How can Blood Stabilizers be used for Validation of New Instruments?

Quality

- Pathology is essential for about 60% of all diagnoses. Thus, quality of pathology services is critical for good care and management.

- In health care there are increasing numbers of methods we can use to monitor quality:
  - Audit (internal and external)
  - Staff Training
  - Internal Quality Control
    - Standards (control or reference material)
  - External Quality Control
    - Unknown samples (peer to peer comparison, method means, group comparisons)

Quality

- One of the key components to enable the monitoring of quality is:

Standardisation

Standardisation

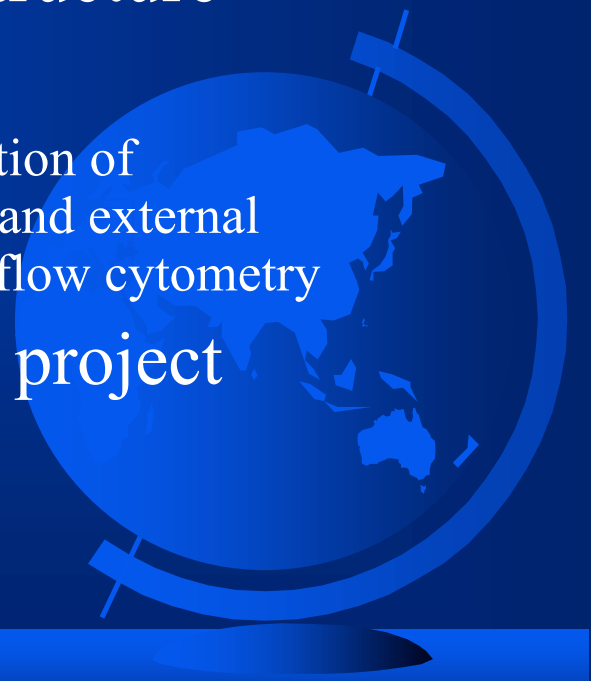
- Quality serves as a basis or example or principle to which others conform **or should conform** or by which the accuracy of others is judged.
  - Oxford Dictionary

Eurostandards

- What was it?
- Why did we do it?
- Who did it involve?
- What did it achieve?

Eurostandards What was it?

- EC Quality of Life and Management of Living Resources Proposal (Fifth Framework)
- Area Support for Research Infrastructure
- Designed to create:
  - Central facility for the production of stabilised reference standards and external quality assessment in clinical flow cytometry
- 3 year funded (2.8 million Euros) project started 1st January 2001



Eurostandards Why do it?

- Use of flow cytometry in the clinical arena has increased dramatically in the last 15 years.
- Currently, a conservative estimate puts the number of flow cytometers in clinical use at over 5,000 in European establishments.
- The European cost for providing such a service is estimated to be 2000 million Euros per annum.
- Interestingly in the UK the average amount spent on EQA is 390 Euros per annum (Equivalent to <0.3% of the facilities annual budget).
- Better standardisation could reduce the estimated expenditure on repeat testing by as much as 300 million Euros.

Eurostandards
Who did it involve?

- It involves 11 sites in 8 countries.
  - Sheffield, Rotterdam, Urbino, Regensburg, London, Salamanca, Milan, Stockholm, Lisbon, Athens, Potters Bar
- Sheffield coordinating centre
- Group met twice per annum and communicated with regular telephone conference calls & e-mail

Eurostandards What have we achieved?

Eurostandards
What did it achieve?

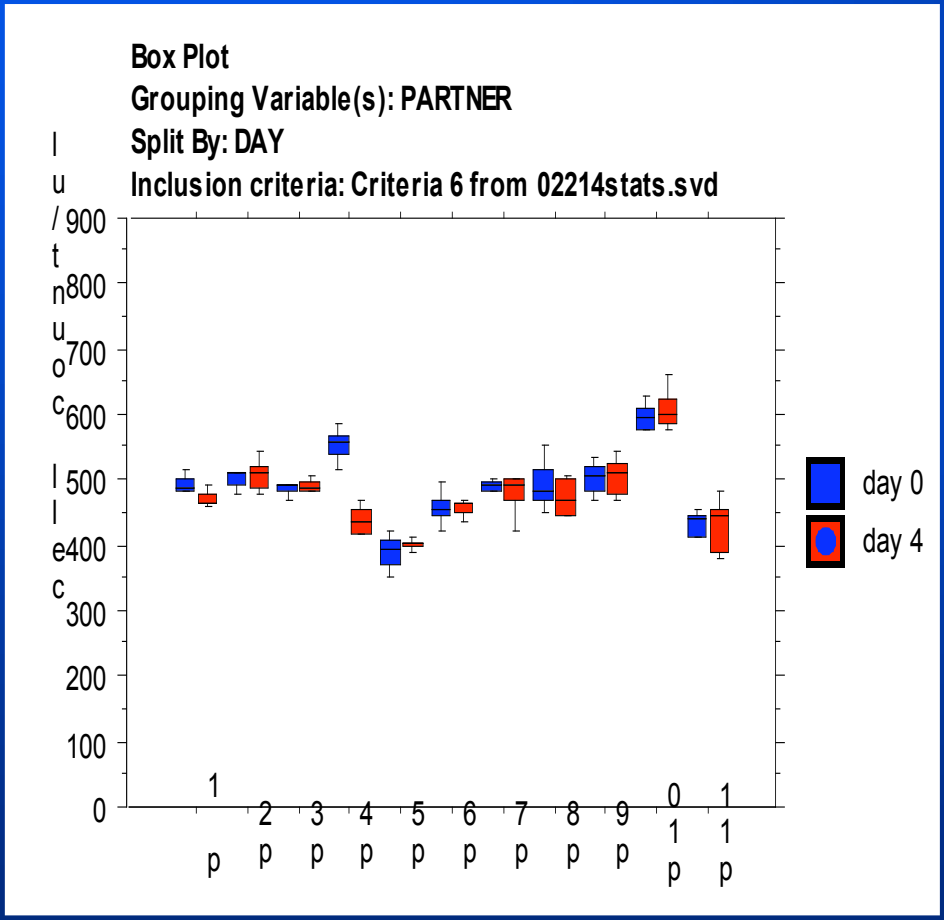
- ***"Proof of Principle"***

- Establish European & National EQA programmes.

Summary of degradation studies

- Approximately 15-18months shelf life (depending on specimen type).
- Will need to use appearance and general feel of material as an indication of shelf life, this however varies between materials and can be subjective.
- Ideally need to send samples out: soon after filling, and then at 6-8 monthly intervals for evaluation, in order for 3 or 4 assays to be completed within 18 months to have large scale data on sample degradation and have agreement on shelf life.

Work package 1
02/ 214 CD4 counts



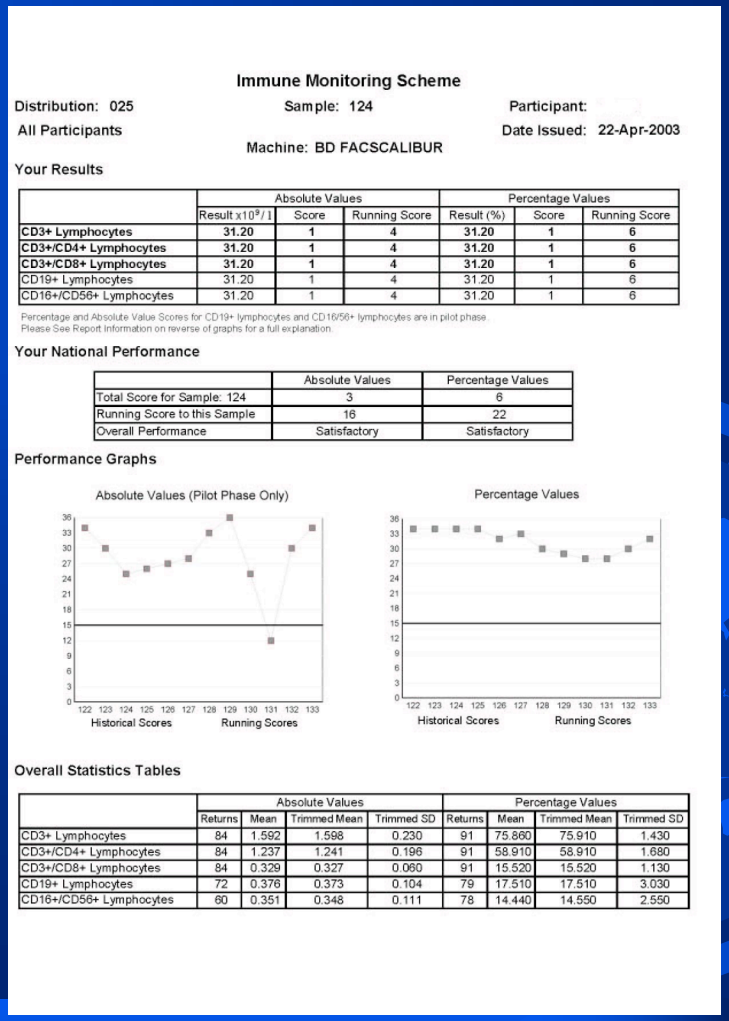
- **Partner 1 and partner 4 had significant difference between days.**
  - Overall mean = 481 cells/ul
  - Overall CV = 11.7%

UK NEQAS/IQA Partnership

UKNEQAS / IQA International Partnership

UKNEQAS Team Provides QA/QC Send out to NIH Supported Sites

IQA Team Monitors Site Performance and Develops Site Specific Training



Immune Monitoring Scheme

Distribution: 025 Sample: 124 Participant:

All Participants Date Issued: 22-Apr-2003

Machine: BD FACSCALIBUR

Your Results

	Absolute Values			Percentage Values		
	Result x10^9/l	Score	Running Score	Result (%)	Score	Running Score
CD3+ Lymphocytes	**31.20**	**1**	**4**	**31.20**	**1**	**6**
CD3+/CD4+ Lymphocytes	**31.20**	**1**	**4**	**31.20**	**1**	**6**
CD3+/CD8+ Lymphocytes	**31.20**	**1**	**4**	**31.20**	**1**	**6**
CD19+ Lymphocytes	31.20	1	4	31.20	1	6
CD16+/CD56+ Lymphocytes	31.20	1	4	31.20	1	6

Percentage and Absolute Value Scores for CD19+ lymphocytes and CD16/56+ lymphocytes are in pilot phase.
Please See Report Information on reverse of graphs for a full explanation.

Your National Performance

	Absolute Values	Percentage Values
Total Score for Sample: 124	3	6
Running Score to this Sample	16	22
Overall Performance	Satisfactory	Satisfactory

Performance Graphs

Absolute Values (Pilot Phase Only)

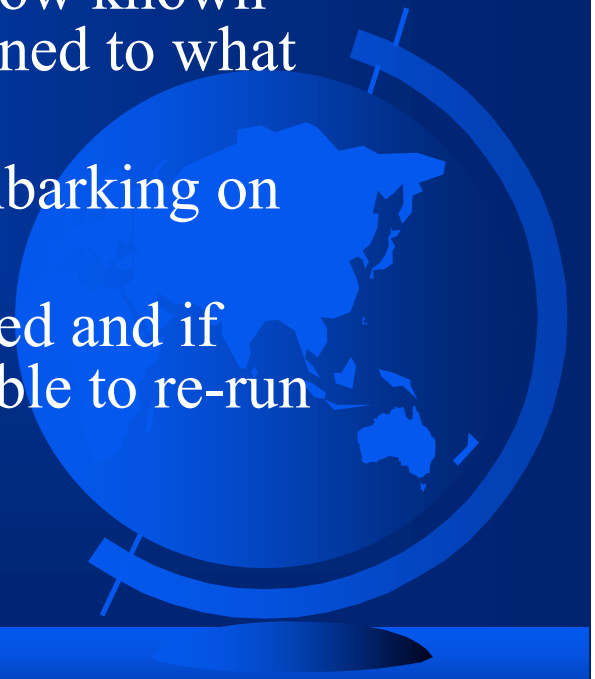
Percentage Values

Overall Statistics Tables

	Absolute Values				Percentage Values			
	Returns	Mean	Trimmed Mean	Trimmed SD	Returns	Mean	Trimmed Mean	Trimmed SD
CD3+ Lymphocytes	84	1.592	1.598	0.230	91	75.860	75.910	1.430
CD3+/CD4+ Lymphocytes	84	1.237	1.241	0.196	91	58.910	58.910	1.680
CD3+/CD8+ Lymphocytes	84	0.329	0.327	0.060	91	15.520	15.520	1.130
CD19+ Lymphocytes	72	0.376	0.373	0.104	79	17.510	17.510	3.030
CD16+/CD56+ Lymphocytes	60	0.351	0.348	0.111	78	14.440	14.550	2.550

UK NEQAS/IQA Initiative

- Panel of stabilised samples devised to examine the individual laboratories ability to measure $CD4^+$ T lymphocytes
- Involves a panel of 20 samples with CD4 ranges from 80 - >800 $CD4^+$ cells/µl
- The technology confidence limits are now known and individual performance can be aligned to what is expected.
- Qualifying round of samples before embarking on EQA
- During course of EQA centres monitored and if show out of consensus results will be able to re-run specimens to determine problem area

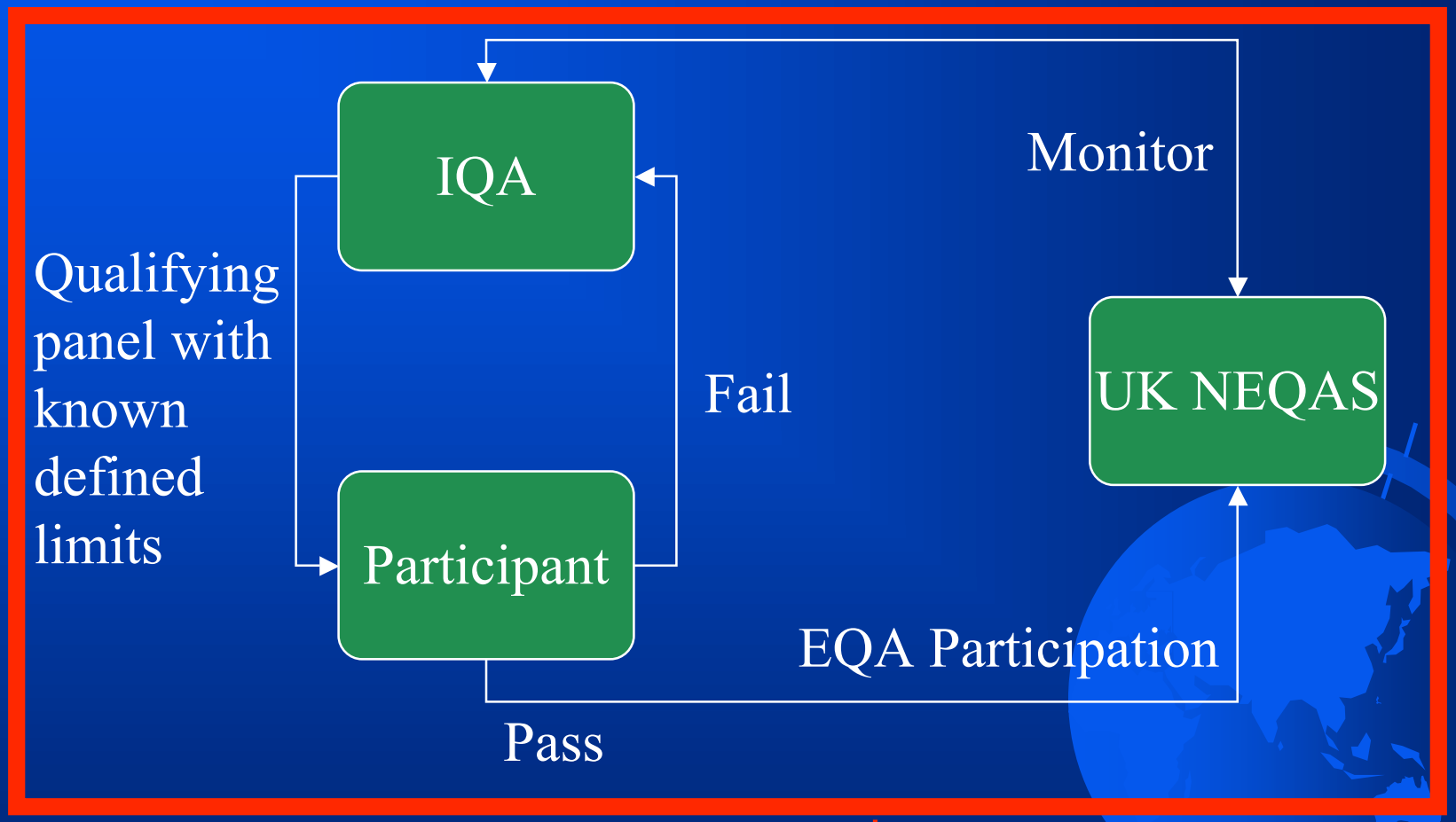


UKNEQAS / IQA International Partnership

- *Veritas* – Latin for Truth
- Verification Panel
- Quality Assessment

"VERIQAS"

UK NEQAS/IQA Initiative



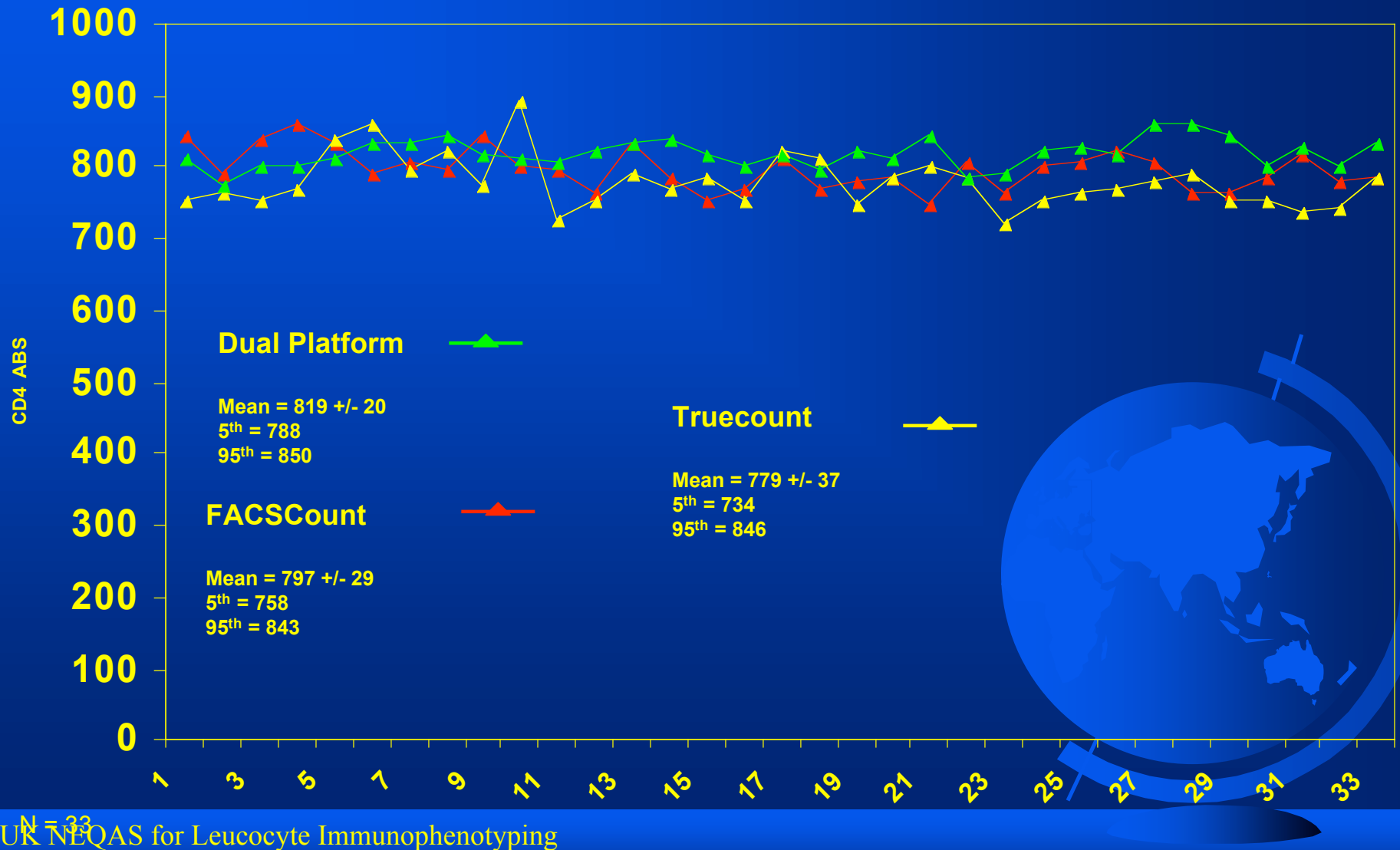
VERIQAS

- **Panel Layout**
  - 20 samples
    - single samples
    - replicate samples

  Whole blood with CD4 absolute range engineered ~ 80 to 850 cells/uL

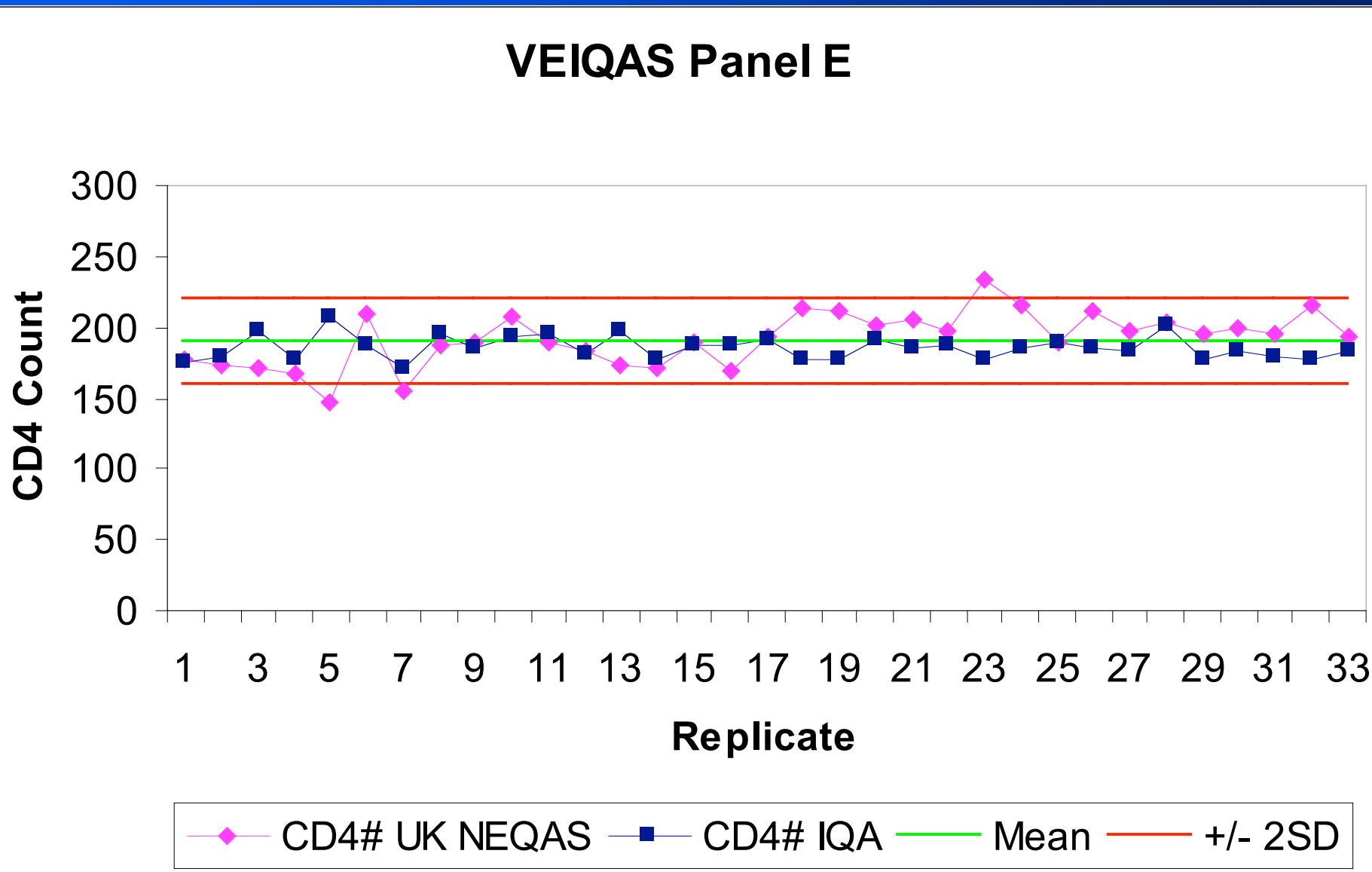
- **Assessment Protocol**
  - Ability to meet QA sample target zone (e.g. 150 CD4 cells)
  - Reproducibility target zone (e.g., CV of replicates)

Validation Panel CD4 Results Donor A
Target CD4 > 350

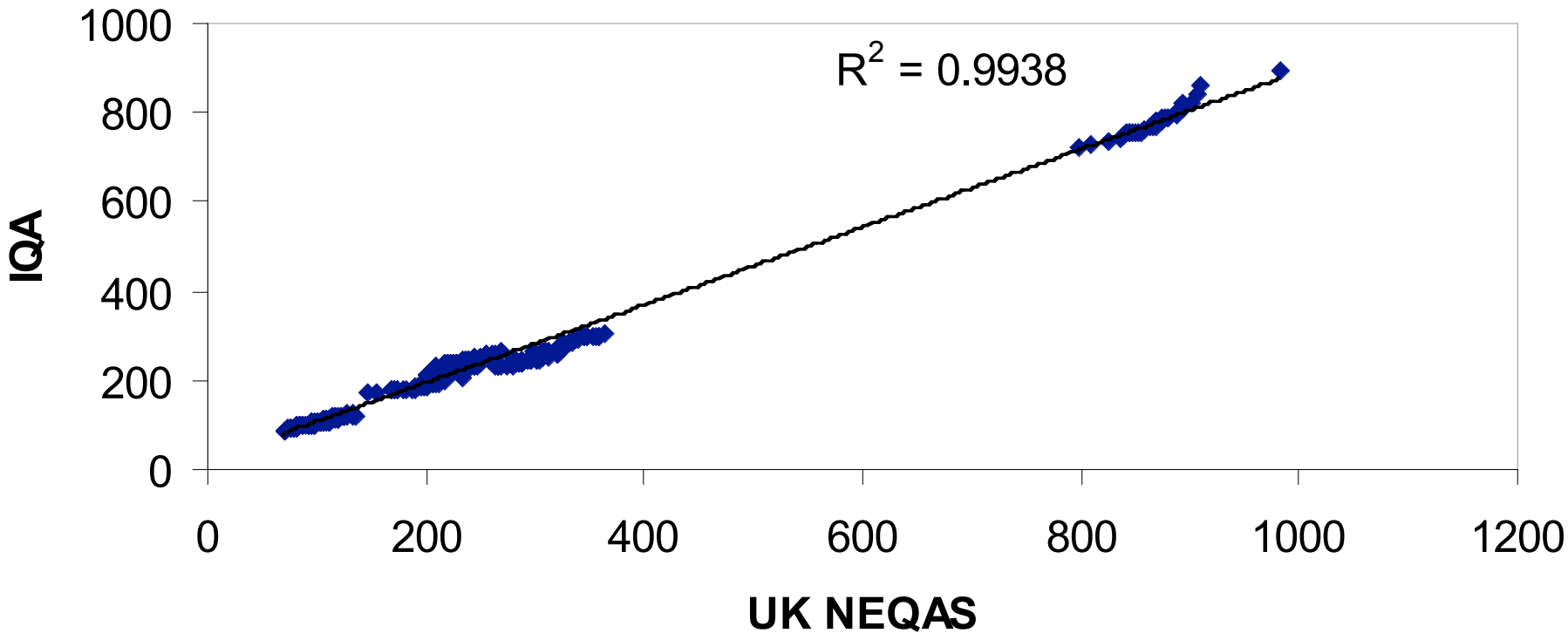


UKNEQAS + IQA Data Set

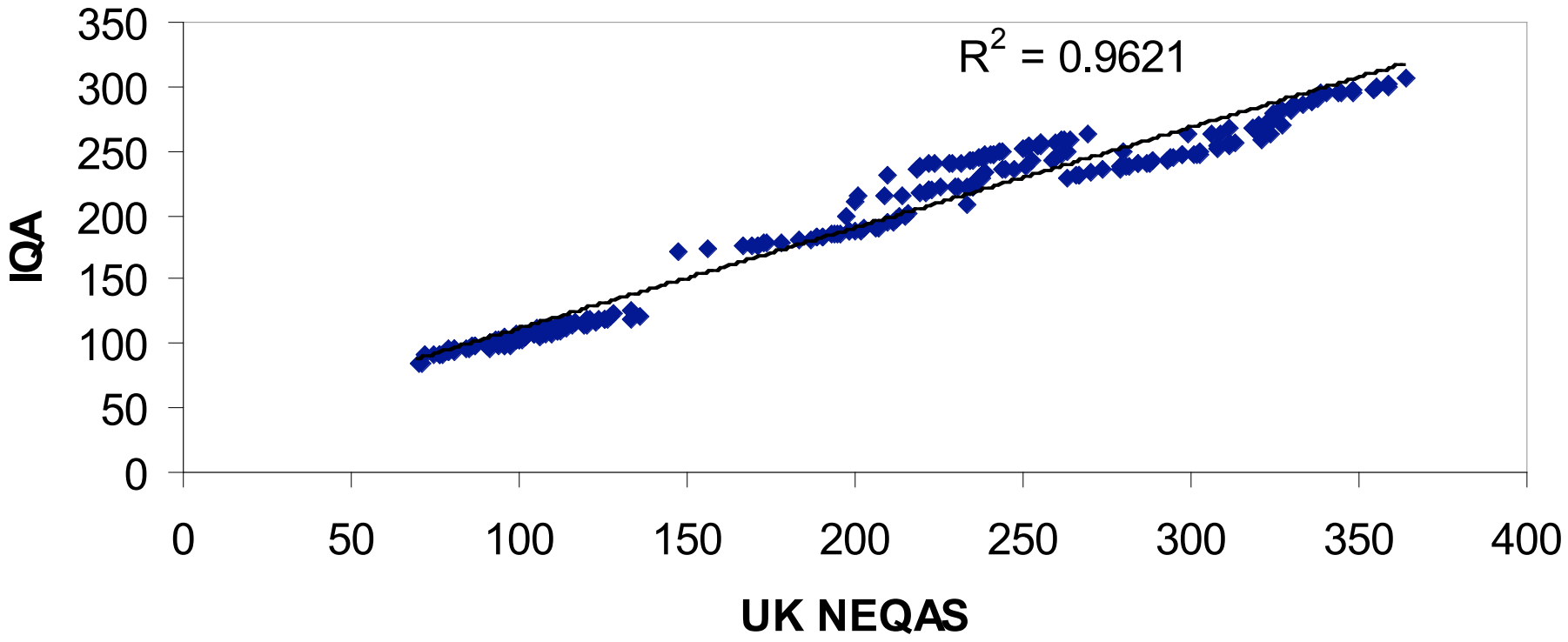
	Mean	Minimum	Maximum	SD	+2SD	-2SD
Sample A	823	724	983	56	936	710
Sample B	92	70	108	10	112	73
Sample C	109	79	136	10	130	88
Sample D	108	94	133	9	127	90
Sample E	189	147	233	15	219	159
Sample F	231	197	280	16	263	198
Sample G	245	210	269	12	269	221
Sample H	269	229	327	29	326	212
Sample I	308	262	364	29	365	250


VEIQAS Panel E
CD4 Count
300
250
200
150
100
50
0
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
33
Replicate
CD4# UK NEQAS
CD4# IQA
Mean
+/- 2SD

Comparison of Absolute CD4 Counts in Two Centres (Cells/ul)



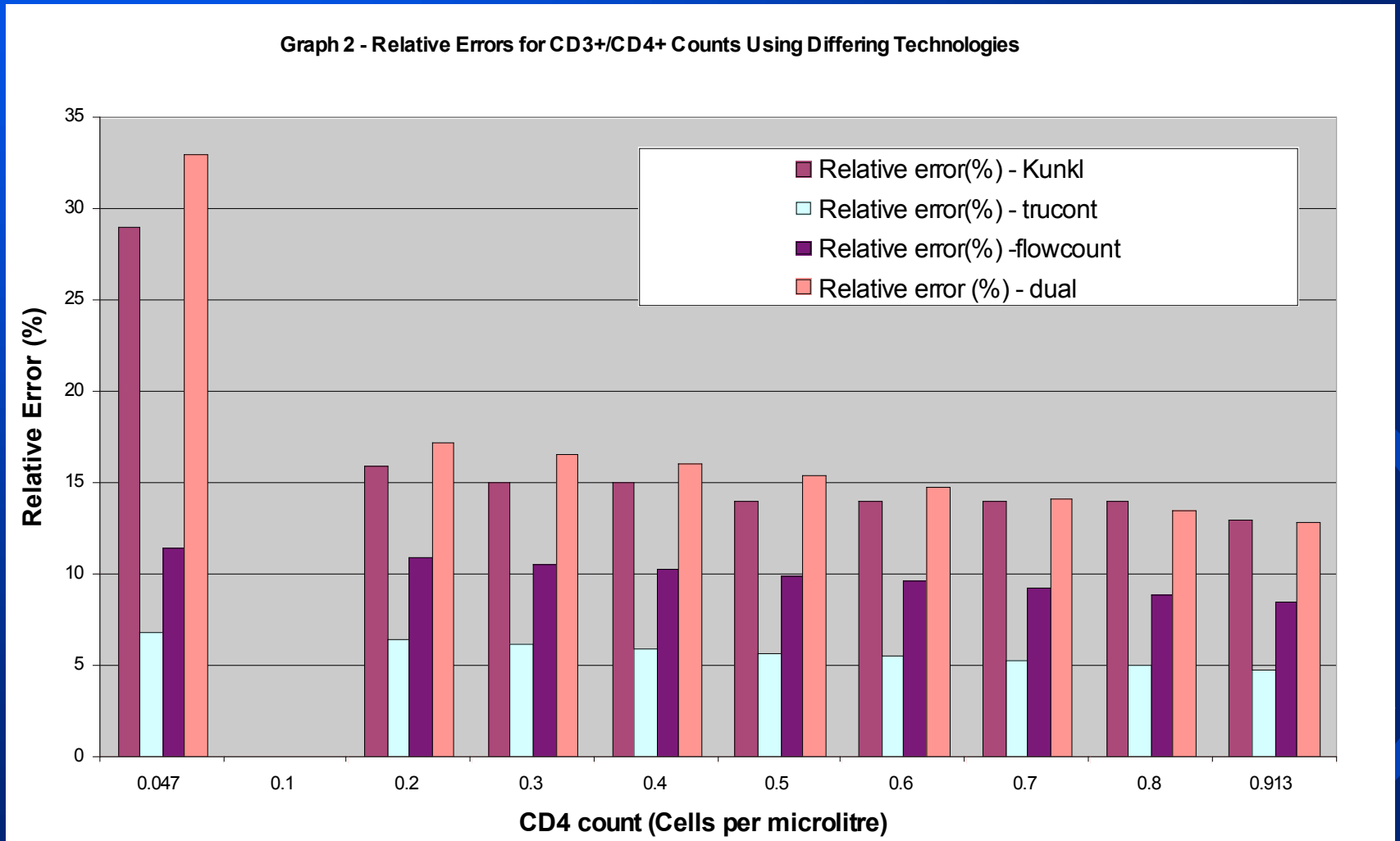
Comparison of Absolute CD4 Counts in Two Centres (Cells/ul)



Technology Confidence Limits and how can we use them?

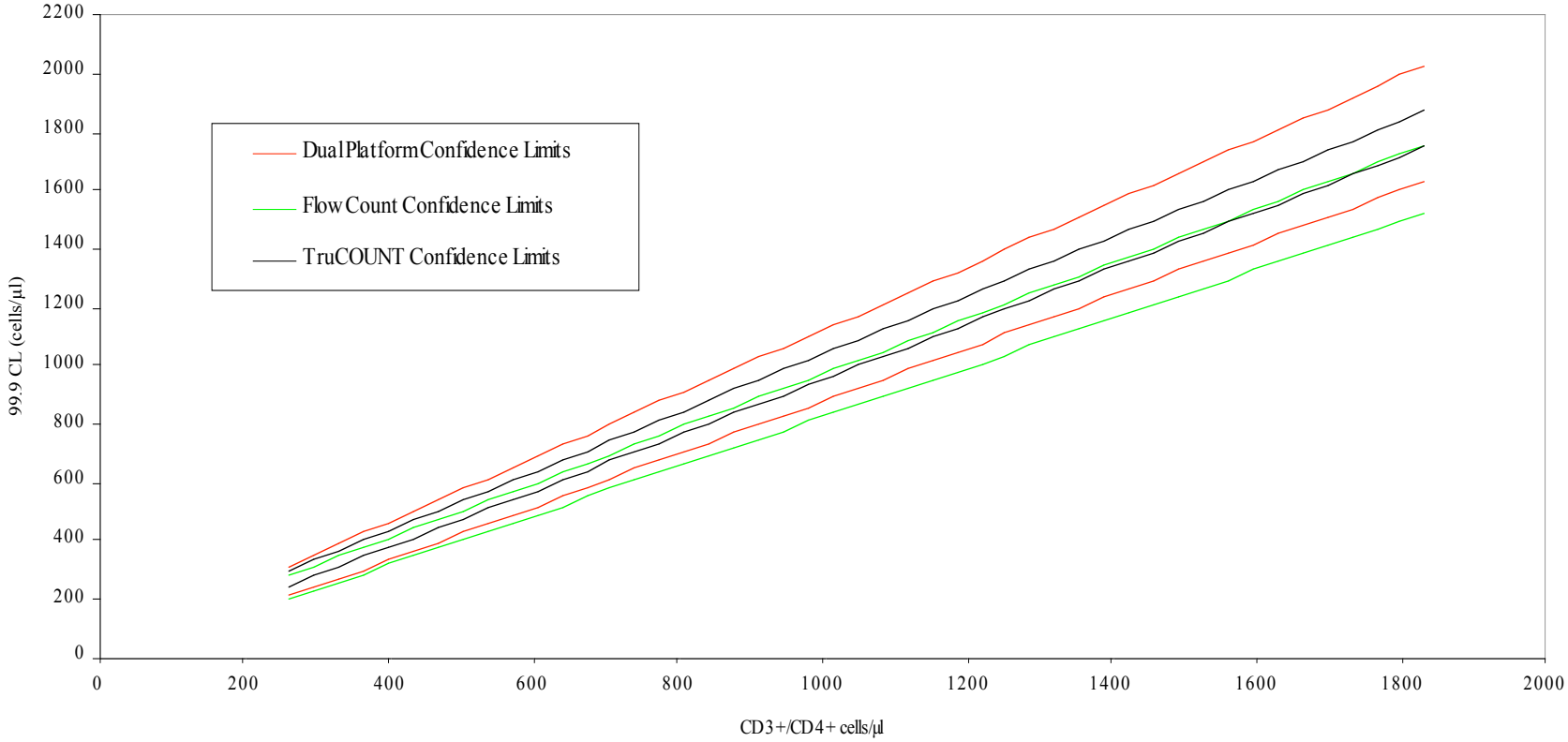
Relative Error for CD4+ T cells

(results based on 48 send outs to 387 laboratories)



Relative Error for CD4+ T cells (results based on 48 send outs to 387 laboratories)

Figure 1- 99.9% Confidence Limits for CD3+/CD4+ Lymphocyte Enumeration by Single and Dual Platform Technologies.



99.9% confidence limits for CD3+/CD4+ counts at a level of 500 cells/µl

Method	Lower 99.9% Confidence Limit	Upper 99.9% Confidence Limit	Confidence Limit Range
Dual Platform	425	580	155
Flow Count (Single platform)	425	530	105
TruCOUNT (Single platform)	480	540	60

Sample Stabilizers

- Transportation introduces two important factors: **Time & Sample Integrity**
- Several haematology and immunology guidelines have published specific time frames for time to completion of analysis.
- Increased burden on the staff, budget and courier costs

Cyto-Chex

Advantages

- Preserves light scatter characteristics
- Simple to use
- Up to 10 days sample preservation
- Allows whole blood lysis

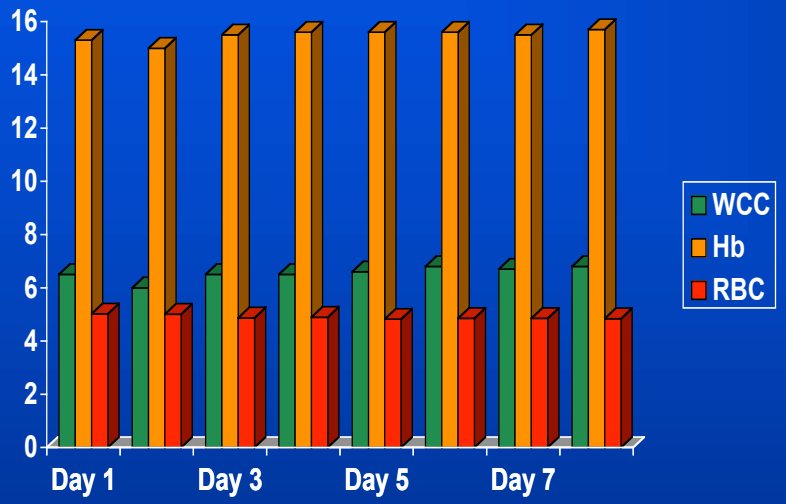
Disadvantages

- Dilution factor (1:1)
- Antigen susceptibilty
- Cannot be used on haematology analysers
- Cannot use on single platform
- Cannot extract DNA/RNA

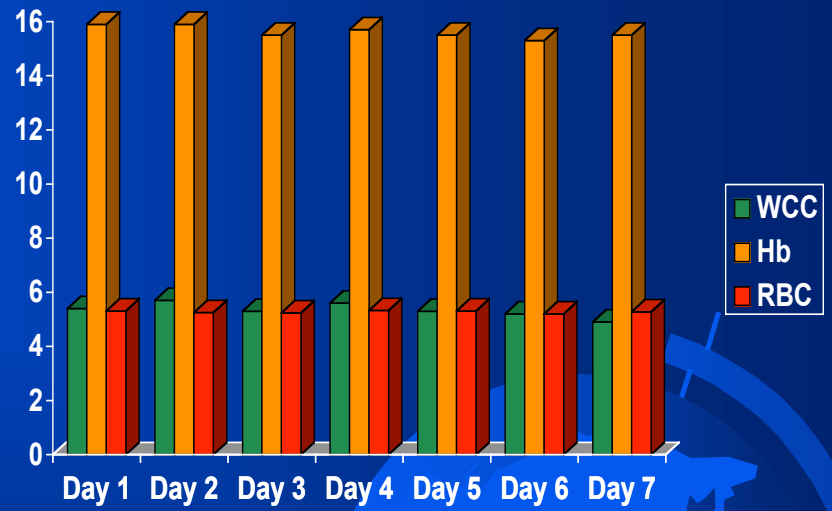
TransFix

- Termed TRANSFix because it allows TRANSportation of FIXed Specimens.
- Minimal dilution effects (10ul per 1ml Sample).
- Can be used on normal, leukaemia and HIV patients.
- Facilitates haematological analysis.
- Minimal effect on antigen density.
- Samples stable for up to 10 days*. All samples kept at 4°C and allowed to warm to R.T. (approx. 1 hour) before use.
- Facilitates flow cytometric analysis.
- Can be added immediately after draw or produced in an anticoagulated form.

TransFix Haematology Analysis

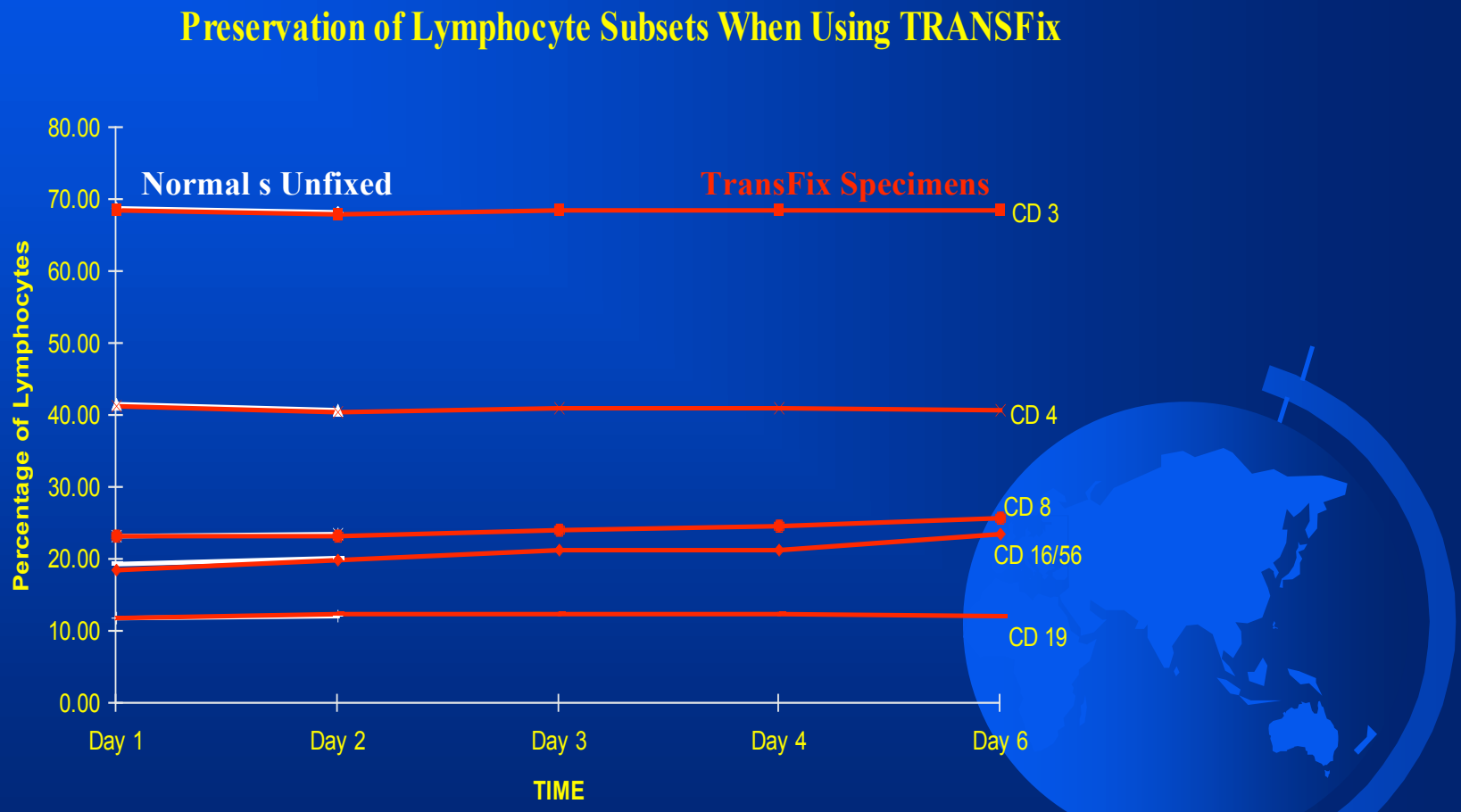


Coulter VCS



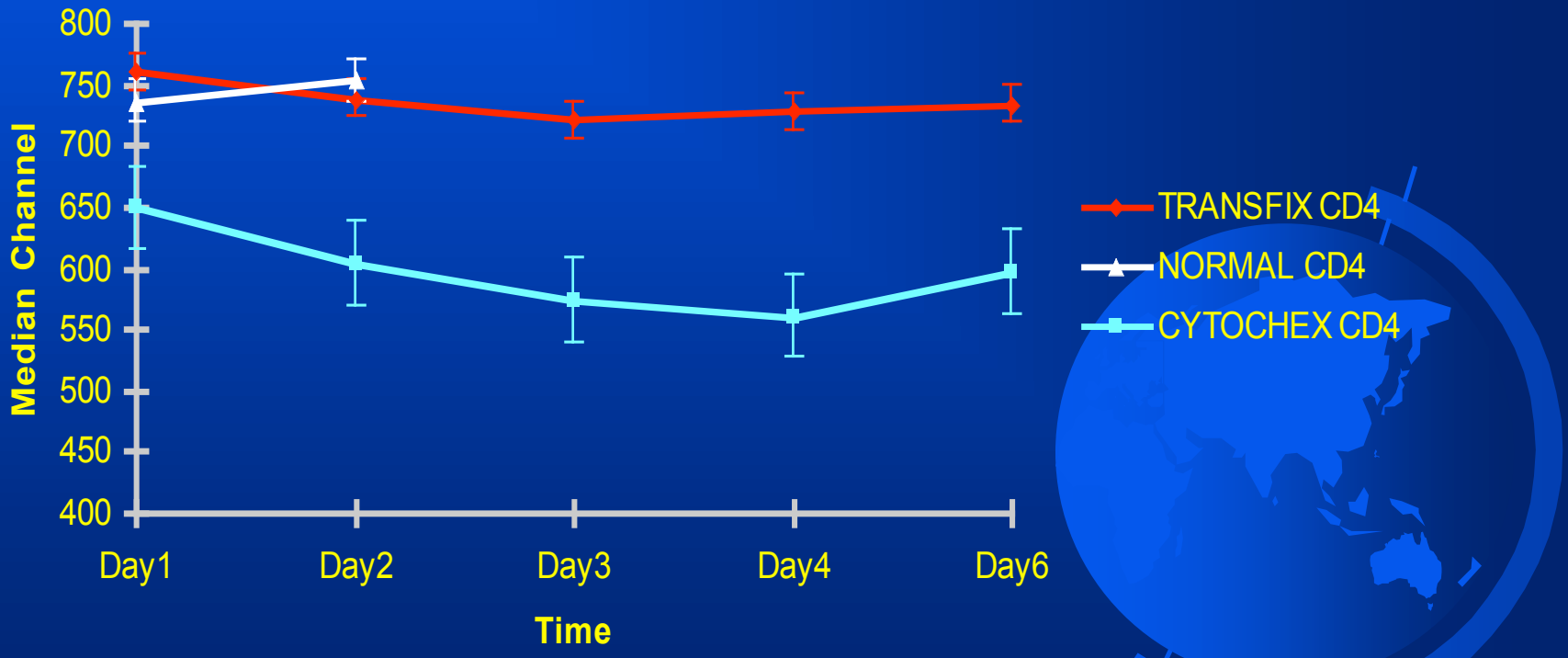
Sysmex NE1500 Analysis

TransFix Flow Cytometric Analysis



TransFix
Flow Cytometric Analysis

Effect of TransFix and Cytochex on CD4 Median Channel Fluorescence



Conclusions
Disadvantages

- Stabilised material not suitable for manual technologies such as Dynabeads.
- Not suitable for obtaining haematology differentials.
- Scatter can be a little confusing for new laboratories.

Conclusions
Advantages

- Stabilised material offer a superior alternative than fresh blood to validate different single platform instrument technologies.
- Longitudinal studies easily conducted.
- Individual laboratory performance easily monitored.
- Can be used for teaching, training and troubleshooting.
- Samples can be engineered to have varying lymphocyte subset counts. Do not have to be HIV+

TransFix Publications

- **Evaluation of leukocyte stabilisation in TransFix^{R}-treated blood samples by flow cytometry and transmission electron microscopy.** Canonico B, et al (2004) J Immunol Methods. 29:67-78

- **CD45 assisted Pan*LeucoGating* for Accurate, Cost Effective Dual Platform CD4+ T cell enumeration.** Glencross, D et al (2002) Cytometry (Communications in Cytometry) 50:69-77

- **Affordable CD4+ T cell counts by flow cytometry. II. The use of fixed whole blood in Resource-poor Settings.** Jani I., et al (2001) Journal of Immunological Methods 257:145-154

- **Evaluation of TransFix, a commercial whole blood stabilizing reagent. This product reduces HIV replication.** *Kim et al: Cytometry Clinical Cytometry (2002) 50: 281*

- **Evaluation of stabilised blood cell products as candidate preparations for quality assessment programs for CD4 T-cell counting.** *M. Bergeron, A.Shafaie, T.Ding, S.Phaneuf, N.Soucy, F. Mandy, J.Bradley & J.Fahey Cytometry (2002) 50, 86-91*