

Validation of the Sysmex XE2100 Haematology Analyser

A laboratory experience

What did we Validate?

- Fully automated high throughput haematology analyser capable of processing 150 samples/hour.
 - **Sysmex XE2100**
- Utilises a combination of flow cytometry and lysing agents to generate leucocyte differentials and flagging of abnormal cells.

Validation Considerations

■ *Why Validate?*

- To access the performance, advantages and limitations of this instrument.

■ *Validation Standard*

- ICSH guidelines published in Clinical Laboratory Haematology 1984

‘Protocol for evaluation of Automated Blood Cell Counters’

■ *Extent of Validation*

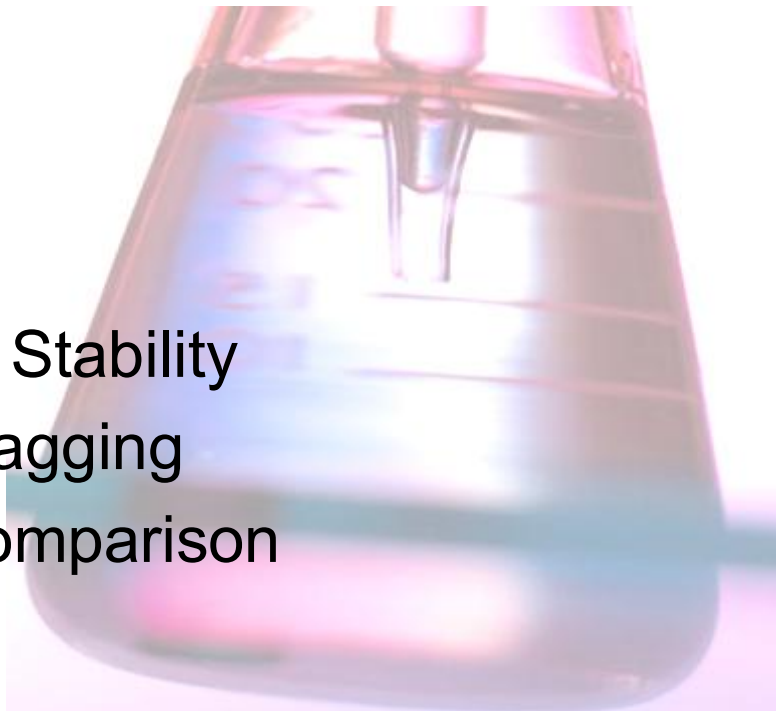
- 332 Blood samples for the evaluation were residual patient samples collected for clinical testing purposes.
- A further 170 utilised in the time & temp stability studies.





Areas Evaluated

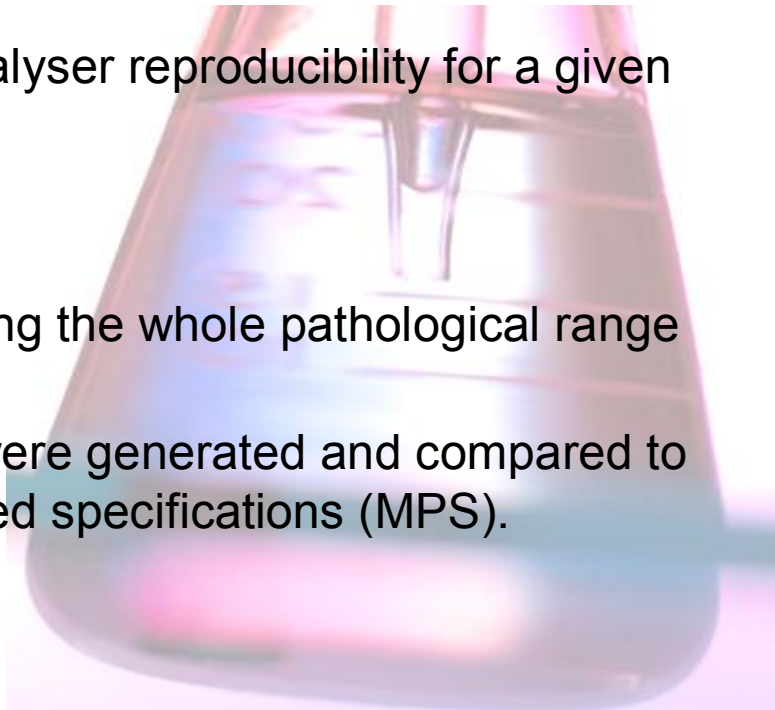
- Precision
- Linearity
- Carryover
- Time & Temp Stability
- Analysis of Flagging
- Differential Comparison





Precision Studies

- **AIM:** Measurement of Analyser reproducibility for a given sample
- **Procedure:**
 - 160 specimens spanning the whole pathological range were each analysed.
 - Variation coefficients were generated and compared to manufacturers published specifications (MPS).



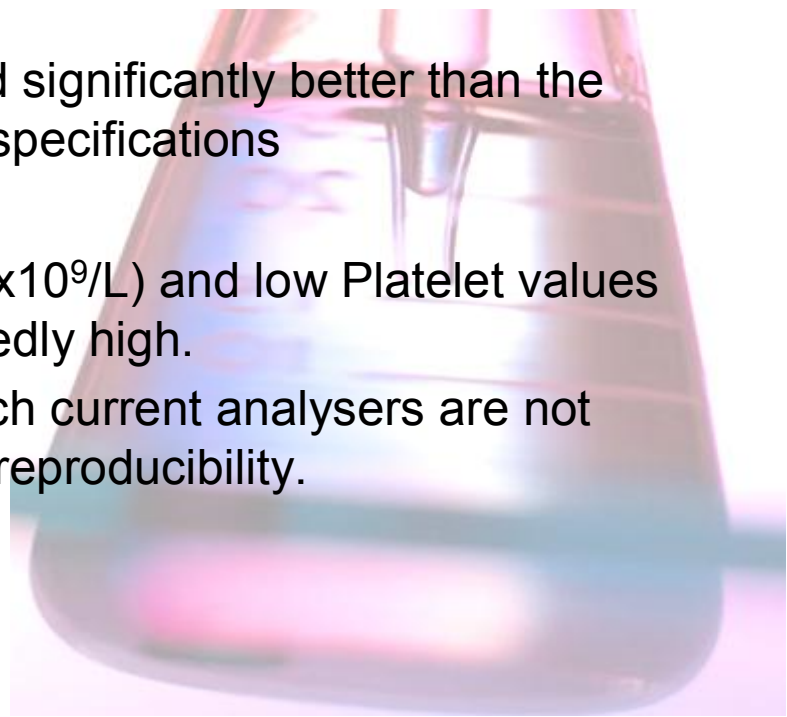
Precision Results

Measurement		XE 1 Auto	XE 1 Man	XE 2 Auto	XE 2 Man	MPS
WCC	<1	6.18	7.60	7.33	5.89	N/A
	1-4	2.36	2.83	3.13	2.42	N/A
	4-11	2.27	2.62	1.37	2.06	3.0
	11-20	1.79	0.81	1.55	1.26	3.0
	>20	1.00	1.61	0.77	0.64	3.0
RCC	<3.8	0.95	0.62	0.81	1.02	N/A
	3.8-6.5	0.53	0.83	1.19	0.62	1.5
	>6.5	0.70	0.37	2.39	0.96	1.5
Hb	<8	0.63	0.87	0.53	0.91	1.0
	8-14	0.61	0.61	0.99	0.63	1.0
	>15	0.77	0.61	0.30	0.47	1.0
Platelets	<10	3.67	7.9	4.66	22.22	N/A
	10-50	4.71	4.51	4.98	7.62	N/A
	50-150	3.27	3.64	3.50	2.31	4.0
	150-450	2.07	2.00	1.87	1.55	4.0
	>450	1.30	0.82	2.50	1.37	4.0
Retics	0.2-2%	8.65	4.99	2.11	8.92	15.0
	>2.0%	5.86	4.65	3.45	5.58	15.0



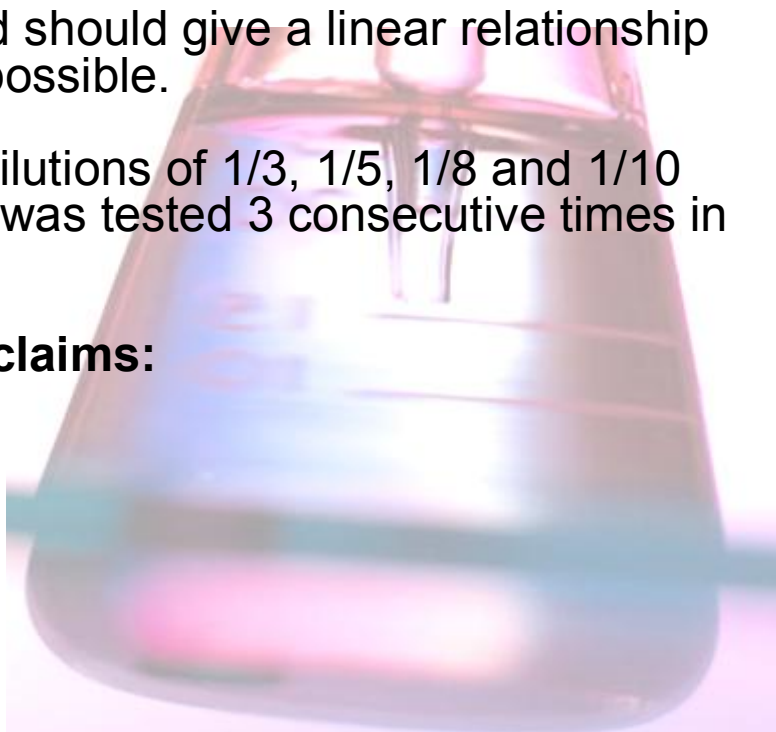
Precision Results

- Precision tests performed significantly better than the manufactures published specifications
- Results for low WCC ($<1 \times 10^9/L$) and low Platelet values ($<50 \times 10^9/L$) were expectedly high.
- Reflects limits below which current analysers are not capable of counting with reproducibility.



Linearity

- **AIM:** An analytical method should give a linear relationship over as large a range as possible.
- **PROCEDURE:** Sample dilutions of 1/3, 1/5, 1/8 and 1/10 were made. Each dilution was tested 3 consecutive times in manual mode.
- **Manufacturers linearity claims:**
 - WCC 0 –100x10³/uL
 - RCC 0-8x10⁶/uL
 - Hb 0-25g/dL
 - Plts 0 1000x10³/uL
 - Retic 0-15%





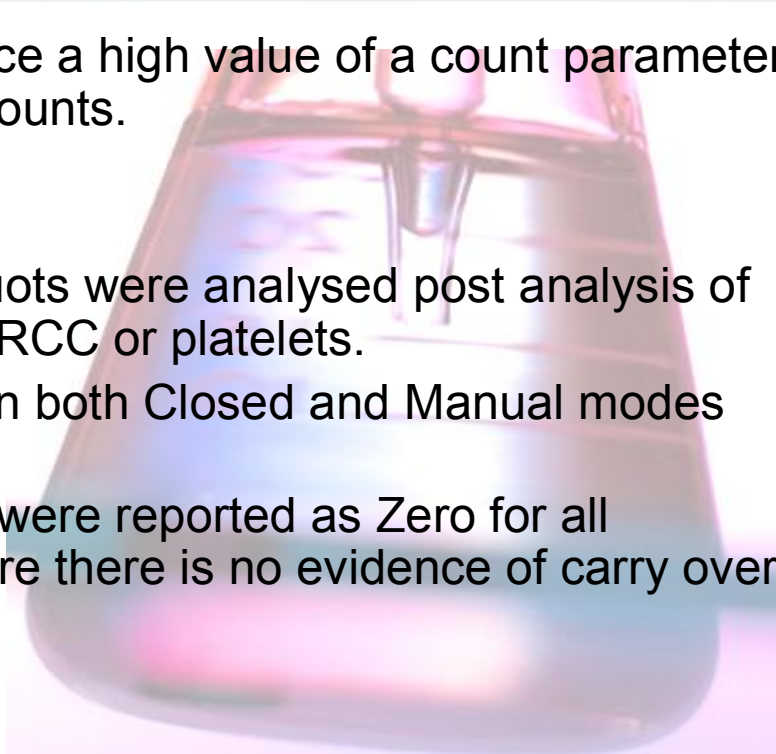
Linearity

Variance Ratio		
	XE1	XE2
WCC		1.45
RBC	8.25	6.99
Hb	0.15	4.18
Platelets	1.24	3.17
Retic	1.06	1.44

$$F_{0.95} \ 2 \ \& \ 8 \ DF = 19.4$$

Carryover

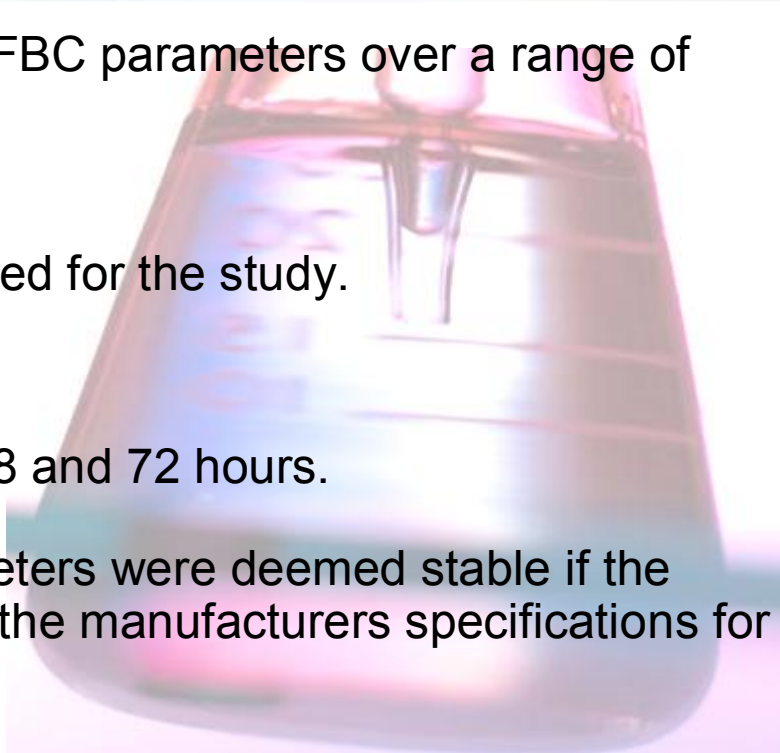
- **AIM:** To analyse any influence a high value of a count parameter might have on subsequent counts.
- **Procedure:**
 - 3 consecutive saline aliquots were analysed post analysis of samples with high WCC, RCC or platelets.
 - Analysis was conducted in both Closed and Manual modes
- **Results:** All saline samples were reported as Zero for all relevant parameters, therefore there is no evidence of carry over within the range tested.





Time & Temperature Stability Study

- **Aim:** Assess the stability of FBC parameters over a range of times and temperatures.
- **Procedure:**
 - 170 samples were analysed for the study.
 - 86 were stored at RT
 - 84 at 4°C
 - Analysed at T0, 12, 24, 48 and 72 hours.
- **Evaluation Criteria:** Parameters were deemed stable if the results did not exceed twice the manufacturers specifications for precision.

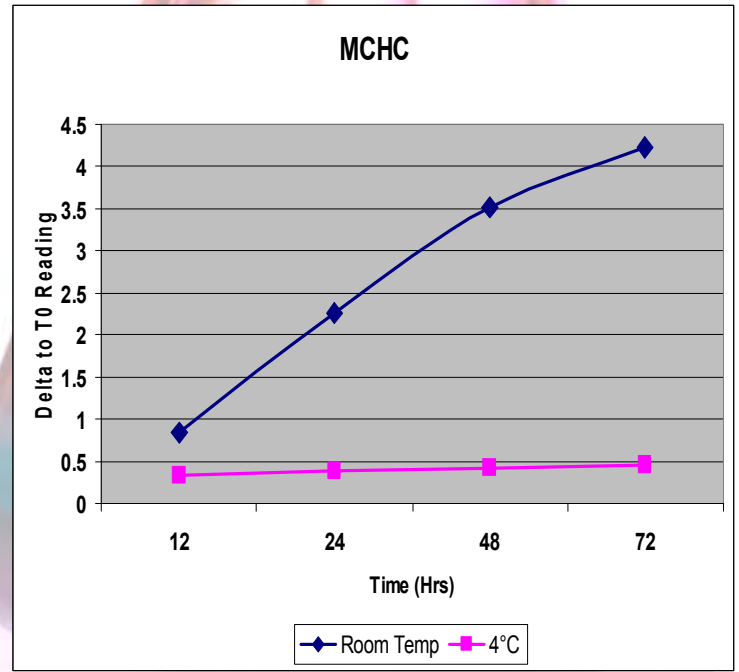
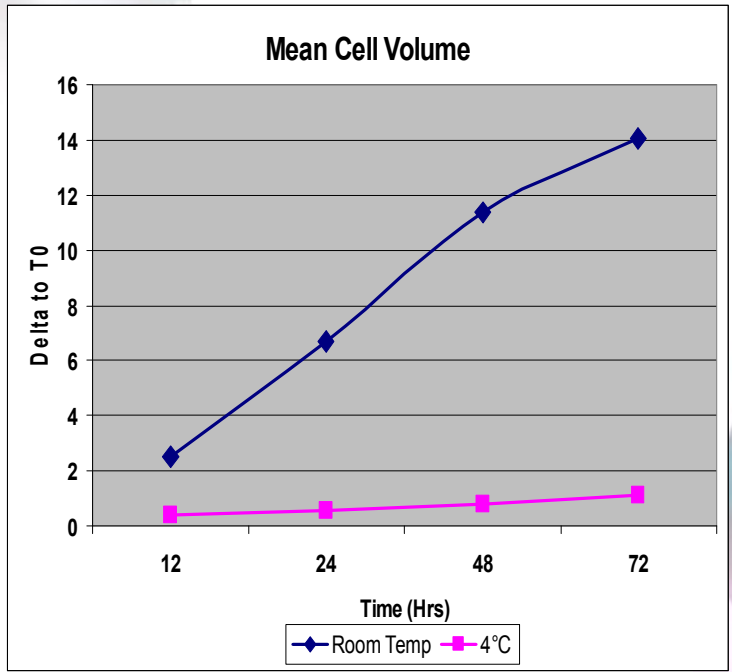




Time & Temperature Stability Study

Parameter	Room Temperature				4°C				2X Precision Limit
	12	24	48	72	12	24	48	72	
WBC	0.15	0.18	0.16	0.24	0.24	0.29	0.3	0.3	+/- 0.48
RBC	0.04	0.04	0.04	0.09	0.03	0.03	0.02	0.03	+/- 0.12
HB	0.08	0.05	0.08	0.1	0.06	0.08	0.07	0.09	+/- 0.22
HCT	0.026	0.025	0.042	0.052	0.003	0.003	0.004	0.005	+/- 0.011
MCV	2.52	6.67	11.4	14.05	0.4	0.54	0.78	1.11	+/- 1.85
MCH	0.43	0.35	0.49	0.45	0.28	0.34	0.28	0.35	+/- 0.88
MCHC	0.83	2.26	3.52	4.23	0.32	0.38	0.41	0.46	+/- 0.96
PLT	6.65	10.84	18.14	24.8	8.33	12.29	17.24	17.38	+/- 21.46

Time & Temperature Stability Study

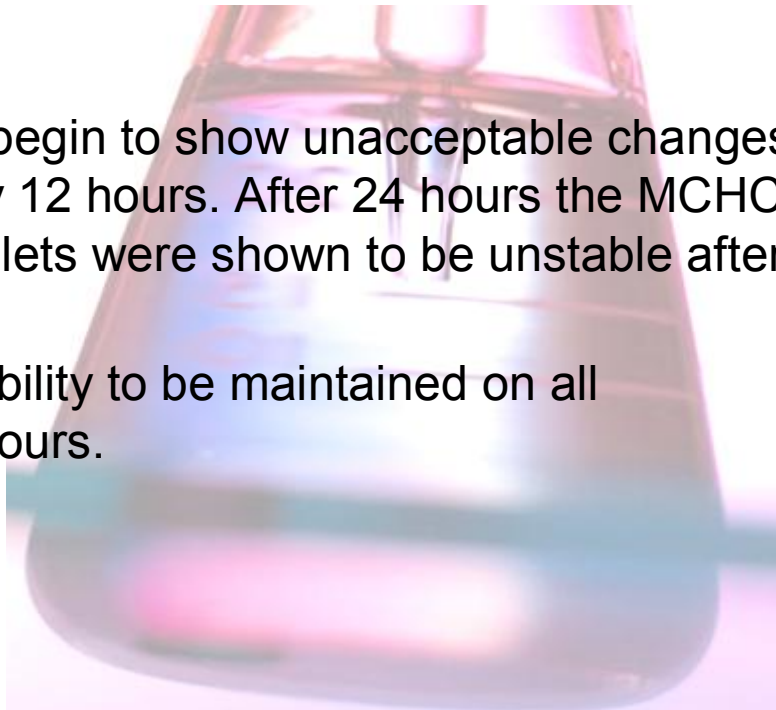




Time & Temperature Stability Study

■ Conclusion:

- Samples stored at RT°C begin to show unacceptable changes in MCV and Hct after only 12 hours. After 24 hours the MCHC also show changes. Platelets were shown to be unstable after 72 hours.
- Storage at 4°C allows stability to be maintained on all parameters for up to 72 hours.



Analysis of Flagging

- **Aim:** To assess the efficiency of the flagging system
- **Procedure:**
 - 157 samples were randomly chosen from in-patients and those attending out patient clinics.
 - Samples were processed through both XE2100 analysers and the SE9500. Blood films were made on each and all flags generated were compared to optical microscopy results.
- **Results:** The true/false-positive and true/false negative rates and efficiency of each analyser were determined and are presented in truth tables. (Next Slide)
- **Conclusion:** High flag-triggering sensitivity which increases the number of manual checks required.



Analysis of Flagging

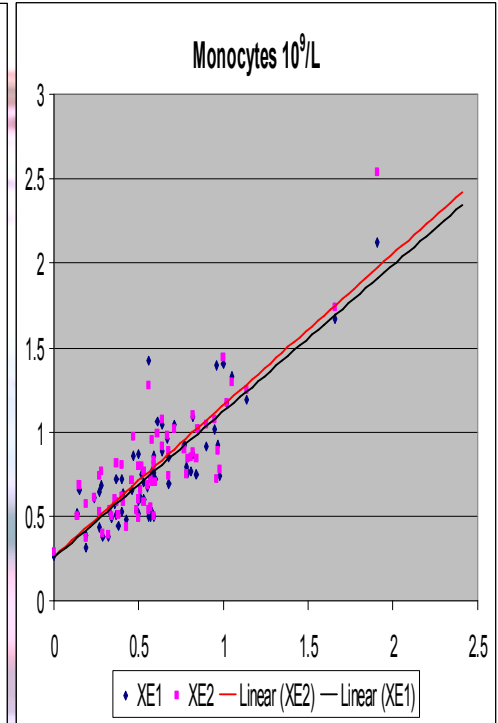
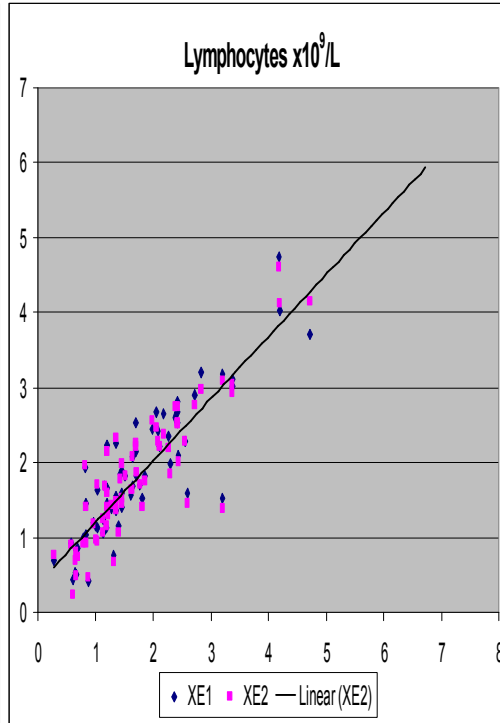
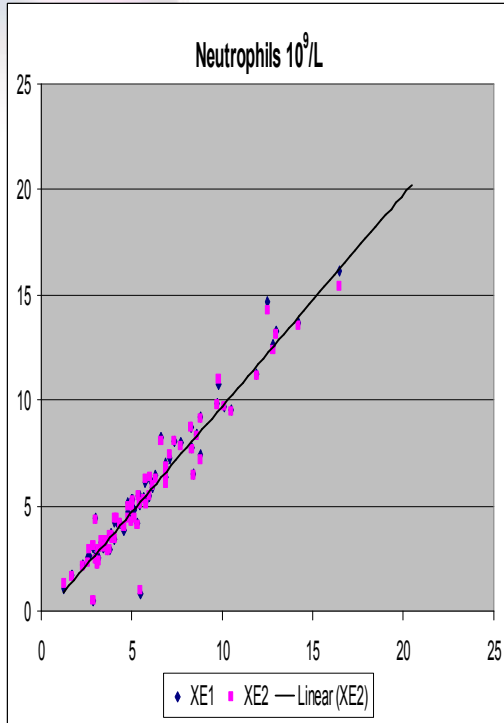
	SE	XE1	XE2
True Positives	46	50	52
False Positives	72	35	44
True Negatives	31	53	48
False Negatives	8	12	11
Sensitivity	0.852	0.806	0.825
Specificity	0.301	0.602	0.522
Predictive Value Negative	0.795	0.815	0.814
Predictive Value Positive	0.390	0.588	0.542
Efficiency	0.490	0.687	0.645

Differentials

- Aim: To assess correlation of WCC differentials to Optical microscopy.
- Procedure: 69 WCC differentials analysed by optical microscopy were compared with those from the XE2100. additional analysis of correlation of the two XE's was also investigated.
- Results:

Correlation Coefficient			
	Optical-XE1	Optical-XE2	XE1 - XE2
Neutrophils	0.97	0.96	1.00
Lymphocytes	0.88	0.88	0.99
Monocytes	0.85	0.85	0.94

Differential Scatter Plots



Validation of the Sysmex Information System (SIS)

- **Aim:** Test the integrity of data transmitted by the XE2100 analyser through the SIS data manager to the laboratory information system
- **Data Tested:**
 - Patient demographics
 - Numerical Results
 - Suspect Flags

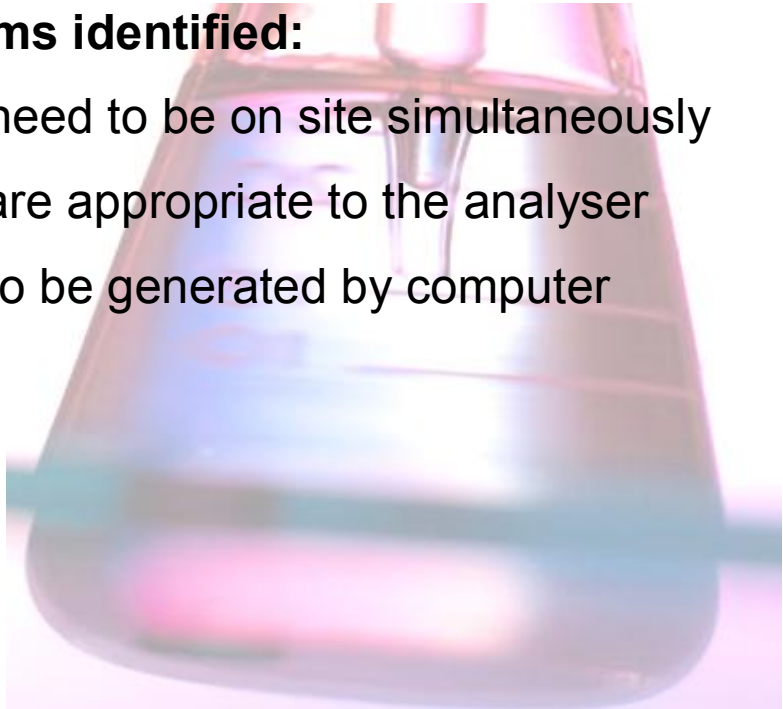




Validation of the Sysmex Information System (SIS)

■ Lessons learned/Problems identified:

1. All relevant personnel need to be on site simultaneously
2. Ensure flags selected are appropriate to the analyser
3. New codes may need to be generated by computer personnel.
4. Contingency planning
5. Back-up PC essential





Conclusions

- Validation showed that the XE2100 performed within manufactures specifications.
- The XE2100 accepted as a suitable replacement for the SE9500, and is currently in service.
- Validation was to ICSH guidelines.

