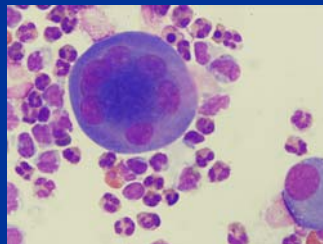


FEARLESS FLUIDS

DEPENDS ON WHAT YOU DREAD THE MOST



YUCKY CELL COUNTS



SCARY CELLS

LET'S TALK ABOUT...

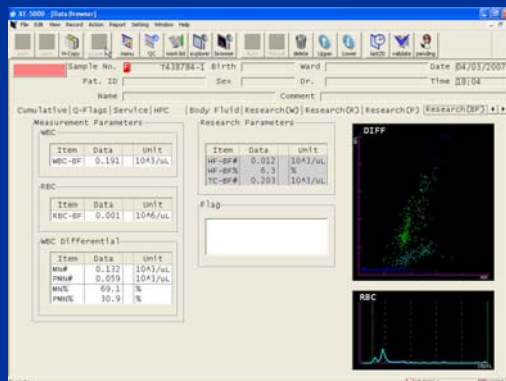
CELL COUNTING

CELL COUNTING (primitive, painful way)

FORMULA - MANUAL

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

CELL COUNTING (better way)



AUTOMATED CELL COUNTING

- better precision
- more efficient
- safer

BEFORE YOU START...THERE ARE SOME
RULES

FIRST...

SYNOVIAL FLUIDS SHOULD BE
TREATED WITH HYALURONIDASE

ORIGINAL REFERENCE - ADD 5 MG TO A 0.5 TO 1 ML
ALIQUOT

IN REALITY...



**SIGMA #H-3757
TYPE VIII BOVINE
STERILE FILTERED**

SECOND....

IT'S A...

TOTAL NUCLEATED CELL COUNT

(MACROPHAGES, MESOTHELIAL
CELLS, etc.)

NOT JUST A WBC

AND THIRD....

IF THERE ARE CLOTS OR FIBRIN GOOBERS...

TAKE THEM OUT

AND

REPORT THE CELL COUNT AS

“APPROXIMATE”

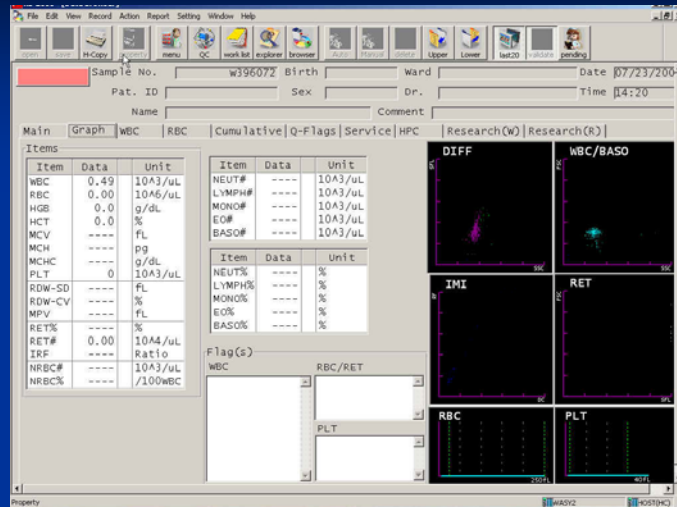
AUTOMATED CELL COUNTS

WHAT'S IMPORTANT ?

- BACKGROUNDS
- PICTURES
- AMR (Analytic measurement range)
- CRR (Clinical reportable range)

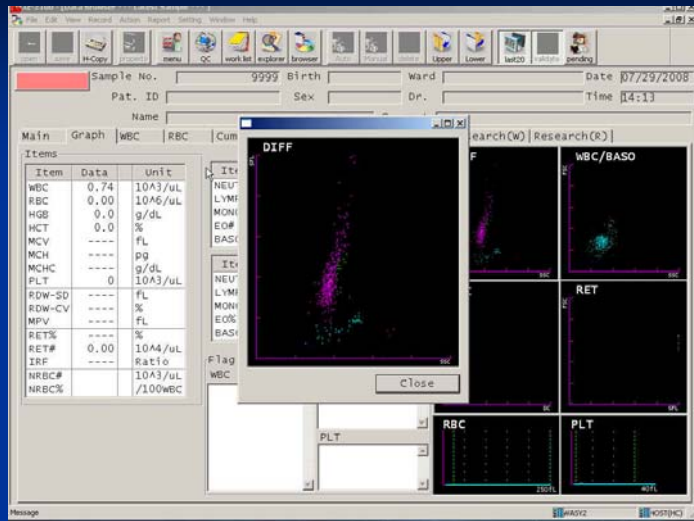
FDA APPROVED ?

PICTURES



SYSMEX XE2100

PICTURES



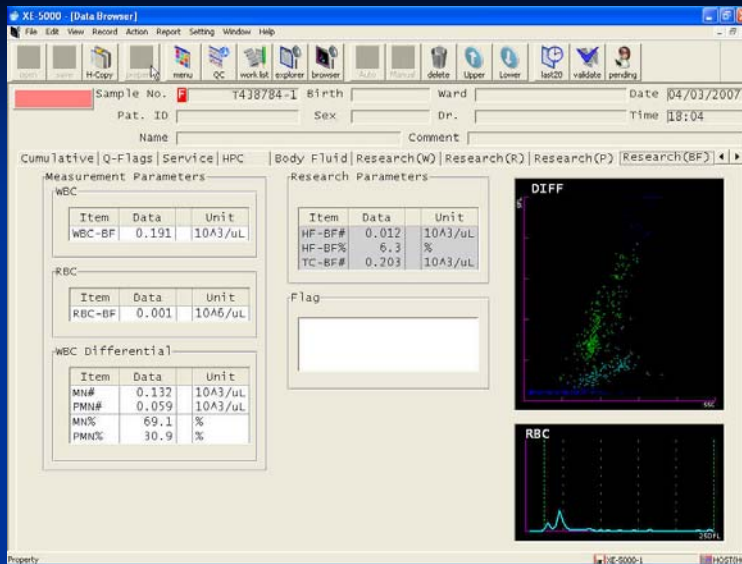
Analyzed Data

WBC#(DIFFch)	0.754 10 ³ /uL
Delta-WBC (DIFF/WBC)	1.014
Laser Current	
LD driver	46.88

SYSMEX XE2100

LINEARITY WBC → 10 / mcl
RBC → 10,000/mcl

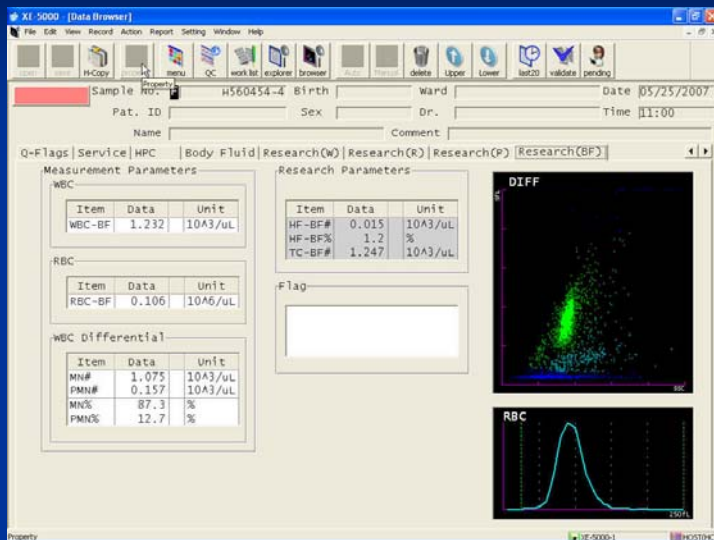
Even better



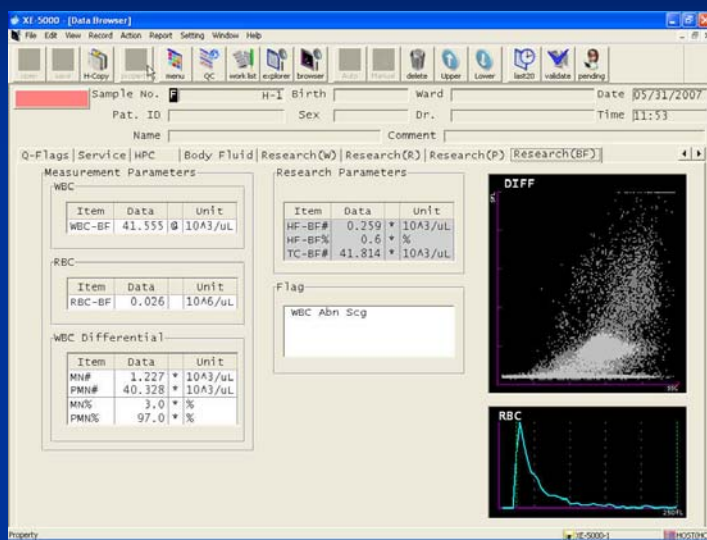
SYSMEX XE5000

LINEARITY WBC → 1 / mcl
RBC → 1,000 / mcl

KNOW WHEN IT'S GOOD..



AND WHEN IT'S NOT...



WHAT YOU HAVE TO DO

- **METHOD COMPARISON**
 - OLD TO NEW
 - csf, effusions, synovial
 - aim for 10 of each
 - low to high counts
- **ESTABLISH THE AMR, CRR**

AMR, CRR, Huh ?

AMR

ANALYTIC MEASUREMENT RANGE

CRR

CLINICAL REPORTABLE RANGE

AMR, CRR, Huh ?

AMR = LINEARITY

How low and how high can the instrument measure

CRR = REPORTABLE RANGE

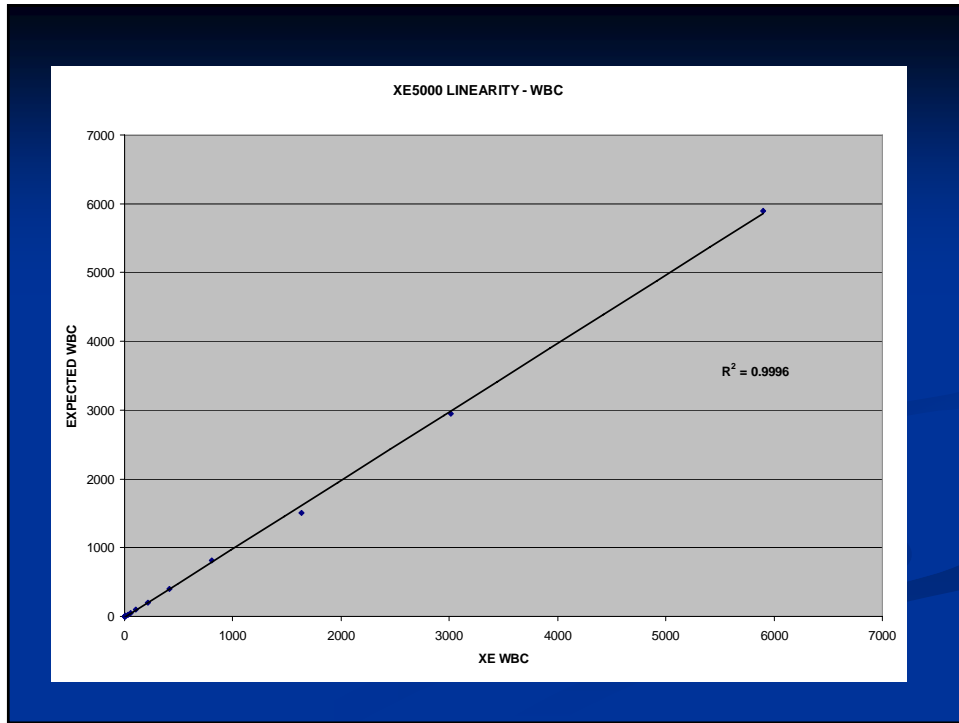
How low and how high can you report results

And why would these be different ???

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	



WHAT IF...

WBC LINEARITY (AMR) IS
10 CELLS - 100,000 CELLS / mcL

CRR could be 10 - 100,000 / mcL

BUT WHAT ABOUT COUNTS < 10 CELLS
OR > 100,000 CELLS

WHAT ABOUT...

COUNTS < 10 CELLS

Manual count or...
CRR could be <10 - 100,000 / mL

COUNTS > 100,000 CELLS

Dilute sample or...
CRR could be <10 - > 100,000

WHAT ELSE DO YOU HAVE TO DO ? METHOD COMPARISONS

SAMPLE ID	FLUID TYPE	NUCLEATED CELL COUNT		MANUAL	RED CELL COUNT		MANUAL
		XE2100	XE5000TC	WBC	XE2100	XE5000	RBC
T451171	CSF		2	1	0.00	0.000	0
X377377	CSF		6	3	0.00	0.000	1
T528363	PER DIALYS		12	5			
W517735	CSF		7	3	0.00	0.000	
W513683	CSF		1	1	0.00	0.000	0
W516236	CSF		5	3	0.00	2000	1450
H504288	CSF		2	1	0.00	0.000	
H510157	PERITONEAL		2	1			
W561348	PERITONEAL		3	4			
C002552586	CSF		5	2	0.00	0.000	
H459839	CSF	26	28	23	10000	10000	13100
T439631	PLEURAL	36	38				
H457953	PERITONEAL	58	65				
T489129	CSF	87	93		0.00	1000	654
W495324	PLEURAL	96	55				

SAMPLE ID	FLUID TYPE	NUCLEATED CELL		RED CELL COUNT	
		COUNT			
		XE2100	XE5000	XE2100	XE5000
H461438	PLEURAL	496	444		
C002536451	SYNOVIAL	502	533		
T512906	PLEURAL	508	494		
W465141	CSF	541	536	20000	14000
S396277	PLEURAL	636	544		
T442428	PERITONEAL	645	644		
F472131	PERITONEAL	711	750		
A002118826	SYNOVIAL	735	716		
W464826	PERITONEAL	817	859		
W495785	PLEURAL	866	735		
C002406093	SYNOVIAL	5238	5545		
H456554	PLEURAL	5364	4043		
H478537	PLEURAL	5597	5321		
C002404493	SYNOVIAL	7257	7773		
F474355	PLEURAL	8894	8568		

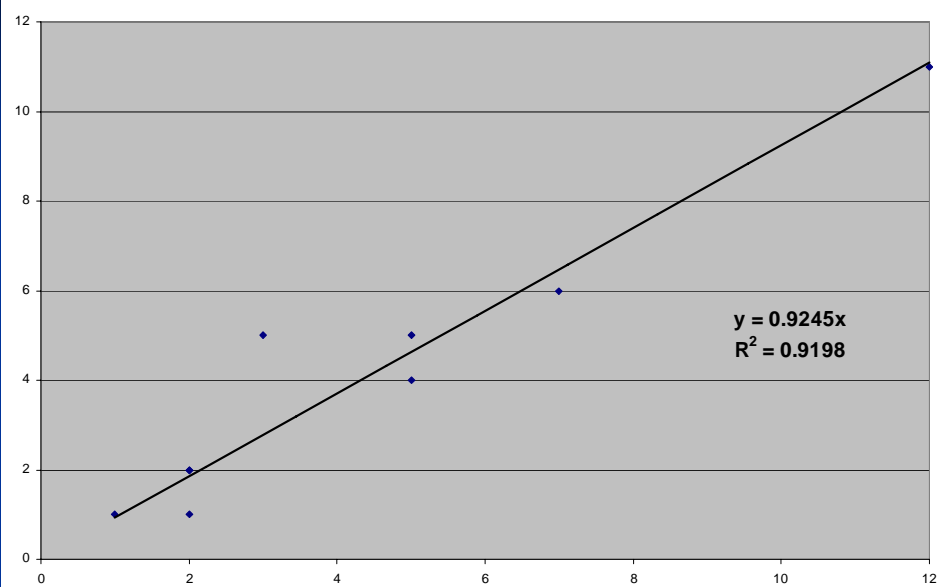
RBC counts are of little clinical use except in CSF

HOW CLOSE SHOULD THE METHODS MATCH ?

Depends on the methods –

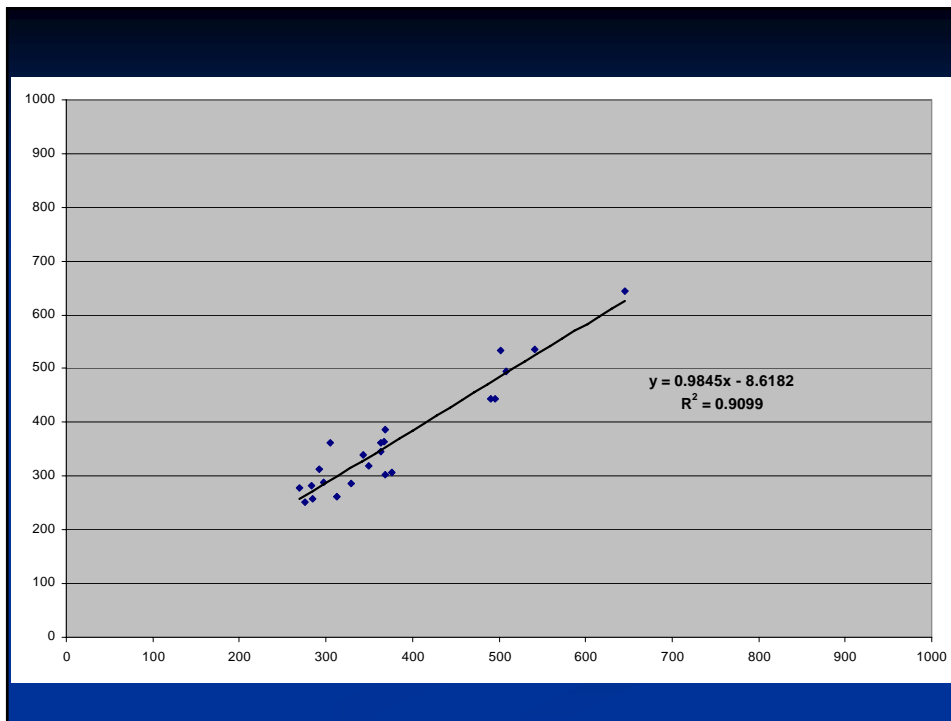
- manual to automated
- automated to automated

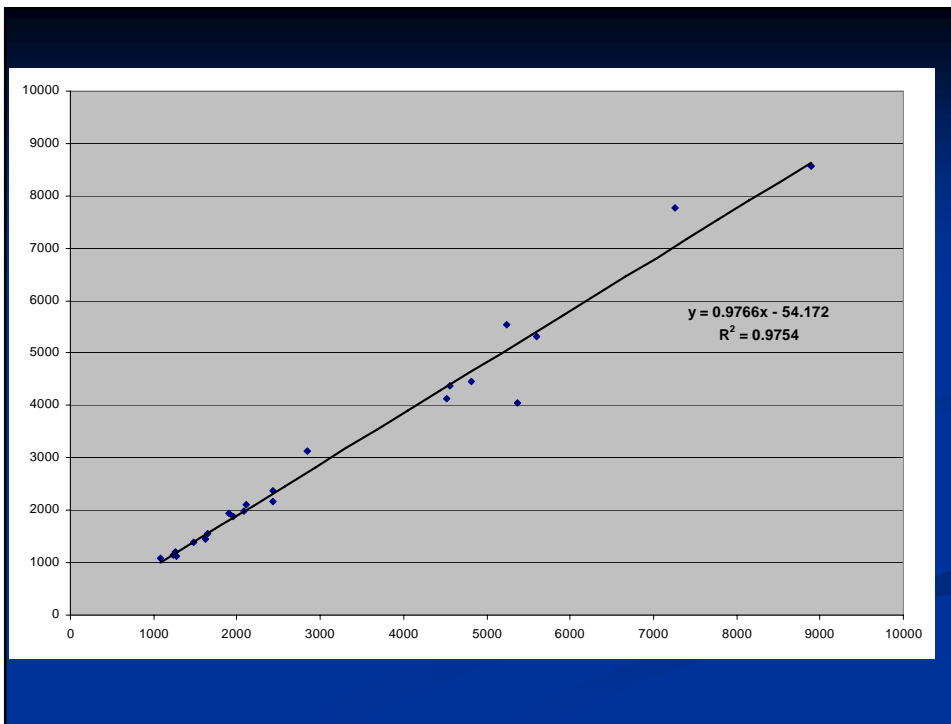
NOT GOOD ENOUGH ?



LOOK AT THE NUMBERS...

SAMPLE ID	FLUID TYPE	NUCLEATED CELL COUNT		MANUAL	RED CELL COUNT		MANUAL
		XE2100	XE5000TC	WBC	XE2100	XE5000	RBC
T451171	CSF		2	1	0.00	0.000	0
X377377	CSF		6	3	0.00	0.000	1
T528363	PER DIALYS		12	5			
W517735	CSF		7	3	0.00	0.000	
W513683	CSF		1	1	0.00	0.000	0
W516236	CSF		5	3	0.00	2000	1450
H504288	CSF		2	1	0.00	0.000	
H510157	PERITONEA L		2	1			
W561348	PERITONEA L		3	4			
C002552586	CSF		5	2	0.00	0.000	
H459839	CSF	26	28	23	10000	10000	13100
T439631	PLEURAL	36	38				
H457953	PERITONEA L	58	65				
T489129	CSF	87	93		0.00	1000	654
W495324	PLEURAL	96	55				





A DOSE OF REALITY

NUCLEATED CELL COUNTS

“RESULTS ARE RARELY HELPFUL”

“FREQUENTLY NOT HELPFUL”

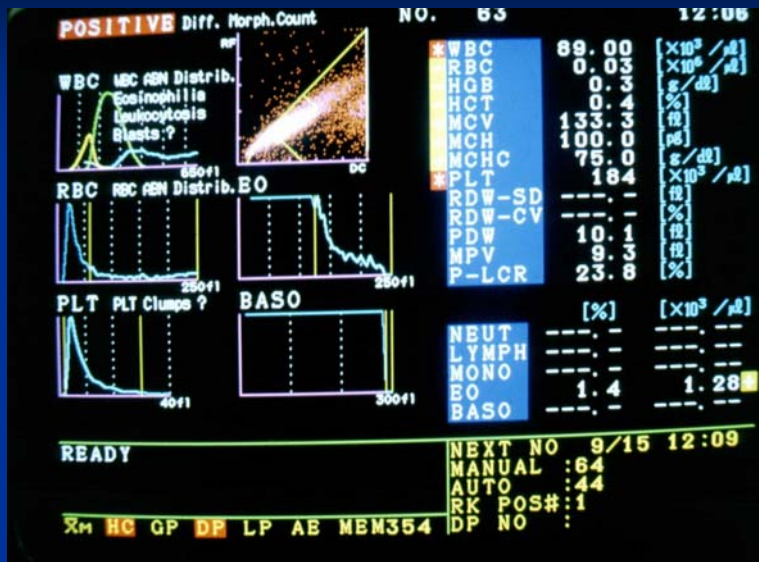
“LACK SPECIFICITY”

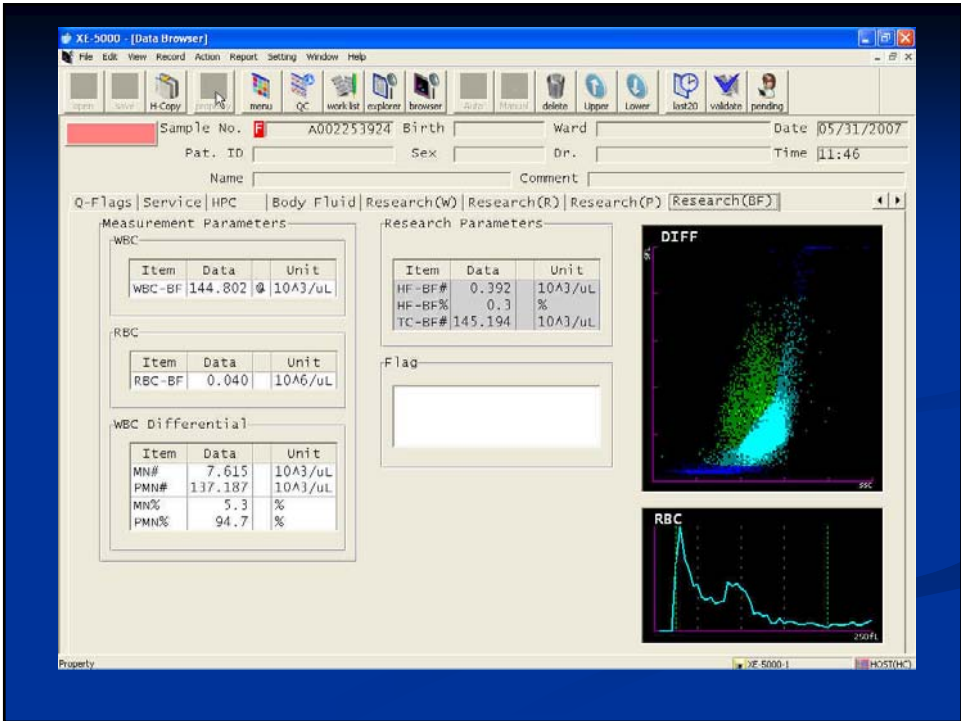
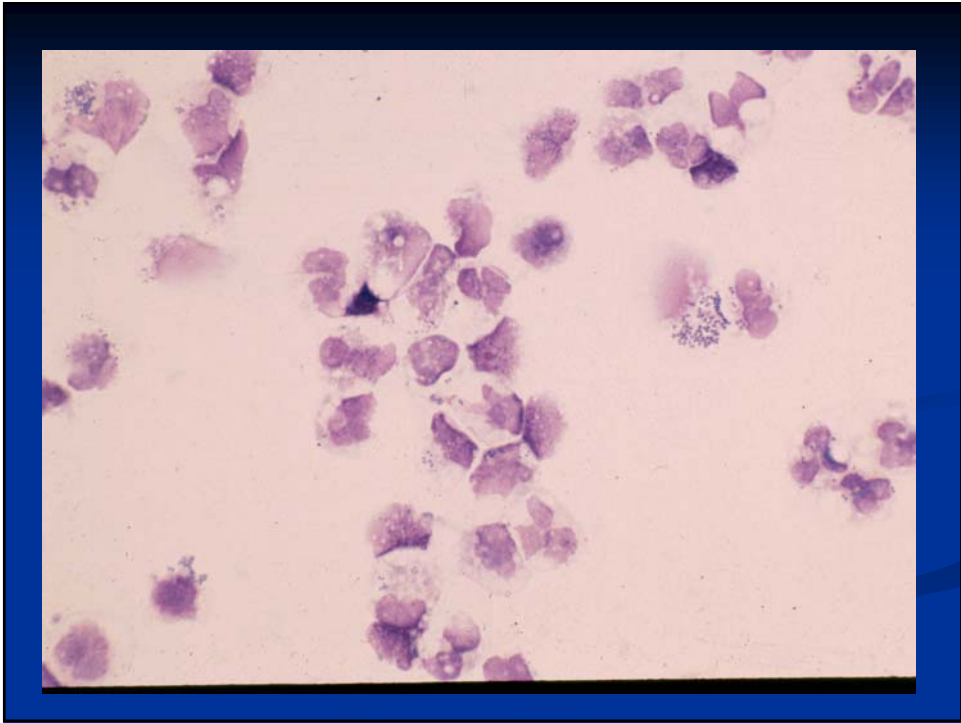
“NEED CLINICAL JUDGEMENT”

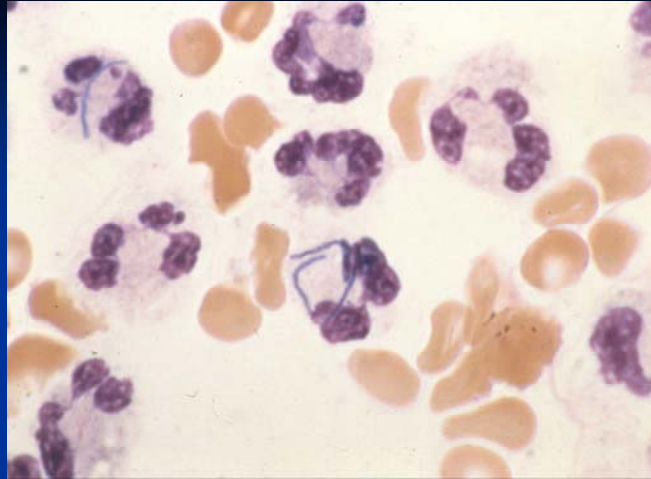
THE TYPE OF CELL PRESENT
IS MORE IMPORTANT THAN
THE CELL COUNT ALONE.

MORE REALITY

CSF







Hmmmm.....

should I dilute this ?



**Do I want to do an
automated or manual cell count ?**

CELLS



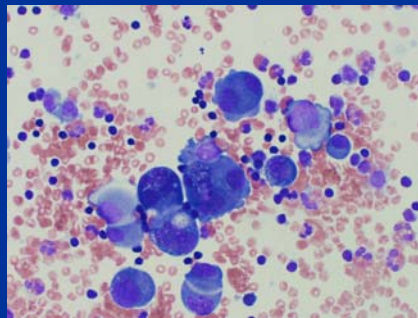
FIRST...YOU NEED ONE OF THESE
or something like it



SECOND.....

EVEN IF IT'S CLOTTED...

YOU CAN DO SOMETHING WITH IT !



CELLS THAT ARE EVERYWHERE

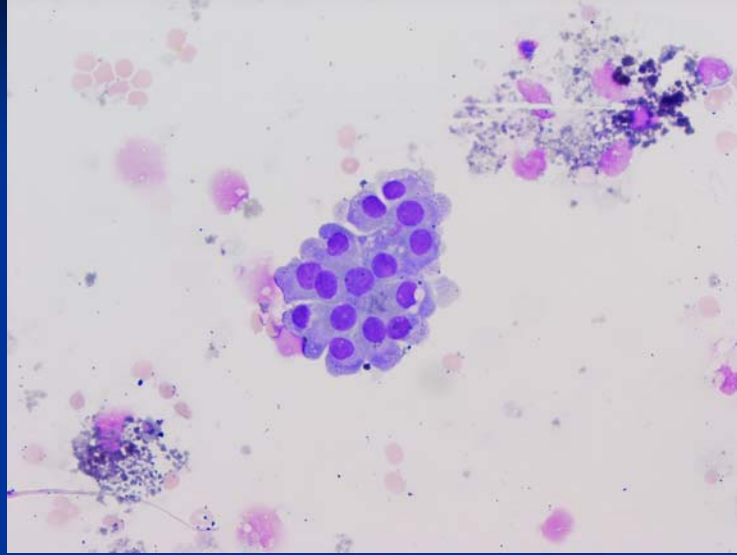
- WBCS, RBCS
- MONOMACROPHAGES
- ALL THE OTHER 'PHAGES'
- MALIGNANT

CELLS CSF

- WBCS RBCS
- EPENDYMAL
- CHOROID PLEXUS
- GERMINAL MATRIX CELLS
- MACROPHAGES
- MALIGNANT



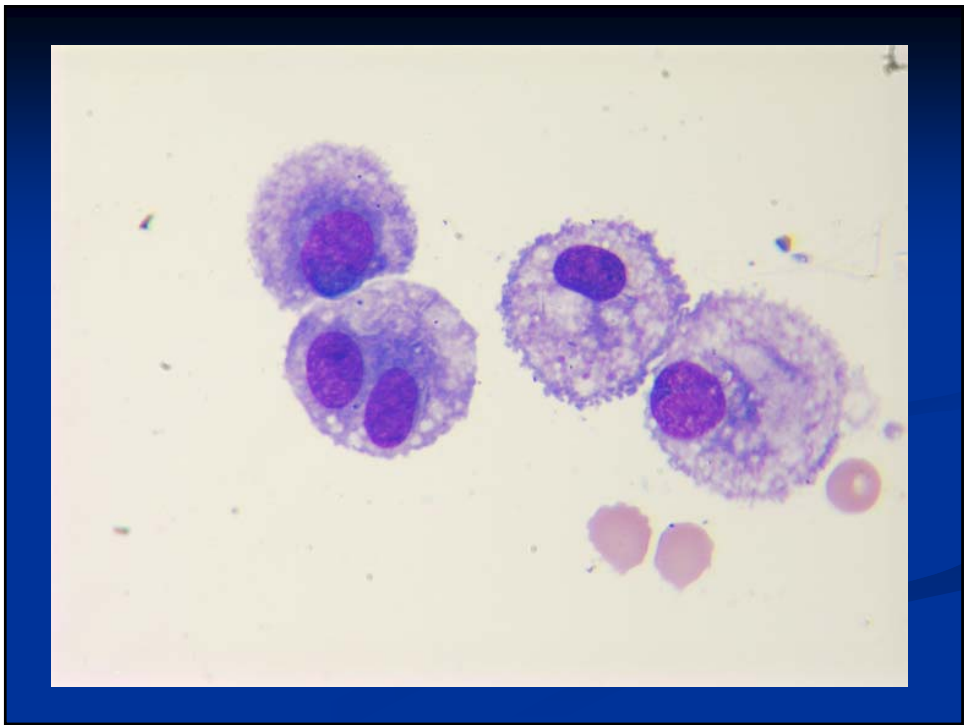
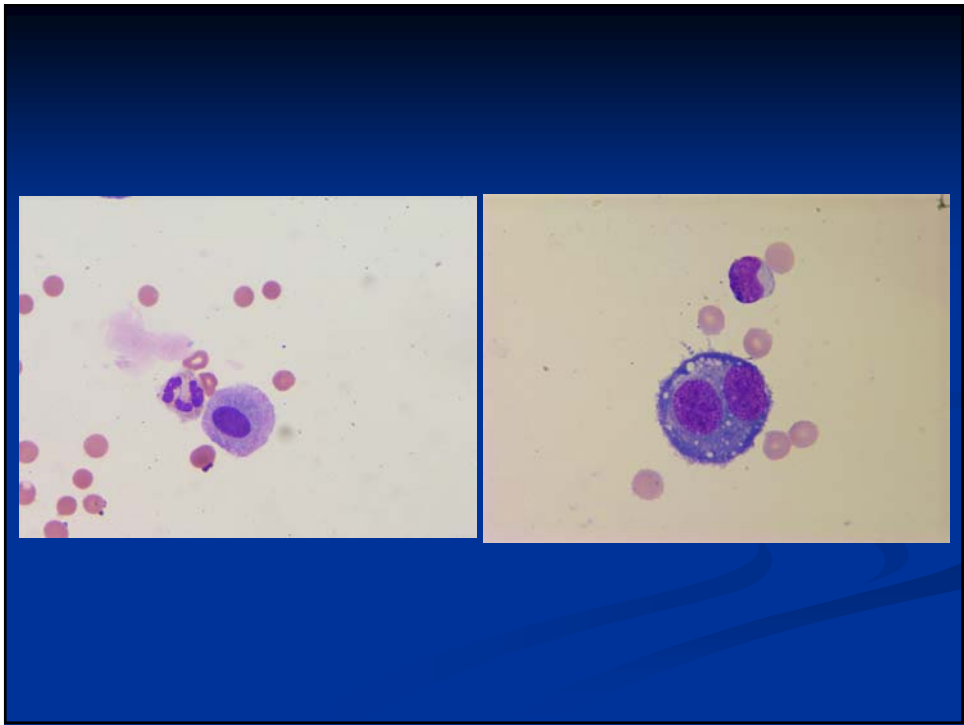
EPENDYMAL, CHOROID PLEXUS, GERMINAL MATRIX ??



= NEUROECTODERMAL CELLS

CELLS SYNOVIAL

- WBCS RBCS
- MACROPHAGES
- SYNOVIAL

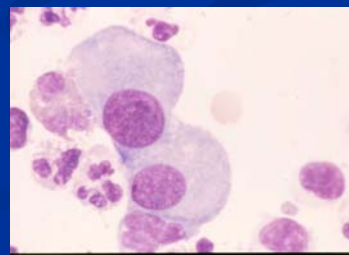
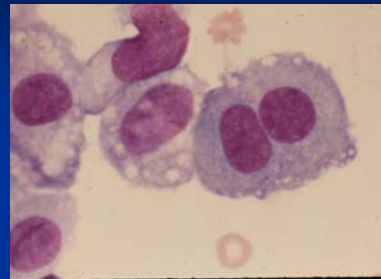
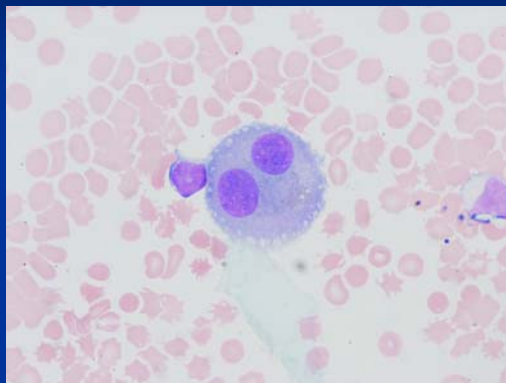


CELLS

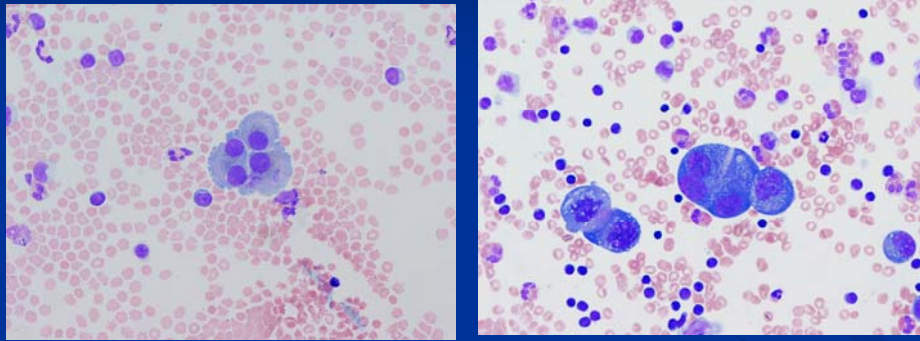
PLEURAL/PERITONEAL

- WBCS RBCS
- **MESOTHELIAL**
- MACROPHAGES
- MALIGNANT

MESOTHELIAL CELLS



DIFFERENTIATING MESOTHELIAL
AND MALIGNANT CELLS



CELLS

MESOTHELIAL
VS
MALIGNANT

CELLS

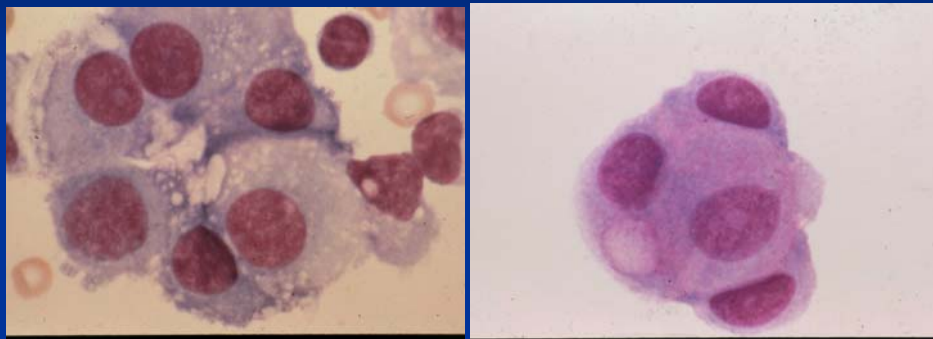
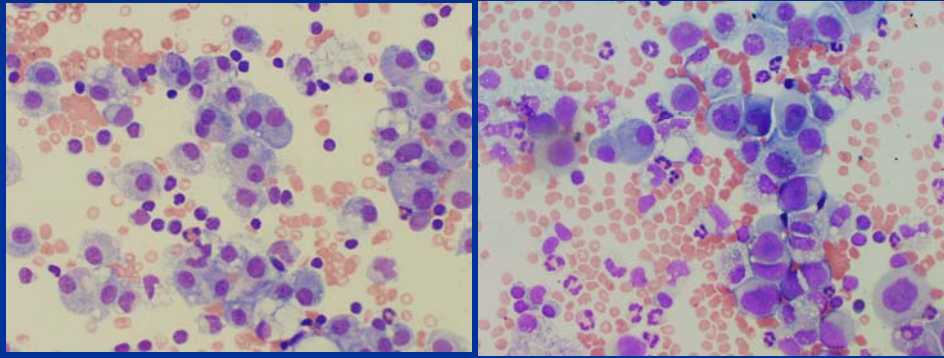
MESOTHELIAL

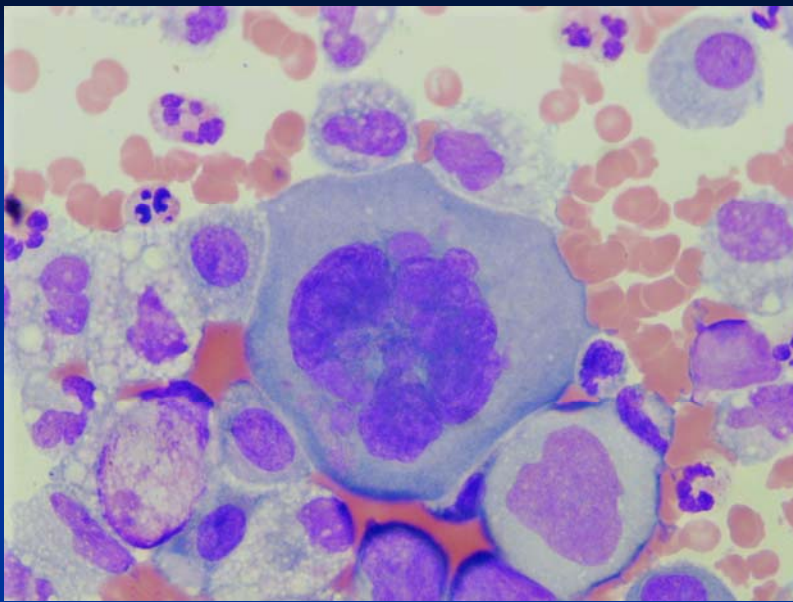
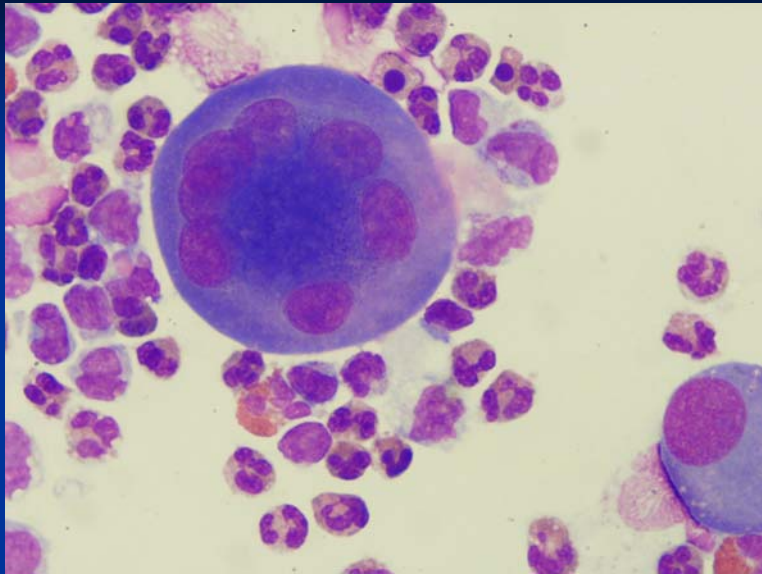
- individual
- uniform
- flat clusters
- n/c ratio low

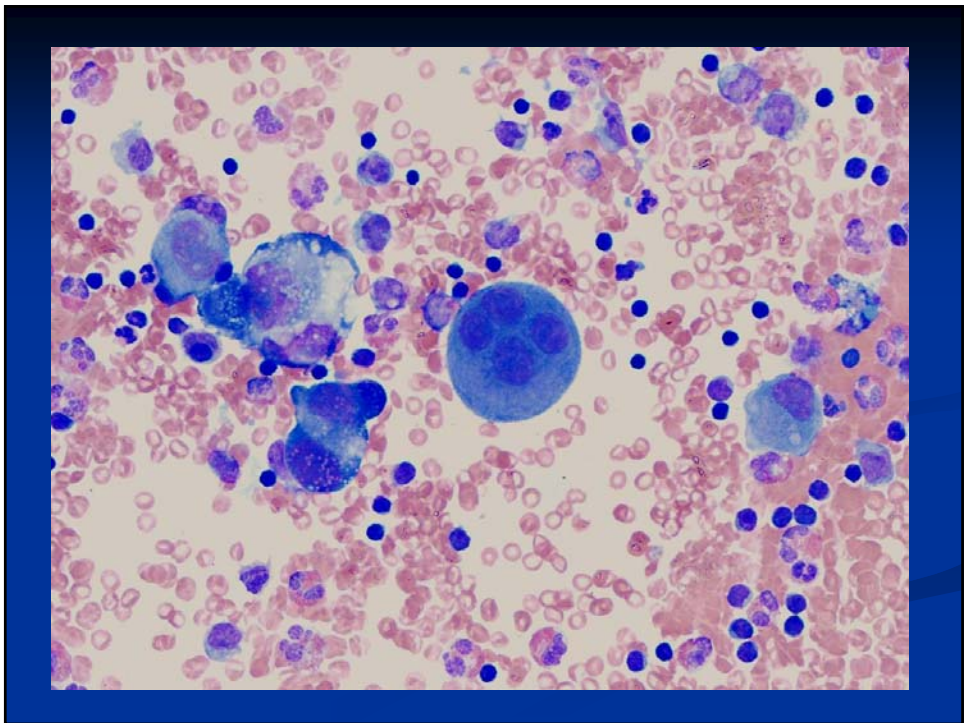
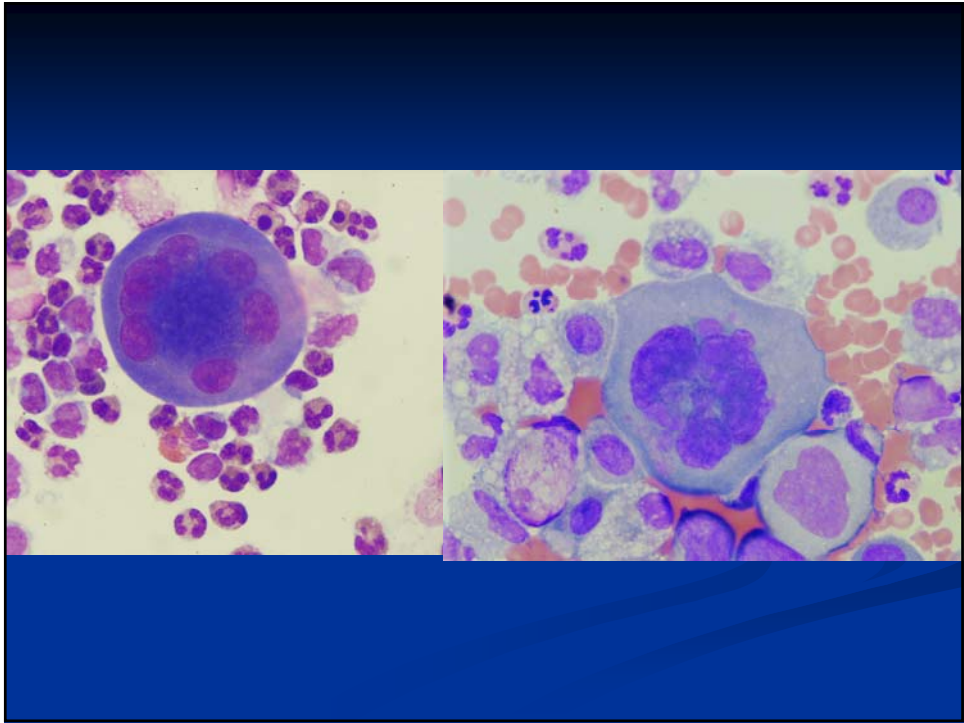
CELLS

MALIGNANT

- cannibalism
- bizarre
- ball-like clusters
- n/c ratio high







CELLS

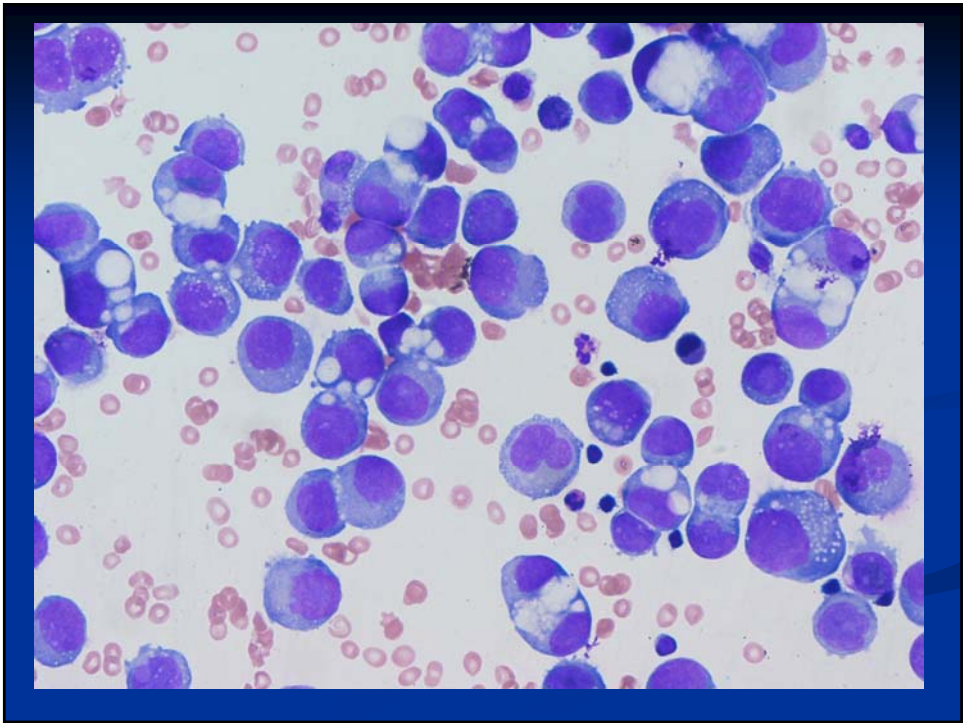
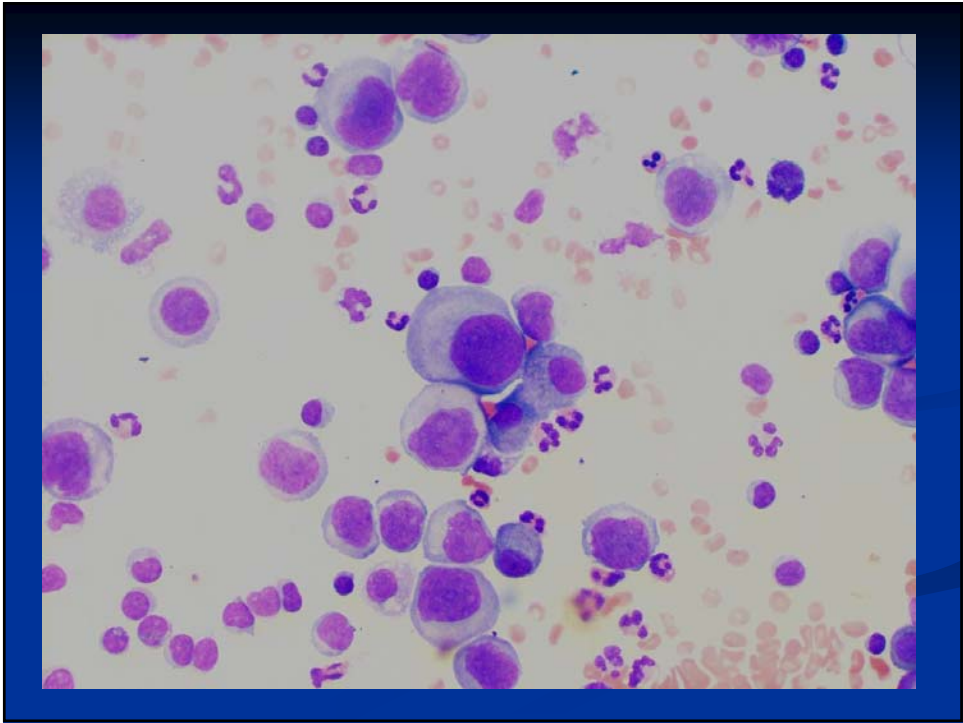
MESOTHELIAL

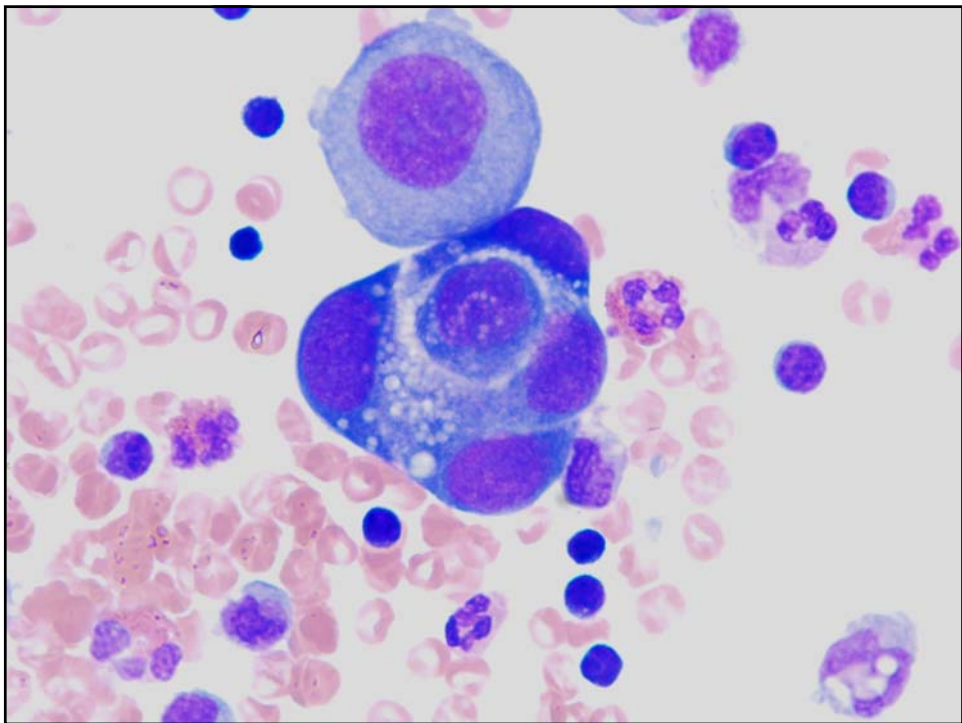
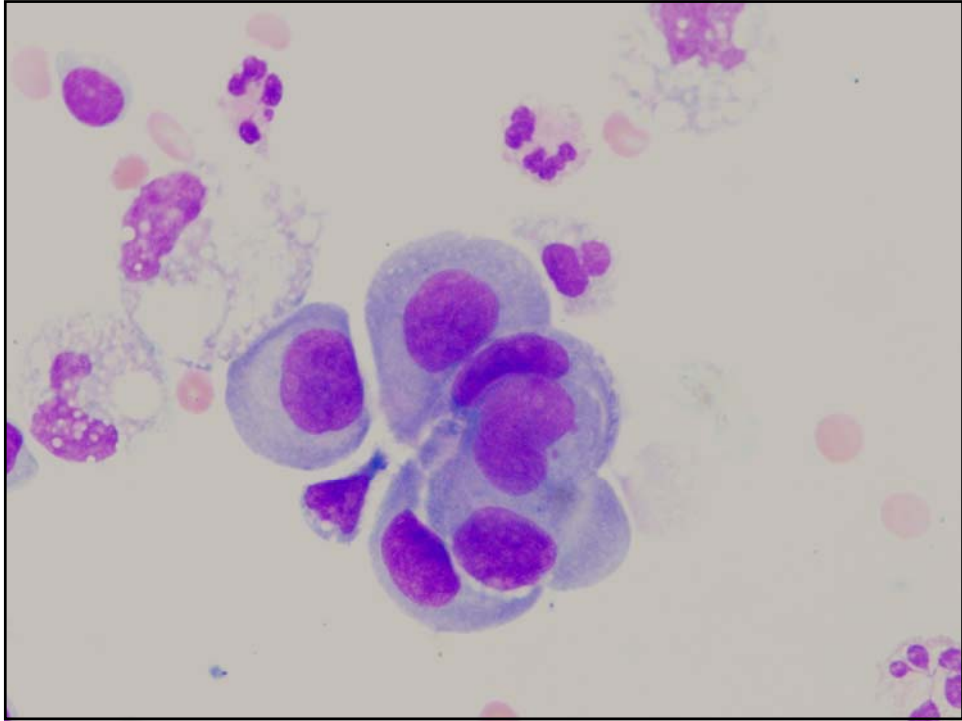
- smooth chromatin
- smooth nuclear membrane
- round/oval nuclei
- if multinucleated-uniform
- minimal vacuolization

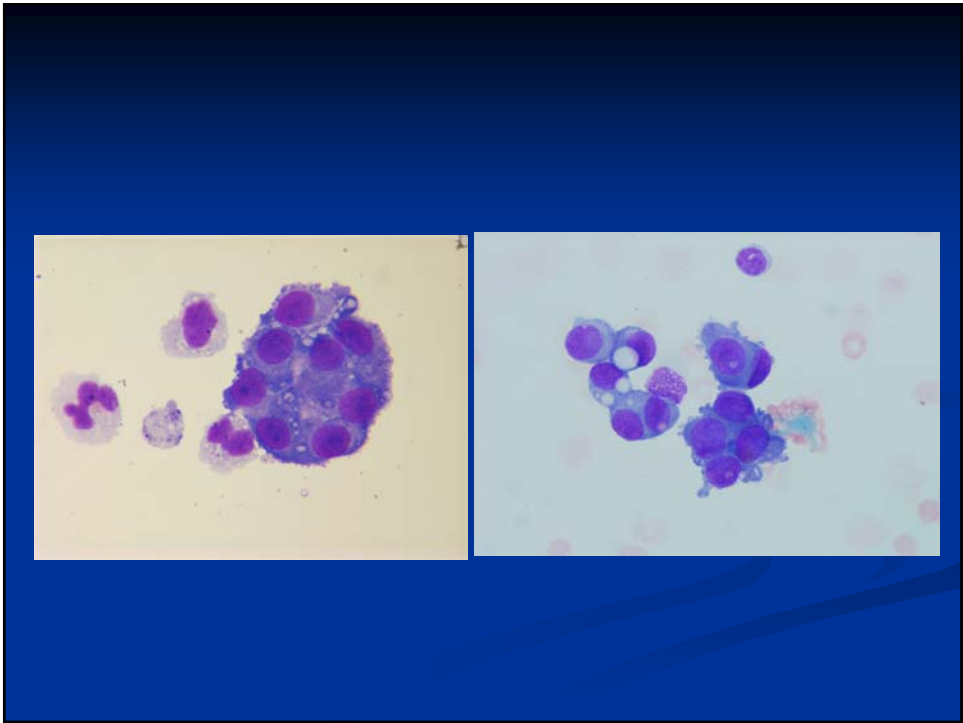
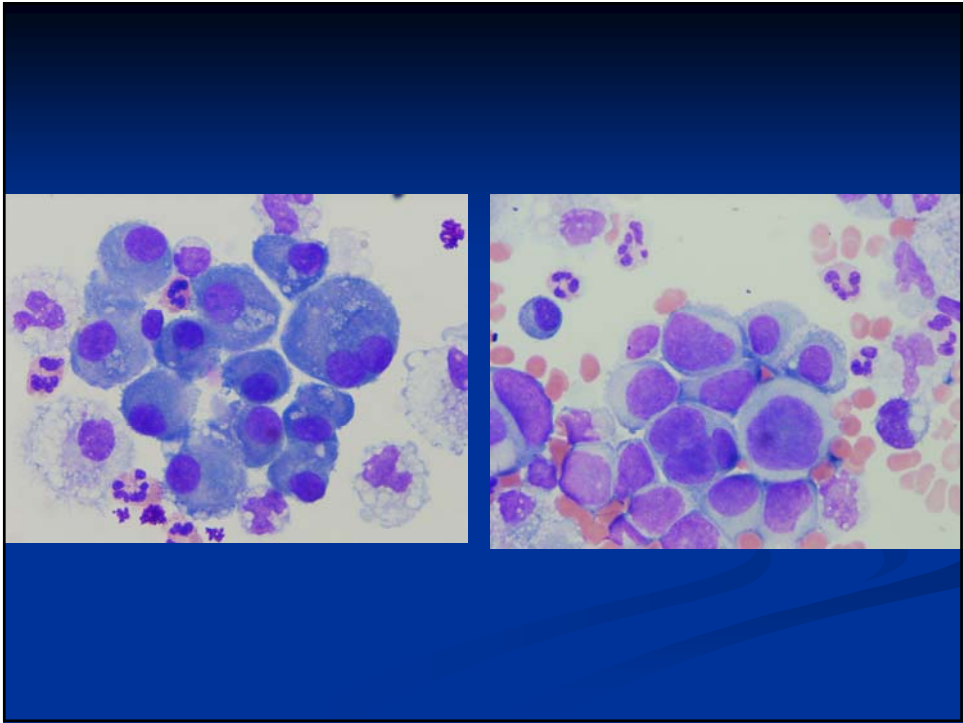
CELLS

MALIGNANT

- uneven chromatin
- irregular nuclear membrane
- nuclear clefting/molding
- multinucleated - non-uniform
- dramatic vacuolization



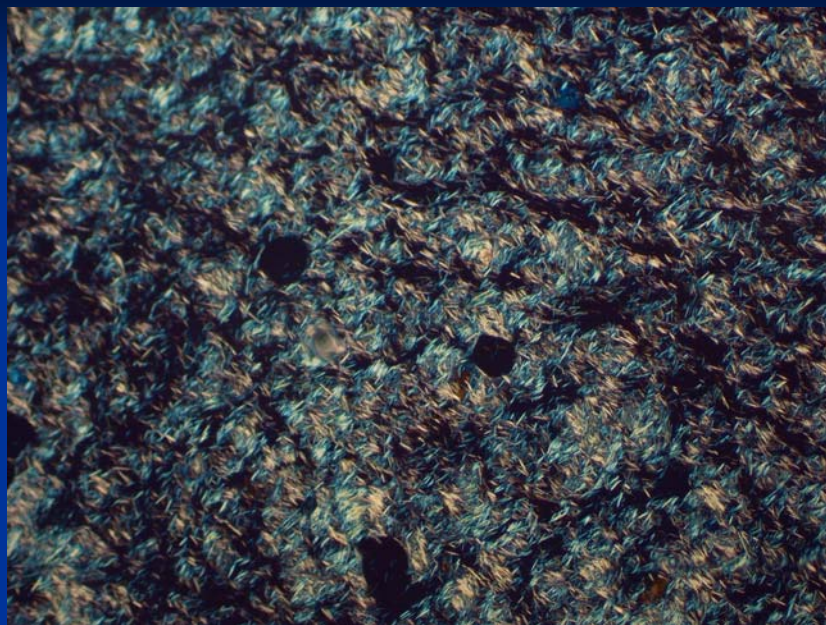




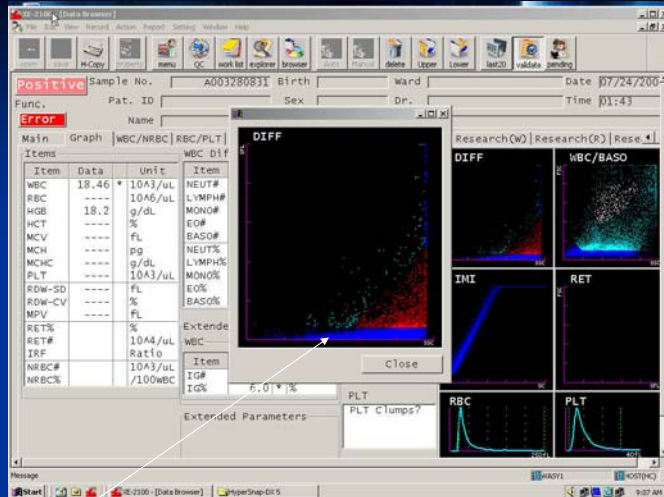
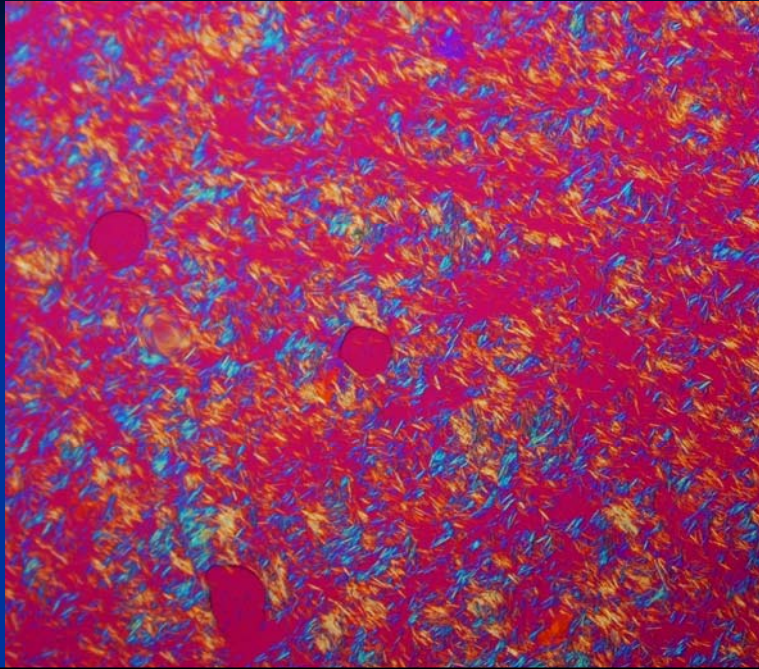
IF YOU'RE WONDERING.....



URIC ACID CRYSTALS - POLARIZED LIGHT

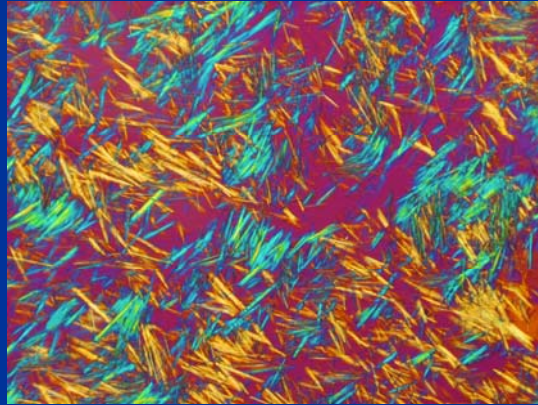


RED COMPENSATOR FILTER



The diff channel on the XE showed interference from the uric acid crystals as did the WBC/BASO channel. A manual count would need to be performed but.....

HOW ARE YOU GOING TO FIND THEM ?
AND DOES IT MATTER ?



If you worry about what this may do to your instrument - don't. It's just fine.

This is the background after running the fluid

The left screenshot displays a patient record for sample 400228031. The 'Main' section shows a list of lab items and their results:

Item	Data	Unit	Item	Data	Unit	Item	Data	Unit
WBC	18.46	10 ⁴ /UL	NEUT#	1.76	10 ⁴ /UL	RBC Lysc Res		
RBC	----	10 ⁶ /UL	LMPH#	0.13	10 ⁴ /UL	WBC Abn Seg		
HGB	18.2	g/dL	MONO#	0.10	10 ⁴ /UL	Baso		
HCT	----	%	IGM	15.66	10 ⁴ /UL			
MCV	----	fL	SADOF	0.83	10 ⁴ /UL			
MCH	----	pg	NEUT%	9.5	%			
MCHC	----	g/dL	LMPH%	0.7	%			
PLT	----	10 ³ /UL	MONO%	0.5	%			
RDW-SD	----	fL	EGN	84.8	%			
RDW-CV	----	%	BAOSO	4.4	%			
MPV	----	fL						

The right screenshot shows the 'DIFF' (Differential) test results for the same sample. It includes a table of counts and percentages for different cell types, and a 'Service Data' section with numerical values:

DIFF	Count	%
DIFF	0	0
DIFF-y	0	0
DIFF-x	0	0
DIFF-w	0	0
DIFF-v	0	0
DIFF-u	0	0
DIFF-t	0	0
DIFF-s	0	0
DIFF-r	0	0
DIFF-q	0	0
DIFF-p	0	0
DIFF-o	0	0
DIFF-n	0	0
DIFF-m	0	0
DIFF-l	0	0
DIFF-k	0	0
DIFF-j	0	0
DIFF-i	0	0
DIFF-h	0	0
DIFF-g	0	0
DIFF-f	0	0
DIFF-e	0	0
DIFF-d	0	0
DIFF-c	0	0
DIFF-b	0	0
DIFF-a	0	0

The 'Service Data' section includes the following values:

- DIFF Sampling Data: DIFF-x: 0.0, DIFF-y: 0.0, DIFF-w: 4.7, DIFF-v: 200.0, DIFF-u: 0, DIFF-t: 0, DIFF-s: 0, DIFF-r: 0, DIFF-q: 0, DIFF-p: 0, DIFF-o: 0, DIFF-n: 0, DIFF-m: 0, DIFF-l: 0, DIFF-k: 0, DIFF-j: 0, DIFF-i: 0, DIFF-h: 0, DIFF-g: 0, DIFF-f: 0, DIFF-e: 0, DIFF-d: 0, DIFF-c: 0, DIFF-b: 0, DIFF-a: 0
- Analyzed Data: WBC(DIFFch): 0.000 10⁴/UL, Delta-WBC (DIFF/WBC): 0.000, Number of Abnormal Cell: Area1: 0, Area2: 0, Area3: 0
- Laser Current: LD Driver: 49.72, PMT CV: 231, SPL: 291



QUESTIONS, COMMENTS ?