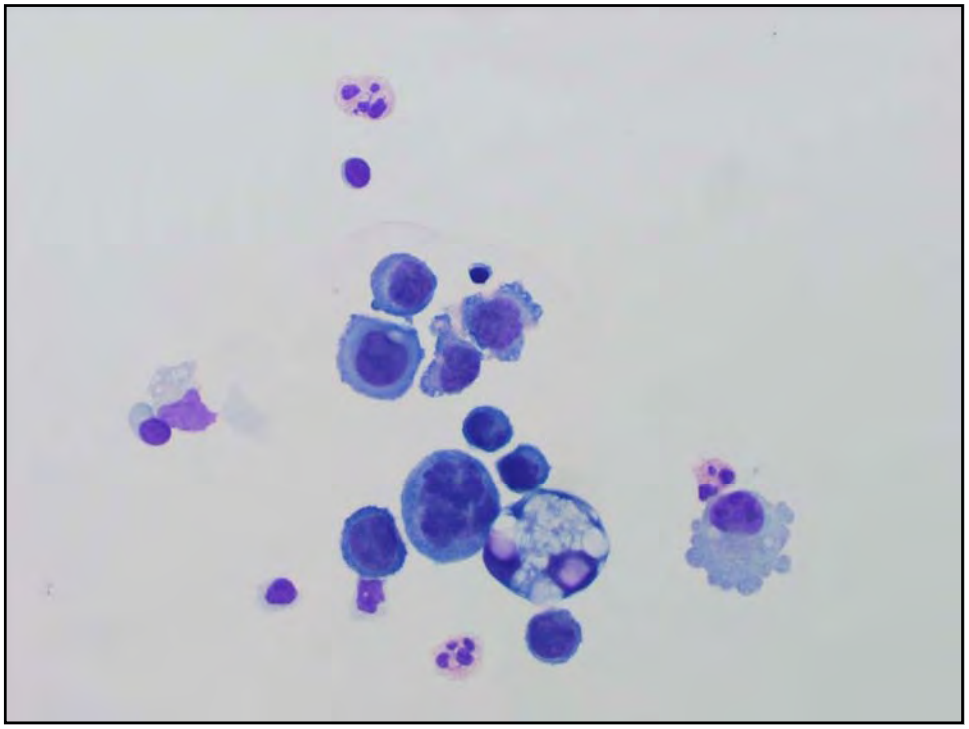
Malignant Cells

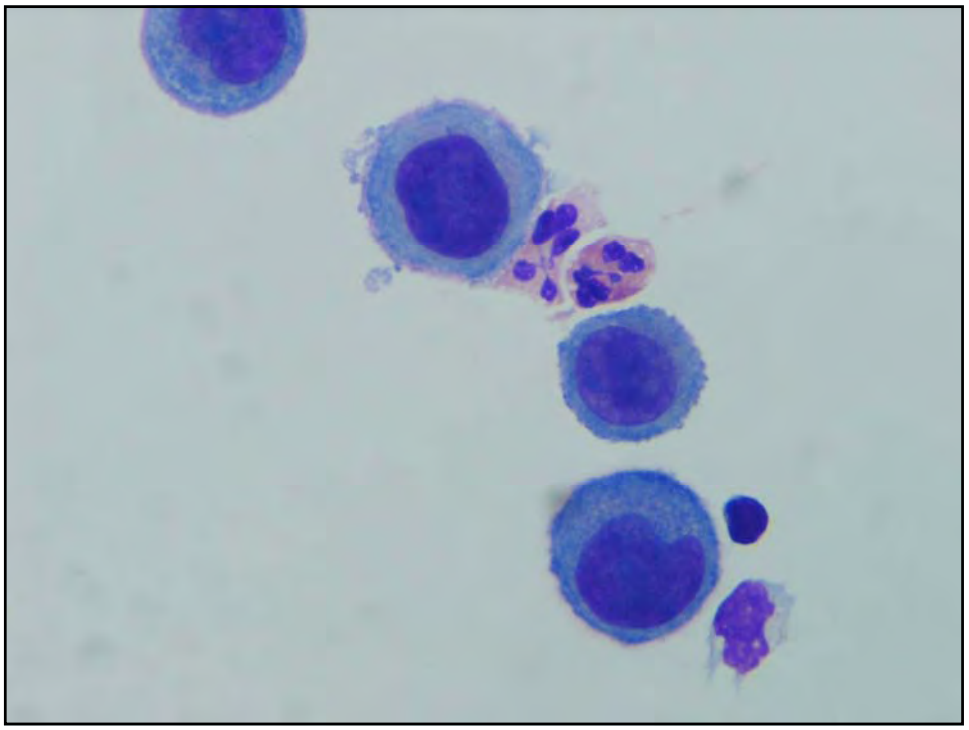


Case Studies

Case Study 1

- 67 YEAR OLD FEMALE
  - MEDICAL HISTORY UNREMARKABLE
  - SEEN IN ER WITH COMPLAINTS OF HEADACHE AND DIZZINESS
- CSF
  - RBC NONE SEEN
  - WBC 135 / mcL

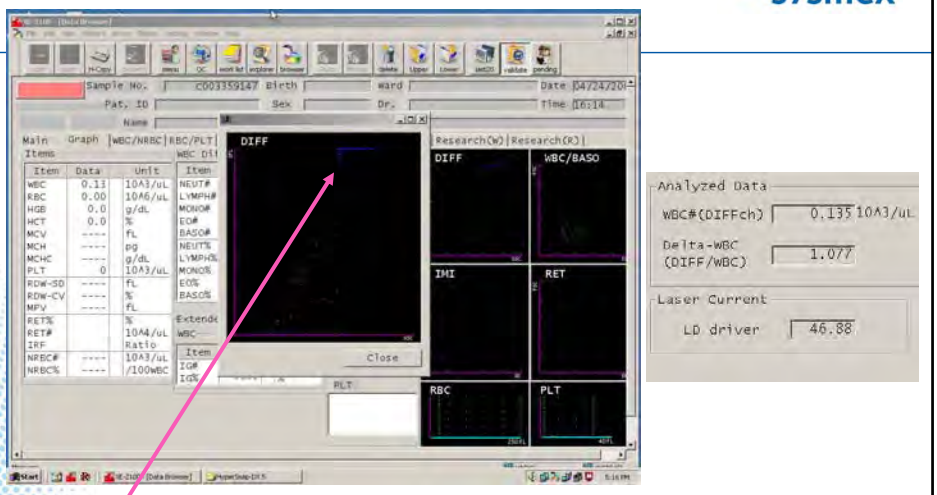




Case Study 1

- Ugly cells
- Cell size
- Irregular chromatin and large vaculoes

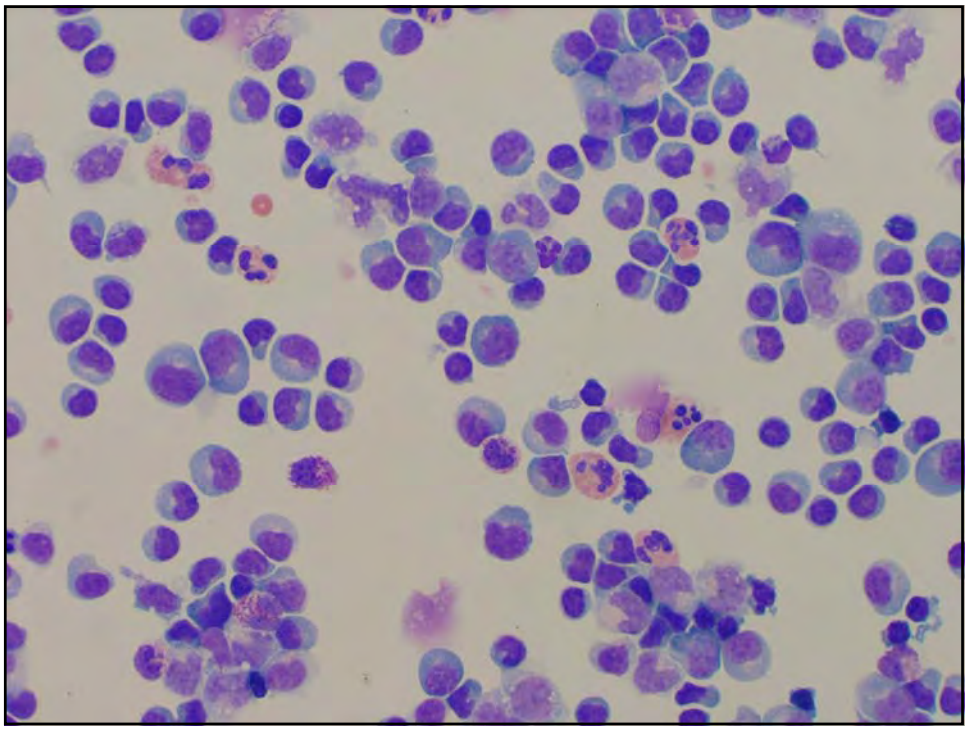
Malignant cells from metastasized breast cancer
Mamograms are important!!



- Very big cells or clusters of cells will appear here because they have a lot of fluorescence.

Case Study 2

- 24 YEAR OLD FEMALE
  - SEEN IN ER FOR HEADACHE AND NAUSEA
  - PREVIOUS HISTORY OF MENINGITIS
- CSF
  - RBC 17 / mcL
  - WBC 754 /mcL

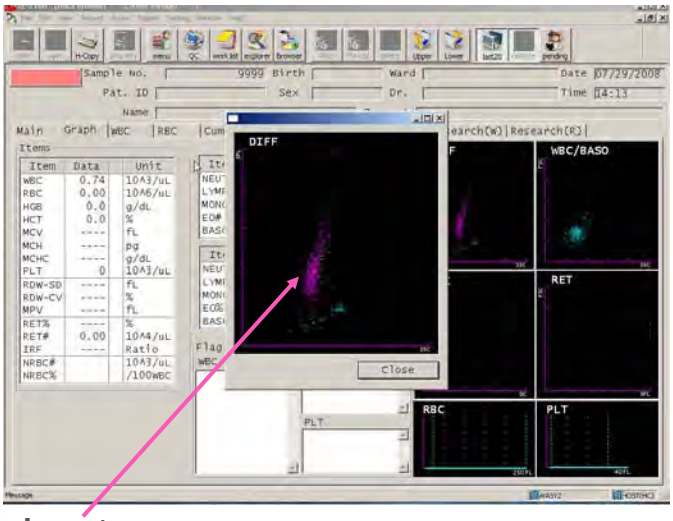


Viral meningitis – more specifically a recurrence of herpes meningitis

She is positive for HSV- Type 2. Recurrent meningitis is known as 'Mollaret's Meningitis'.

Herpes meningitis occurs in 10% of cases of primary genital HSV-2. Women are at higher risk for herpes meningitis than men.

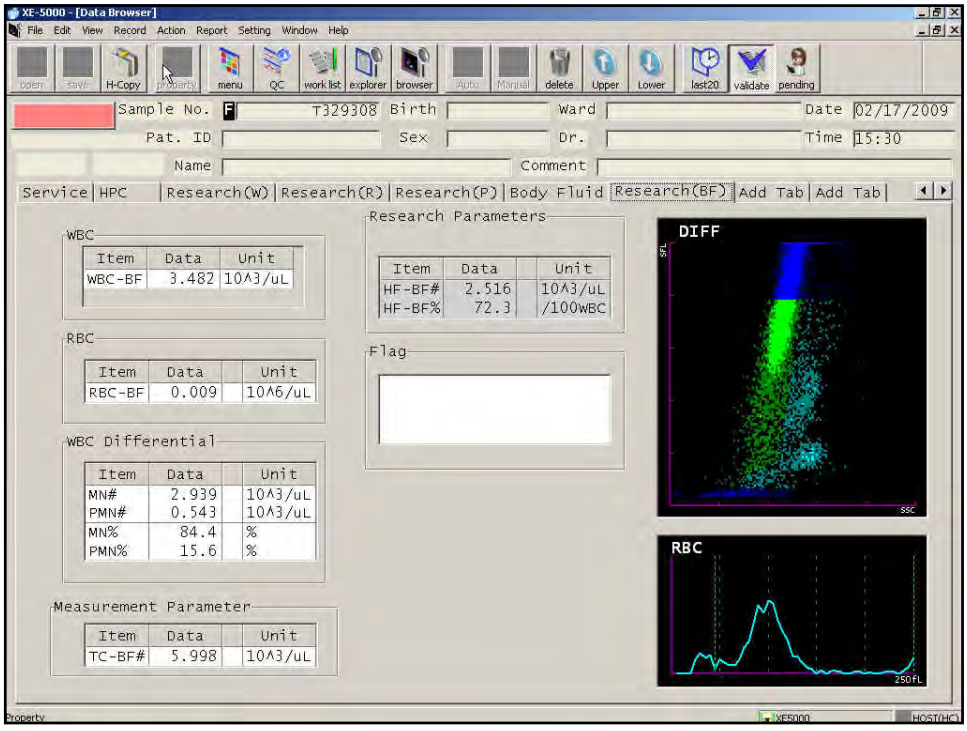
Surprisingly, herpes meningitis resolves without complications in 7 – 10 days but can recur in some patients.

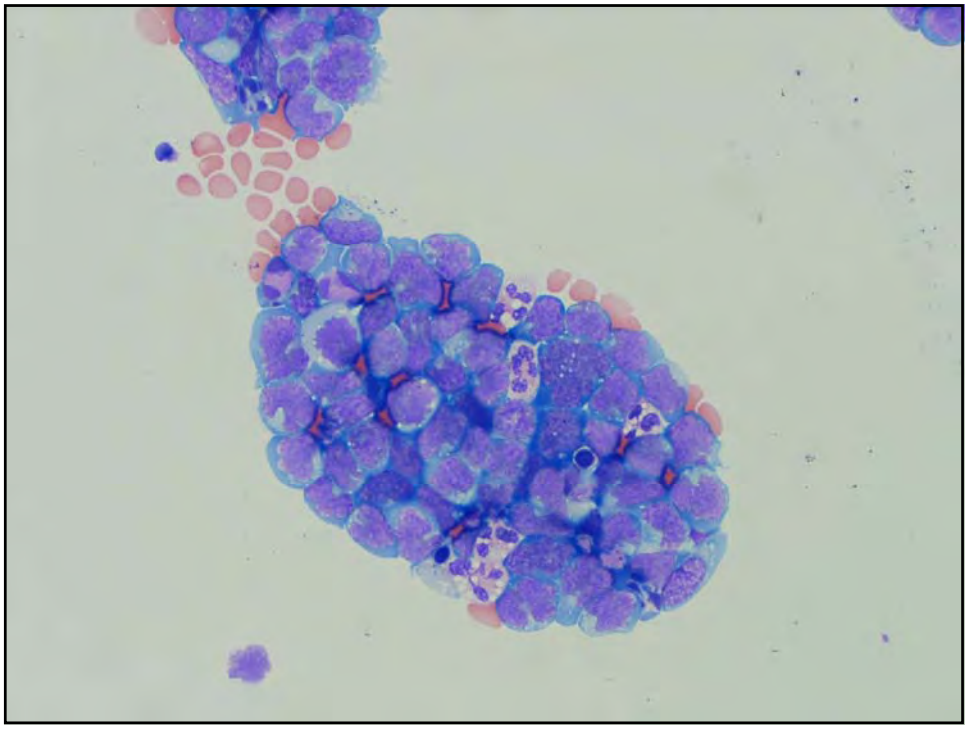


lymphocytes

Case Study 3

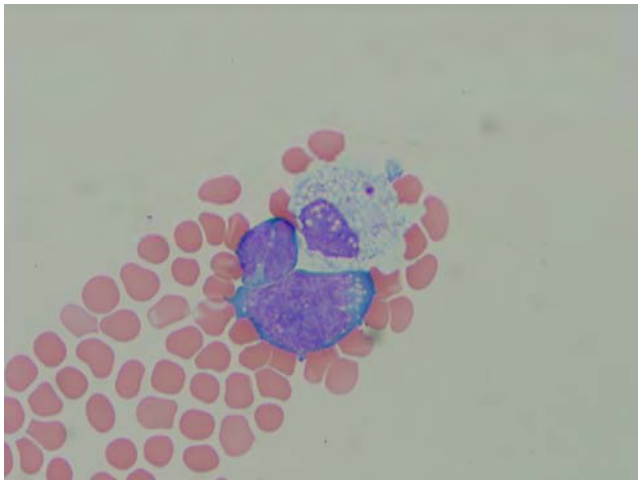
- 67 YEAR OLD MALE
  - 5.5 YRS POST HEART TRANSPLANT
- PLEURAL FLUID
  - NUCLEATED CELLS 5,998 /mcL





Burkitt's lymphoma

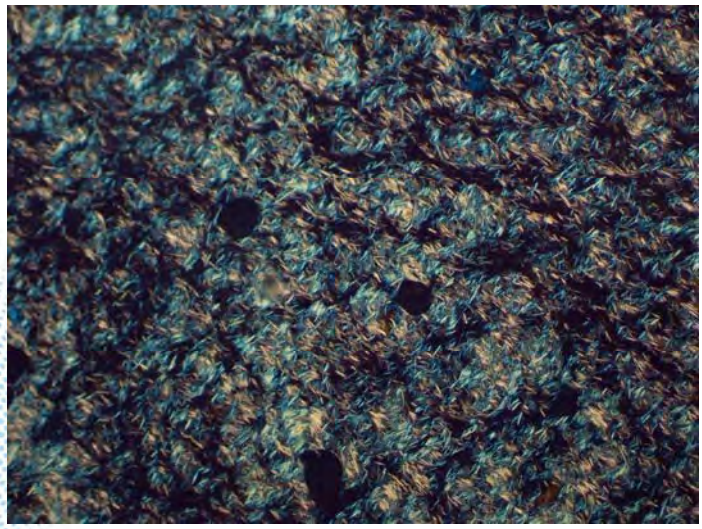
Monomorphic post transplant lymphoproliferative disorder



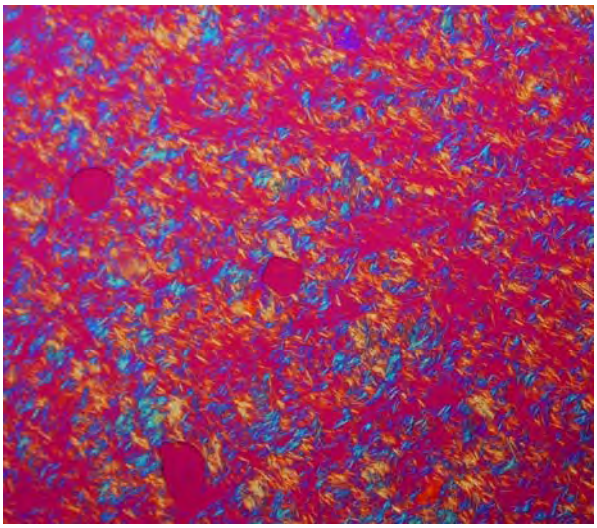
Case Study 4

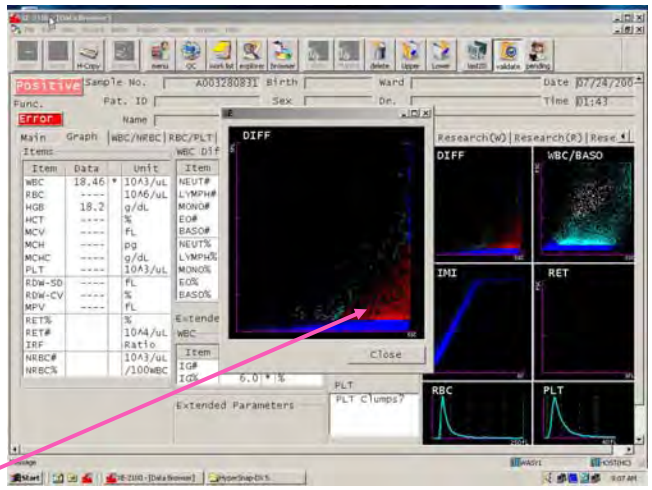
Synovial Fluid

Uric Acid Crystals – Polarized Light



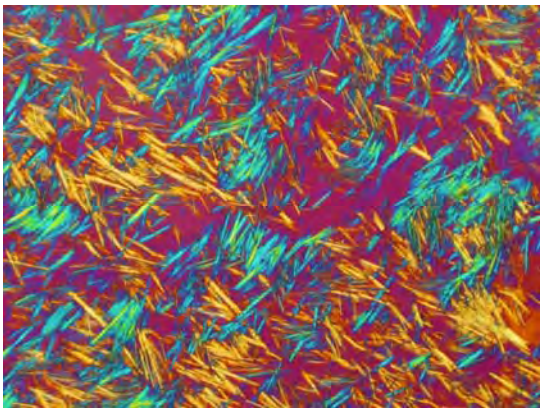
Red Compensator Filter





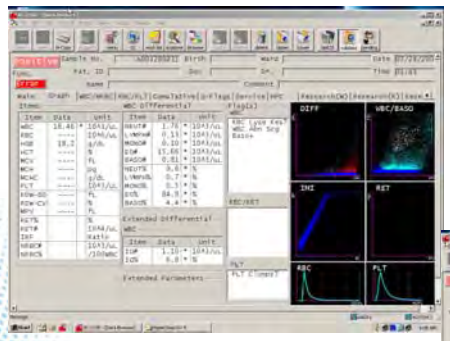
Interference from the uric acid crystals. A manual count would need to be performed but……..

HOW ARE YOU GOING TO FIND THE CELLS ?

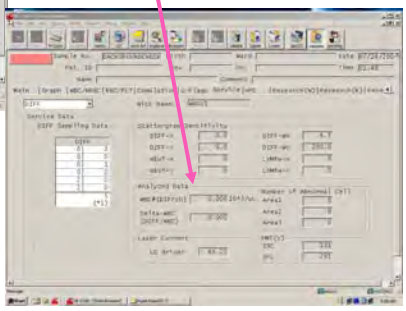


AND DOES IT MATTER ?

Worried About Running This Through The Instrument?

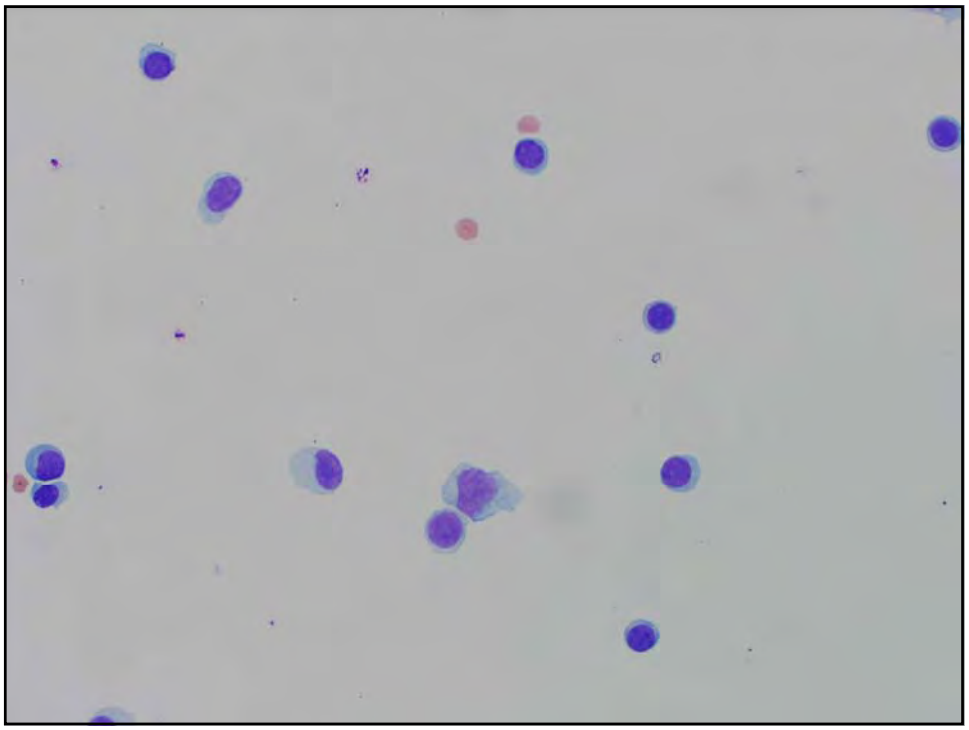


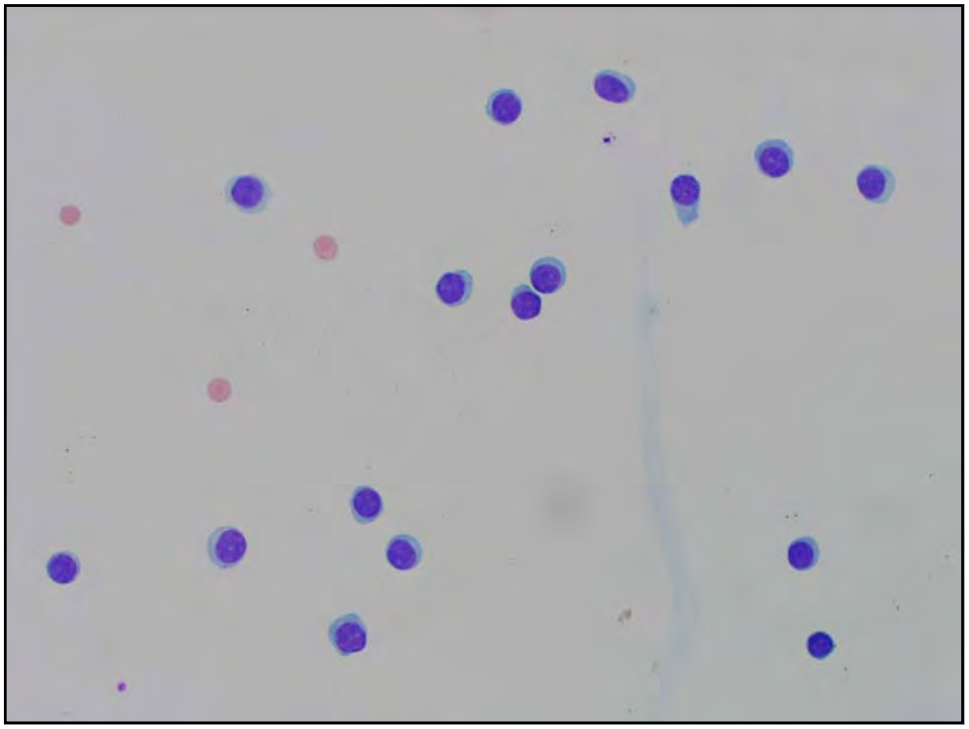
Background count after running the fluid

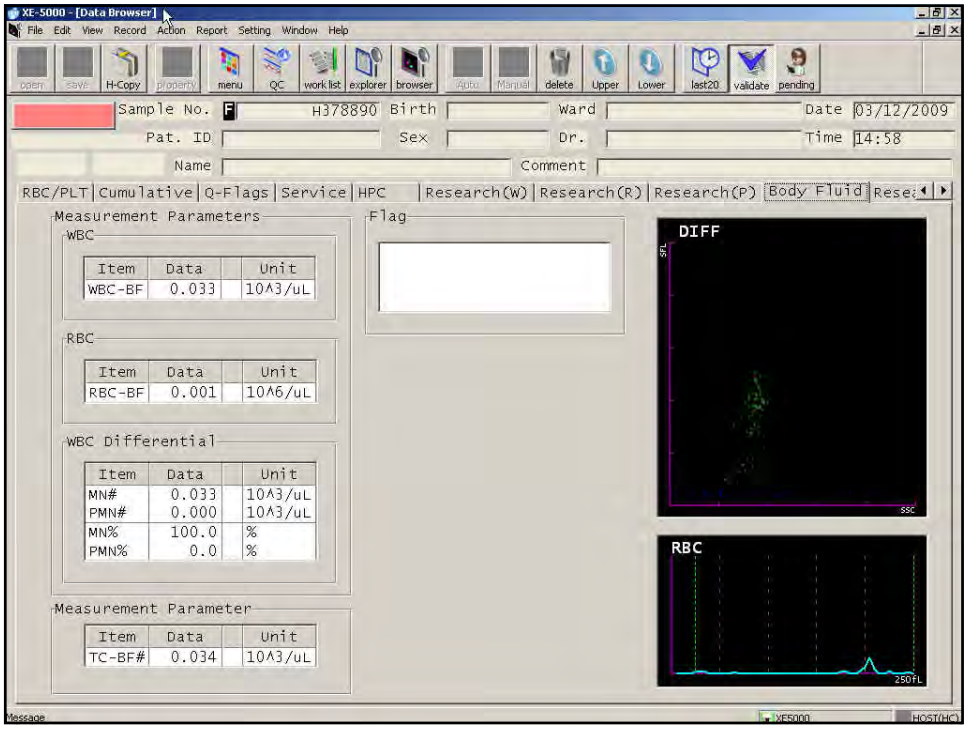


Case Study 5

- 47 YEAR OLD FEMALE
  - ER PATIENT
  - ALTERED VISION ??
- CSF
  - NUCLEATED CELLS 4 / mcL
  - RBC 3 / mcL






XE-5000 - [Data Browser]
File Edit View Record Action Report Setting Window Help
open save H-Copy property menu QC work list explorer browser Auto Manual delete Upper Lower last20 validate pending
Sample No. F H378890 Birth Ward Date 03/12/2009
Pat. ID Sex Dr. Time 14:58
Name Comment
RBC/PLT Cumulative Q-Flags Service HPC Research(W) Research(R) Research(P) Body Fluid
Measurement Parameters
WBC
Item Data Unit
WBC-BF 0.033 10^3/uL
RBC
Item Data Unit
RBC-BF 0.001 10^6/uL
WBC Differential
Item Data Unit
MN# 0.033 10^3/uL
PMN# 0.000 10^3/uL
MN% 100.0 %
PMN% 0.0 %
Measurement Parameter
Item Data Unit
TC-BF# 0.034 10^3/uL
Flag
DIFF
SFL
SSC
RBC
250 FL
Message
XE5000
HOST(HC)

SOMETHING TO REMEMBER….

CYTOCENTRIFUGE CONCENTRATION

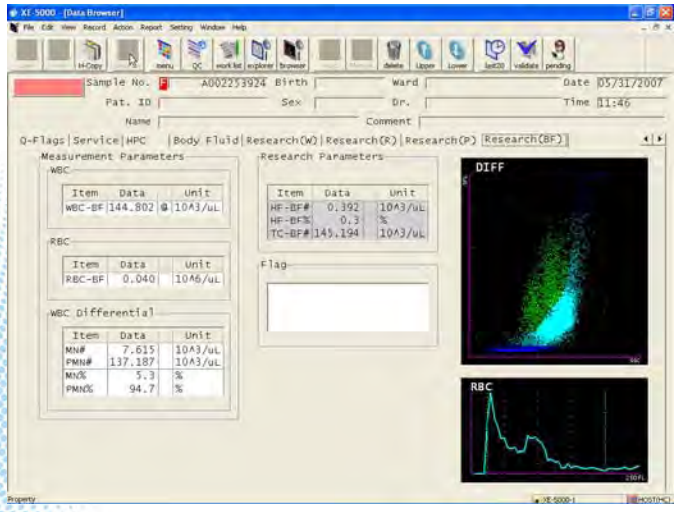
~ 20 FOLD

…IF YOU DO IT RIGHT !

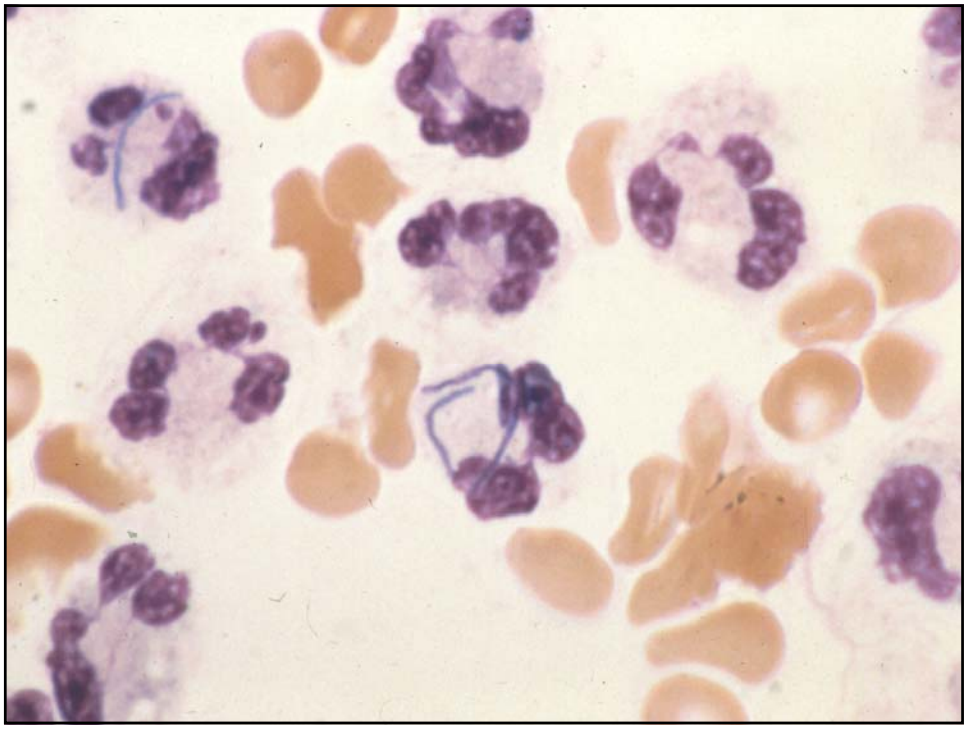
Case Study 5

- 47 YEAR OLD FEMALE
  - ER PATIENT
  - ALTERED VISION ??
- CSF
  - NUCLEATED CELLS 4 / mcL
  - RBC 3 / mcL
  - REPEAT COUNT -NUCLEATED CELLS 29 / mcL

Case Study 6



Should we dilute?



References

The Best Body Fluid References:

Body Fluids, 3rd edition (Kjeldsberg) - ASCP Press
Color Atlas of Body Fluids - CAP

Questions