

# *Review of CD4 Technologies*

**Suzanne Crowe, Burnet Institute**

On behalf of

**Members of the CD4 Working Group,  
Forum for Collaborative HIV Research**



# Laboratory monitoring & the 3x5 program

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- **Cost of ARV Rx in developing countries is potentially exceeded by the cost of laboratory monitoring.**
- **Sophisticated assays often established as part of clinical trial infrastructure, paid for by international grants**
- **HIV+ individuals outside trials must find funds to pay for their own monitoring**
- **Monitoring HIV treatment is complex**
  - **CD4 and VL**
  - **Drug toxicity**
  - **Adherence**
  - **Drug resistance**

# New CD4 monitoring tests and technologies

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## ● Flow based assays

- Guava EasyCD4 System
- Partec CyFlow
- PointCARE
- Panleukogating technology

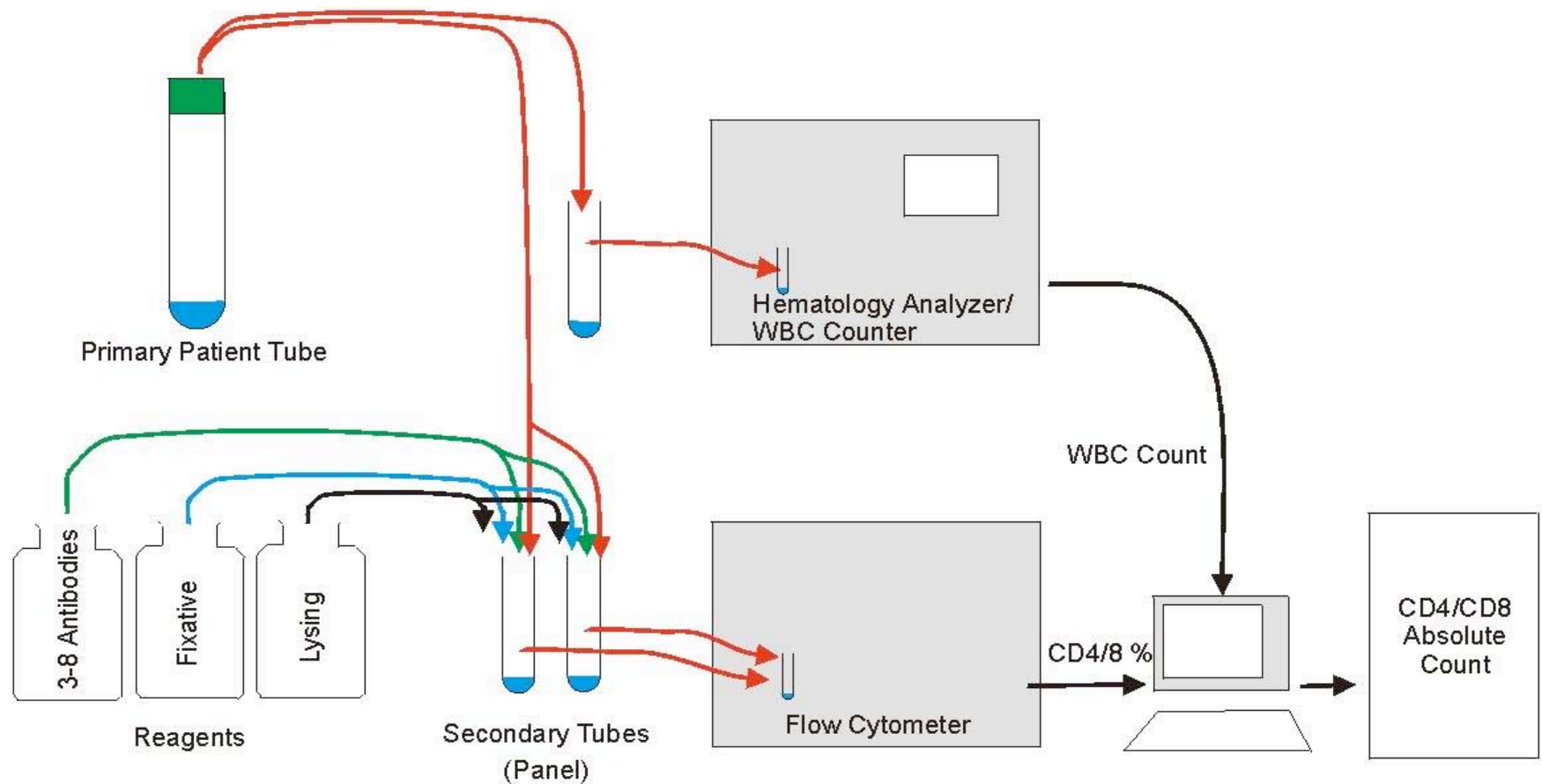
## ● Manual assays

- Dynal immune bead-based assay (microscopy)
- Coulter immune bead-based assay (microscopy)

## ● In the pipeline

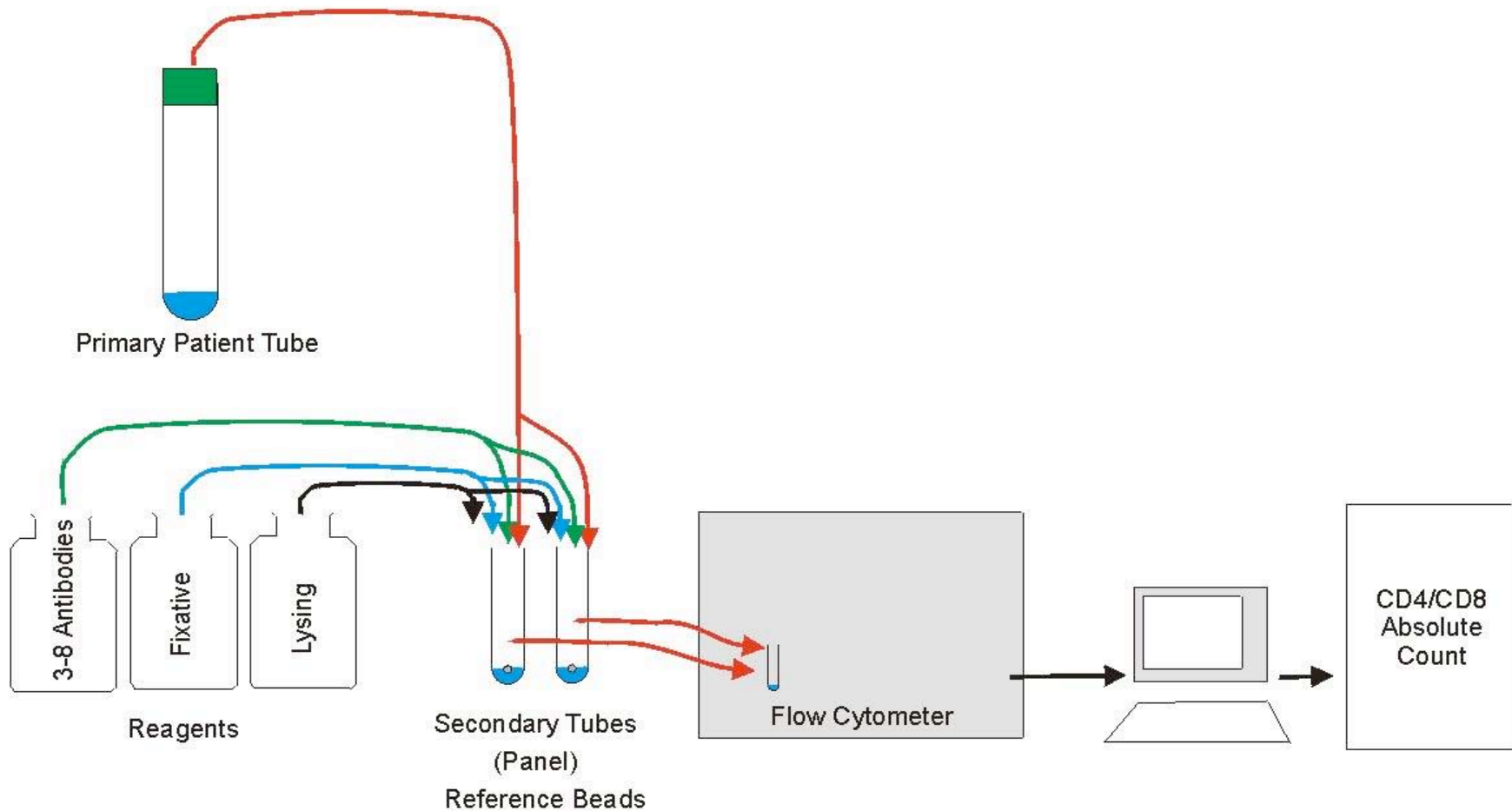
- LabNow microchip technology
- SemiBio slide test (microscopy)
- suPAR (soluble urokinase plasminogen activator receptor)
- CD4 dipstick for remote care

# Dual Platform Flow Cytometry

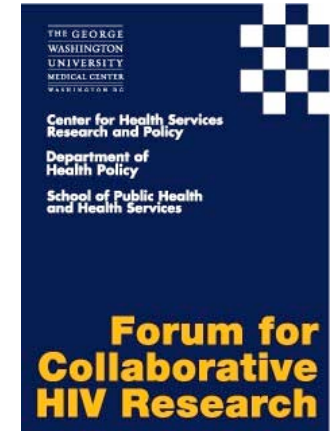


*Slide courtesy of Roland Gohde*

# Single Platform Flow Cytometry



*Slide courtesy of Roland Gohde*

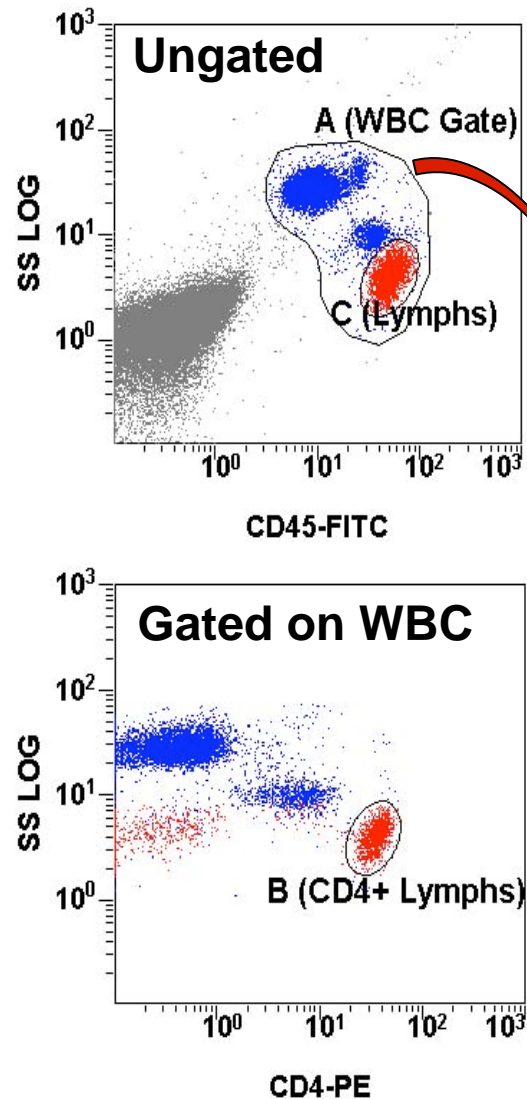


# Pan-leukogate or PLG CD4 Methodology

*Slides courtesy of Angela Vernon and Meryl Foreman, Beckman Coulter*



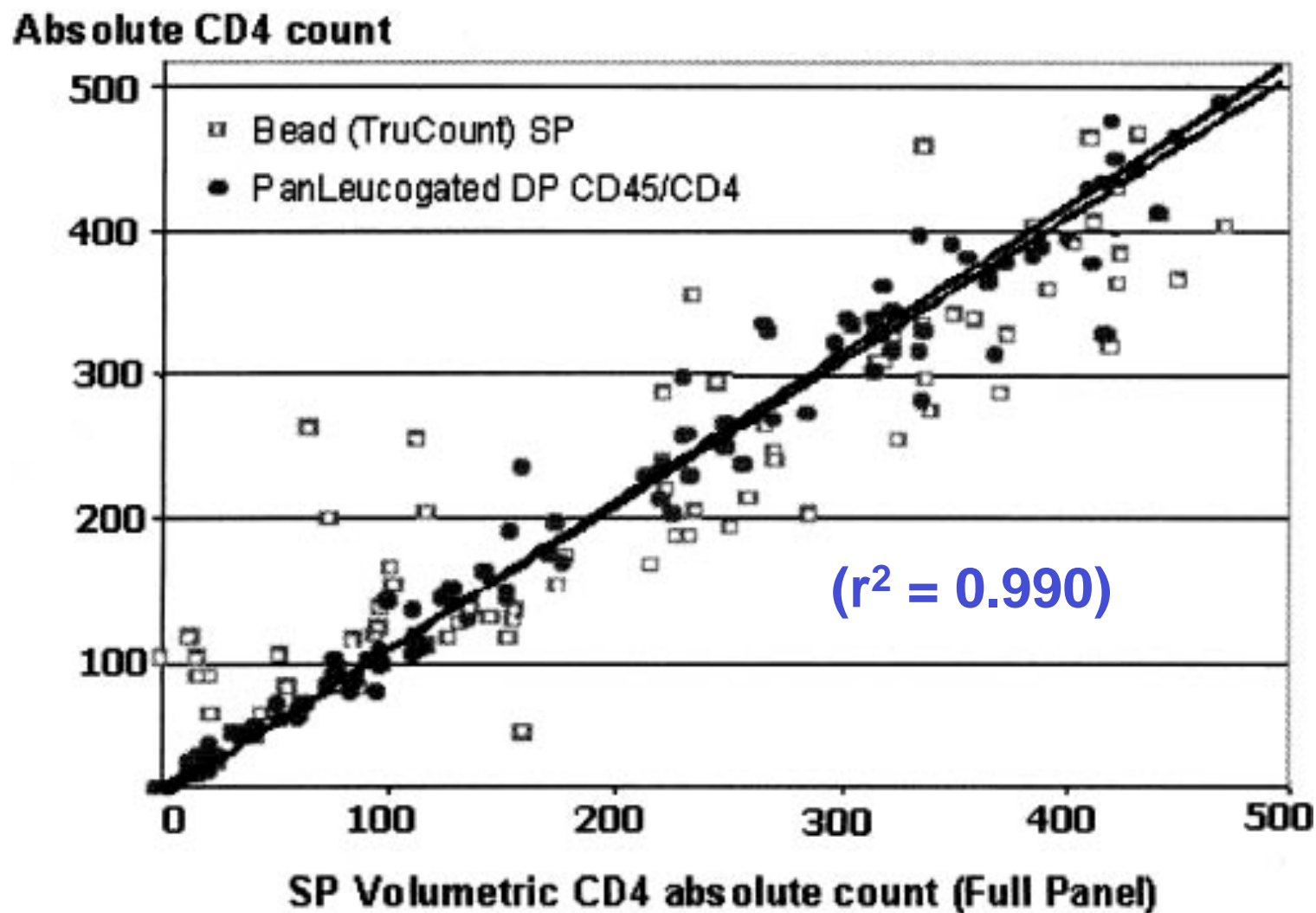
# PLG CD4 Methodology



- Identifies CD4+ lymphocytes based on a pan-leucocyte count
- **WBC count (cells/ul) X CD4 events from region B / CD45 events from region A = Absolute CD4**
- The WBC gate is not affected by EDTA changes that occur with older specimens.
- Hematology lymph % is affected by EDTA, count not reliable beyond 24 hours.

*Slide courtesy of Angela Vernon and Meryl Foreman, Beckman Coulter*

# PLG CD4 Count

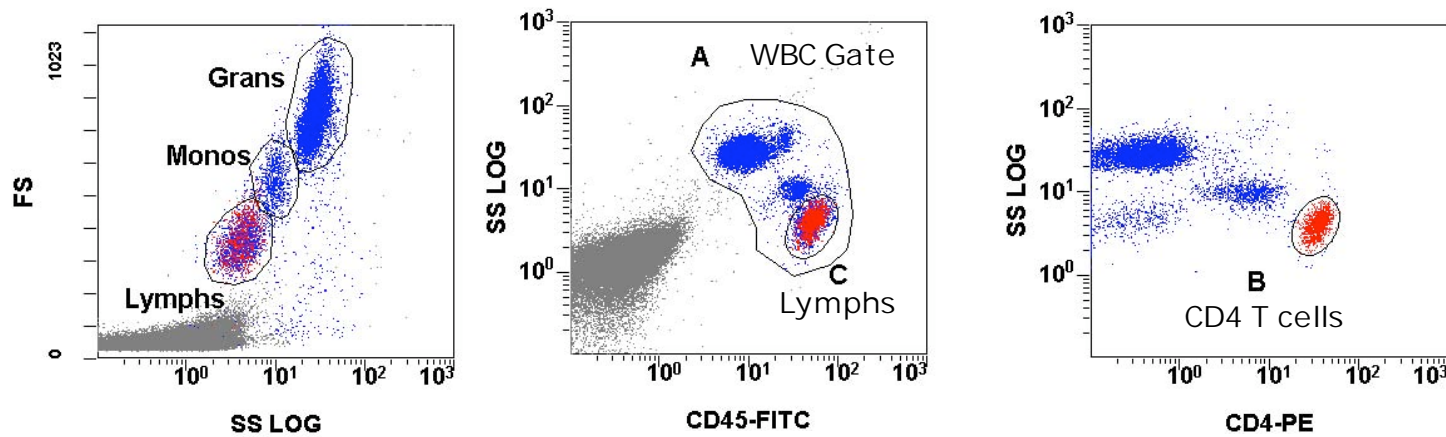


*Glencross et al. Clinical Cytometry, 50:2, 2002*

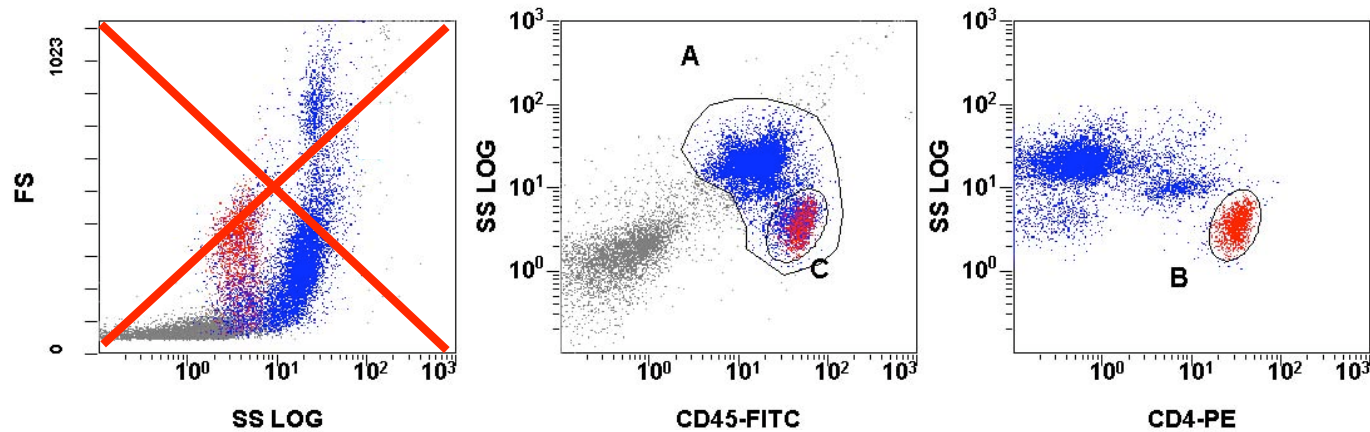
# Aged Specimen Performance – Limitations of Scatter Gating

*Forward Scatter cellular structure lost over time, results in inability to define appropriate gates using scatter alone*

Day 1



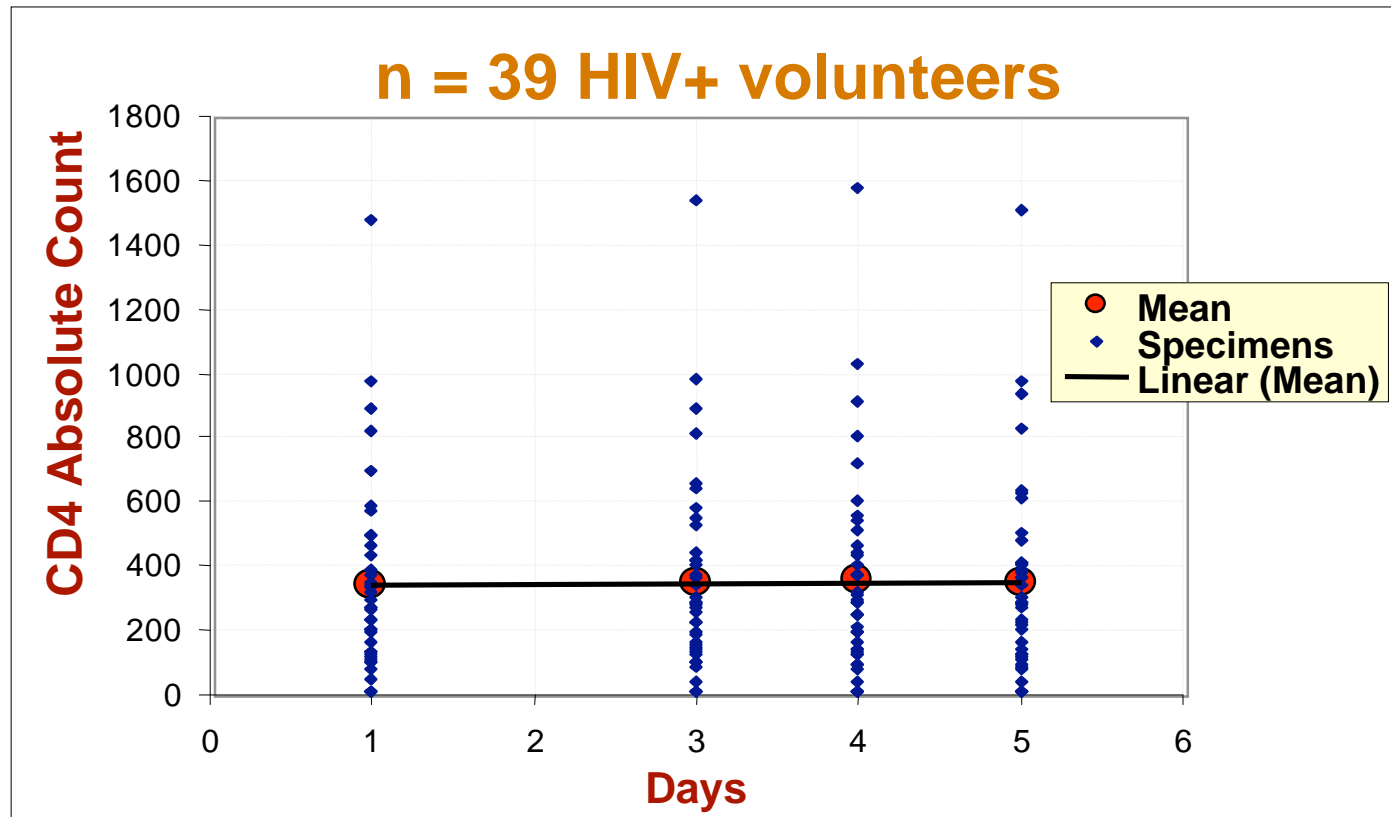
Day 5



*Slides courtesy of Angela Vernon and Meryl Foreman, Beckman Coulter*

# PLG: Aged Specimen Performance

## Beijing, China



- Mixed Model ANOVA for trend over time;  $p=0.8919$
- CD4 Count Range: 7 – 1579 cells/MI; Median CD4 count = 371 cells/μL

*Slide courtesy of Ank Gowans, Beckman Coulter and CDC Beijing*

# Summary: PLG CD4

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- **New flow cytometry-based method**
  - Based on a pan-leukocyte marker
  - uses a 2-color pre-optimized reagent
  - provides **both CD4% and absolute counts**
  - extends sample age beyond 24 hrs to up to 5 days
  - good correlation with “gold standard” flow
  - compatible with most flow cytometers
    - *with 2 color capability & 488 nm laser line*
  - <\$6 per test
- **Licensed by Beckman Coulter from NHLS, South Africa**
- **High capacity: good for high volume centralized labs**



# Guava EasyCD4

*Slides courtesy of Jeff Harvey, Tina Baumgartner, Leonard Buchner*



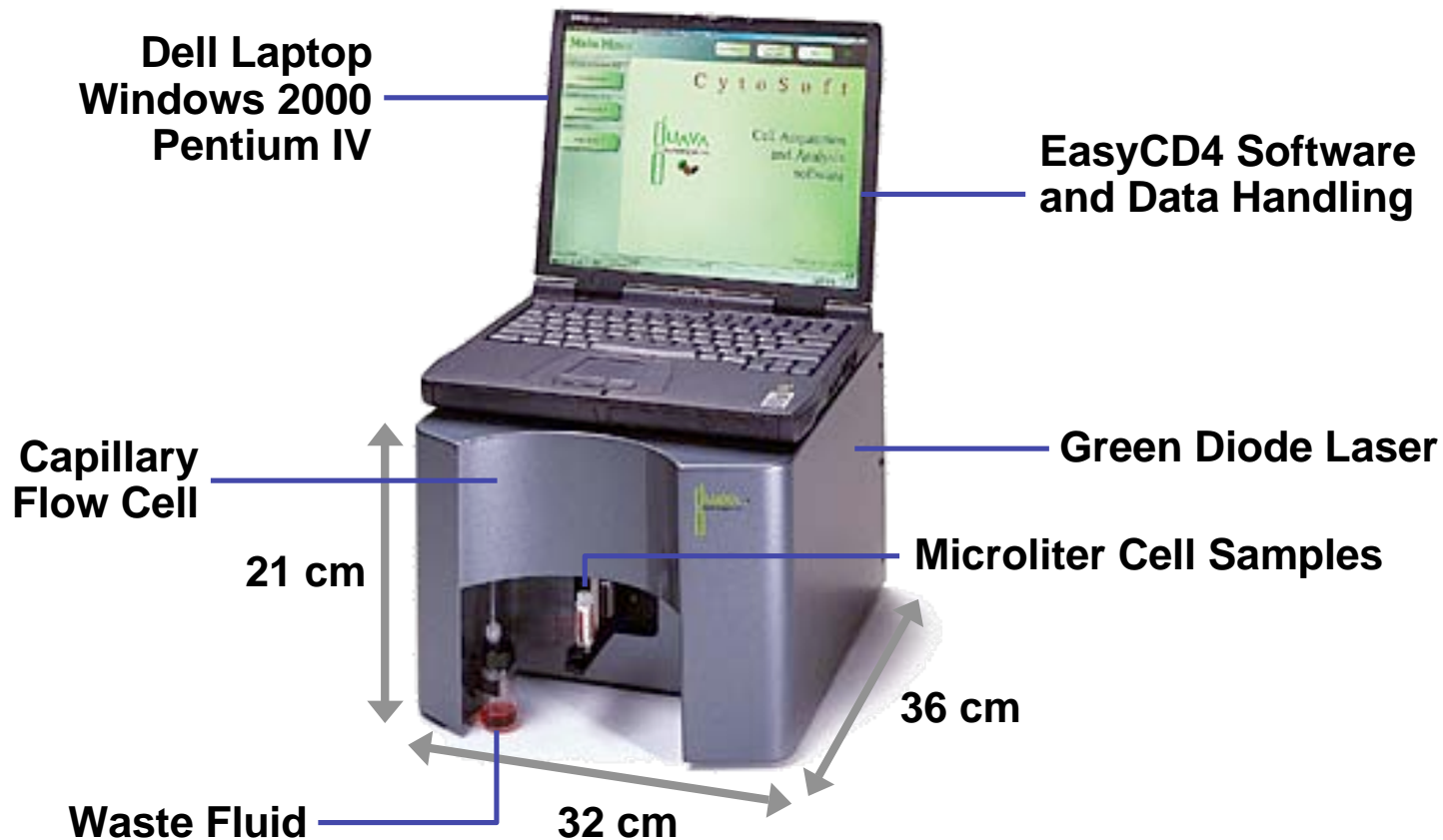
# Guava EasyCD4

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- **Measures absolute CD4 (can measure CD8)**
- **Sample volume:**
  - 10  $\mu$ L of whole blood (EDTA)
- **Reagents**
  - 10  $\mu$ L of antibody cocktail
    - Anti-CD3-PE-Cy5
    - Anti-CD4-PE
  - 180  $\mu$ L of Lyse-Fix solution
- **Components/Software**
  - Dell Laptop computer included
  - Software includes instrument set-up, data acquisition and analysis

# The Guava EasyCD4 System:

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**15.9 kilos with PC**

# Guava EasyCD4 Protocol

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- Add 10uL of antibody cocktail to each tube
- Add 10uL EDTA whole blood to each tube, vortex, **incubate 15min**
- Add 180uL of Lyse/Fix solution, **incubate 15min**
- During sample incubation, turn on power and allow 10 minute warm-up
- Run Guava Check QC procedure **(5 min)**
- Adjust (or recall) instrument settings
- Acquire samples; Analyze results

# Guava EasyCD4

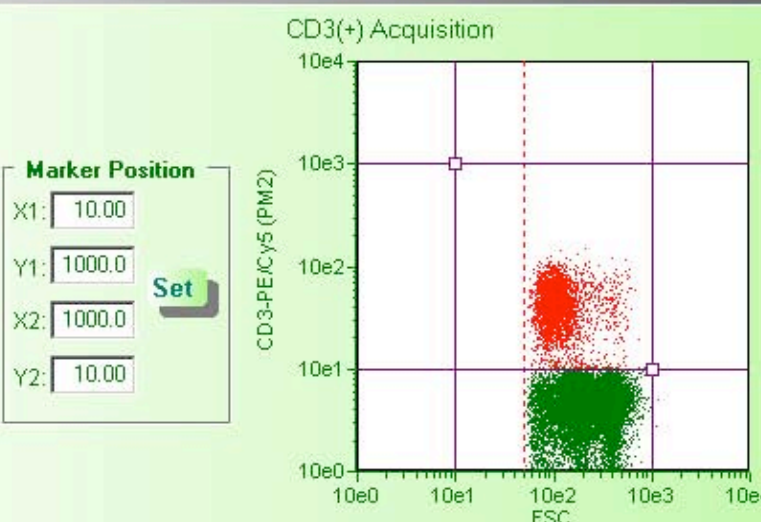
## Analysis

Go to Acquisition

Main Menu



- Unit Control
- Sample Information
- Analysis Sample List
- 0001:#1-1-Stv Ab-FACS-lyse
  - 0002:#1-2-Stv Ab-FACS-lyse
  - 0003:#1-3-Stv Ab-FACS-lyse
  - 0004:#1-1-Gua Ab-FACS-lyse
  - 0005:#1-2-Gua Ab-FACS-lyse
  - 0006:#1-3-Gua Ab-FACS-lyse
  - 0007:#1-1-Stv-Ab-Spain lyse-400ul
  - 0008:#1-2-Stv-Ab-Spain lyse-400ul
  - 0009:#1-3-Stv-Ab-Spain lyse-400ul
  - 0010:#1-1-Gua-Ab-Spain lyse-400ul
  - 0011:#1-2-Gua-Ab-Spain lyse-400ul
  - 0012:#1-3-Gua-Ab-Spain lyse-400ul
  - 0013:#1-1-Stv-Ab-RnD -1:5,180ul
  - 0014:#1-2-Stv-Ab-RnD -1:5,180ul
  - 0015:#1-3-Stv-Ab-RnD -1:5,180ul
  - 0016:#1-1-Gua-Ab-RnD -1:5,180ul
  - 0017:#1-2-Gua-Ab-RnD -1:5,180ul
  - 0018:#1-3-Gua-Ab-RnD -1:5,180ul
  - 0019:#2-1-Stv-Ab-F-lyse,180ul
  - 0020:#2-2-Stv-Ab-F-lyse,180ul
  - 0021:#2-3-Stv-Ab-F-lyse,180ul
  - 0022:#2-1-Gua-Ab-F-lyse,180ul
  - 0023:#2-2-Gua-Ab-F-lyse,180ul
  - 0024:#2-3-Gua-Ab-F-lyse,180ul
  - 0025:#2-1-Stv-Spain-400ul
  - 0026:#2-1-Gua-Spain-400ul
  - 0027:#2-2-Gua-Spain-400ul
  - 0028:#2-3-Gua-Spain-400ul
  - 0029:#2-2-Stv Ab-F-lyse-180ul
  - 0030:#2-2-Stv Ab-F-lyse-180ul
  - 0031:#2-1-Stv Ab-RnD-1:5dil.180ul
  - 0032:#2-2-Stv Ab-RnD-1:5dil.180ul
  - 0033:#2-3-Stv Ab-RnD-1:5dil.180ul
  - 0034:#2-1-GuaAb-RnD-1:5dil.180ul
  - 0035:#2-2-GuaAb-RnD-1:5dil.180ul
  - 0036:#2-3-GuaAb-RnD-1:5dil.180ul



**Marker Position**

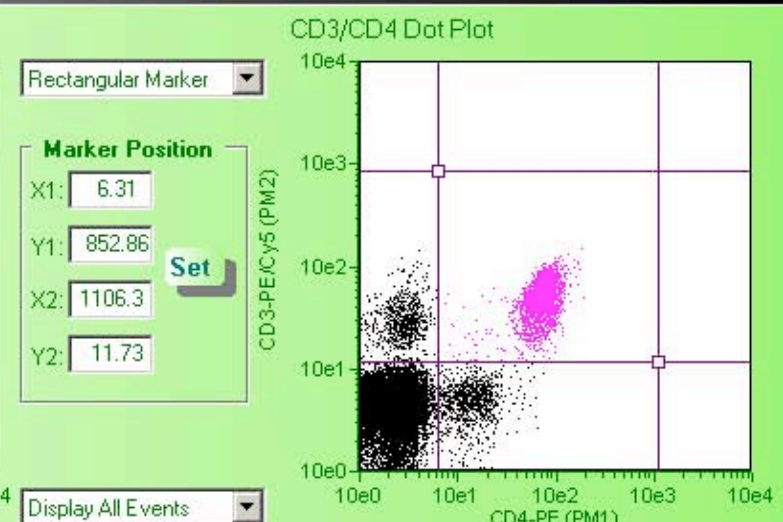
X1:

Y1:

X2:

Y2:

**Set**



Rectangular Marker

**Marker Position**

X1:

Y1:

X2:

Y2:

**Set**

Display All Events

### T Cell Lymphocyte Phenotype Analysis

CD4 T Cells (cells/ $\mu$ L) = 737.8

	Count	% of CD3	% of All	MFI-x	MFI-y
CD3+, CD4+	2170	72.3%	11.2%	75.0	49.5
CD3+	3000	100.0%	15.5%	55.1	43.8

### Analysis Operations

Open Data Set

Log Comment

Export to FCS 2.0

View Event Log

Export to Spreadsheet

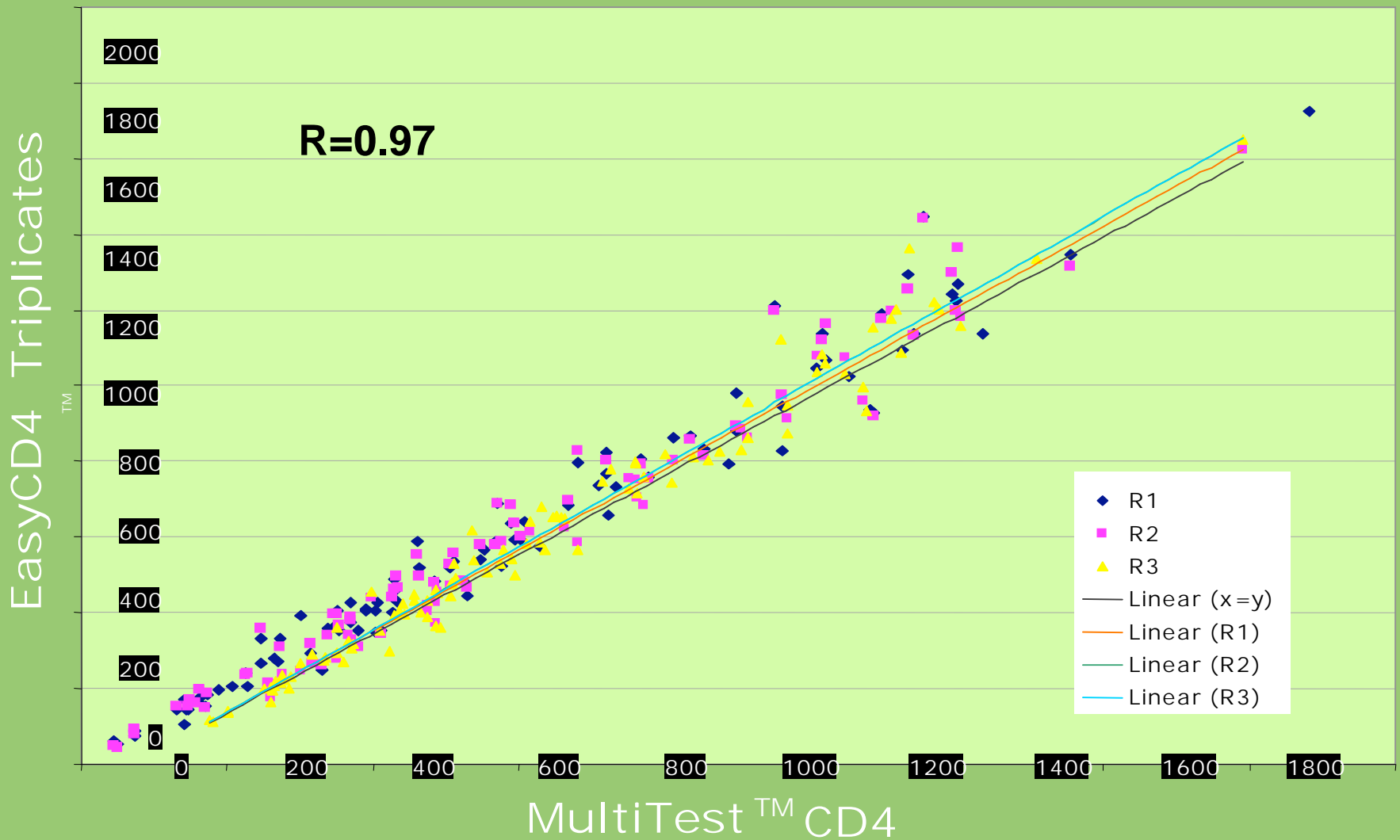
Apply Current Settings to Selected Samples

Print Preview

Print

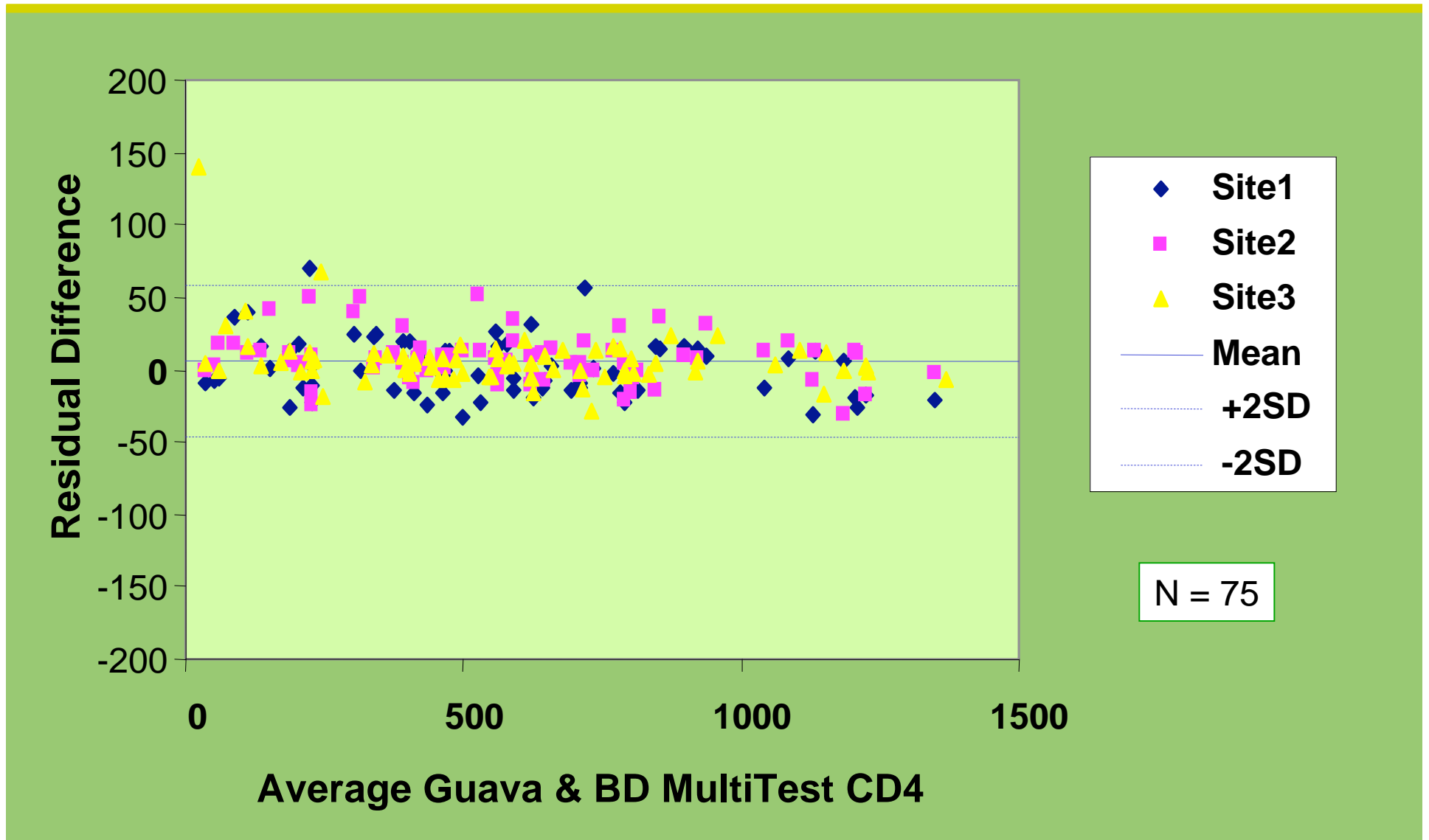
Ready For First Sample 00:00 of 00:00

# EasyCD4 vs MultitestCD4



**UCSF-GCRC/GIVI-CFAR Core Immunology Laboratory**

# North America – California 3 Site Trial



*Slides courtesy of Jeff Harvey, Tina Baumgartner, Leonard Buchner*

# Guava EasyCD4 at YRG CARE



# Summary Easy CD4 (Guava)

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- Quick assay
- Reagent cost is low (\$3/test)
- Capital costs high (\$45,000)
- Existing QA panels not compatible with this technology
- Measures percentage CD4 as %CD3+ T cells
- Still undergoing evaluation



# Partec Cy-Flow

*eg CyFlow Counter1P*

*CyFlow SLGreen 2P*

*CyFlow SL Blue 5P*

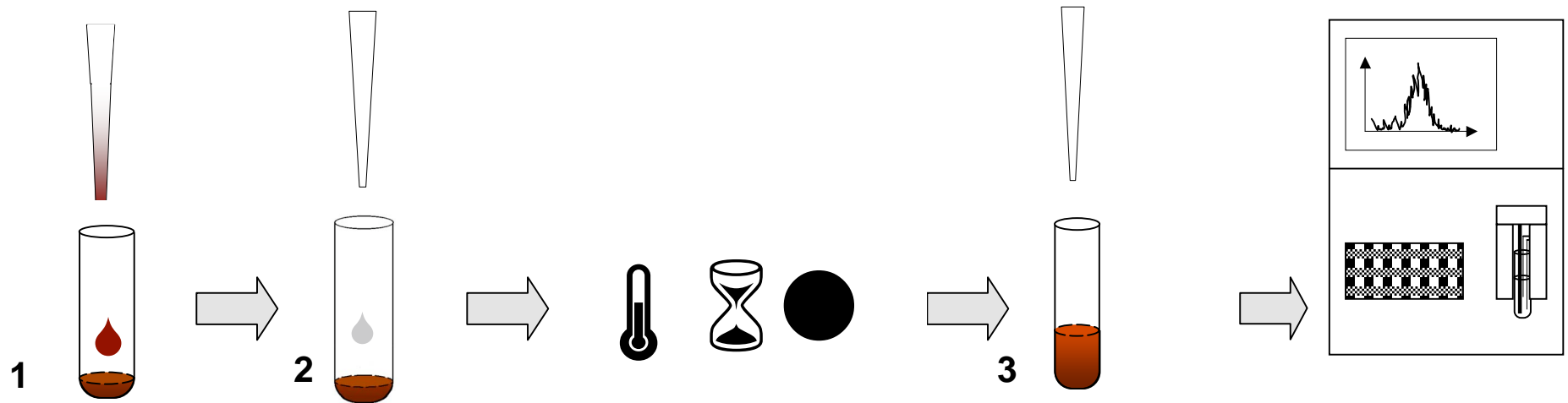
*Slides courtesy of Roland Gohde*



# Cy-Flow

## no lyse - no wash CD4 protocol

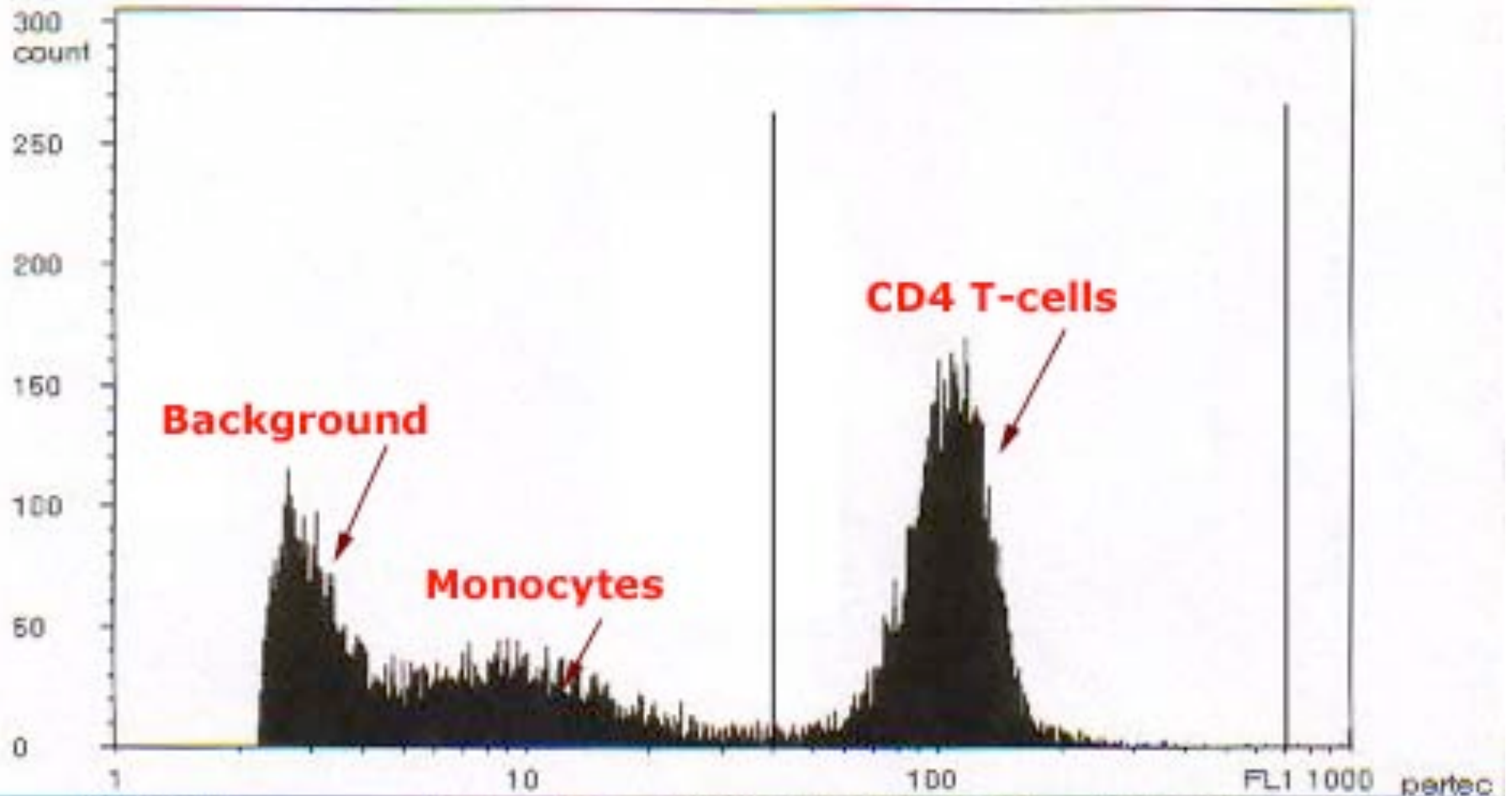
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### 3-Step Protocol

- 50 $\mu$ l blood from the patient into a sample tube
- add 10 $\mu$ l of CD4-PE and **incubate for 10 minutes** at RT in the dark
- add 850 $\mu$ l of the no lyse dilution buffer

File: 427  
17.10.03 16:00:38  
Total Count: 13287  
Gated Count: 13287 (100.00%)  
**691025** cells/ml

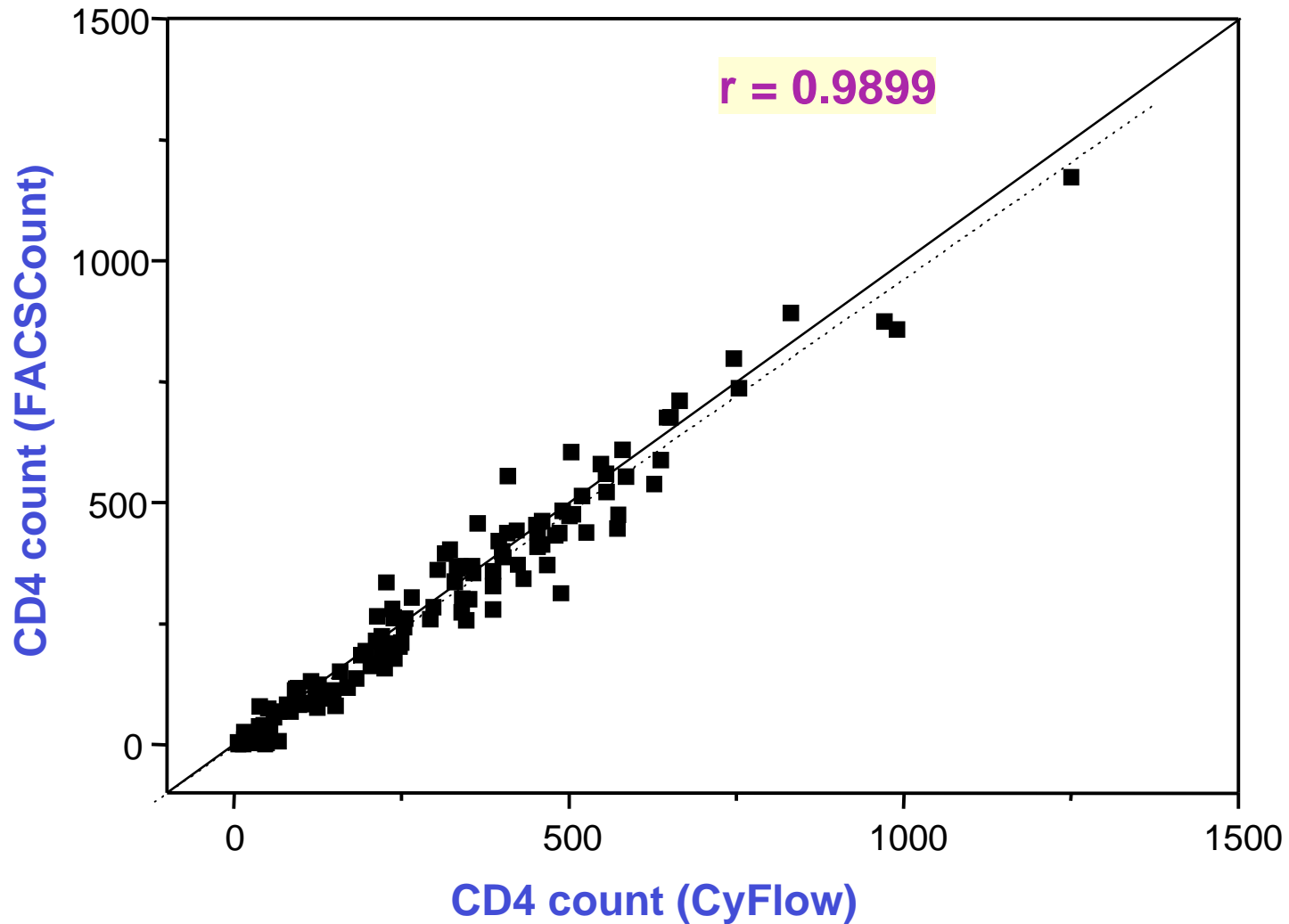


PAR GAIN	L-L	U-L	SPEED [µl/s]	4.00	LAMP [h]	74.3
*I FL1 360.0 Ig1	211	999	RATE [1/s]	281		

Dilut. 21.100

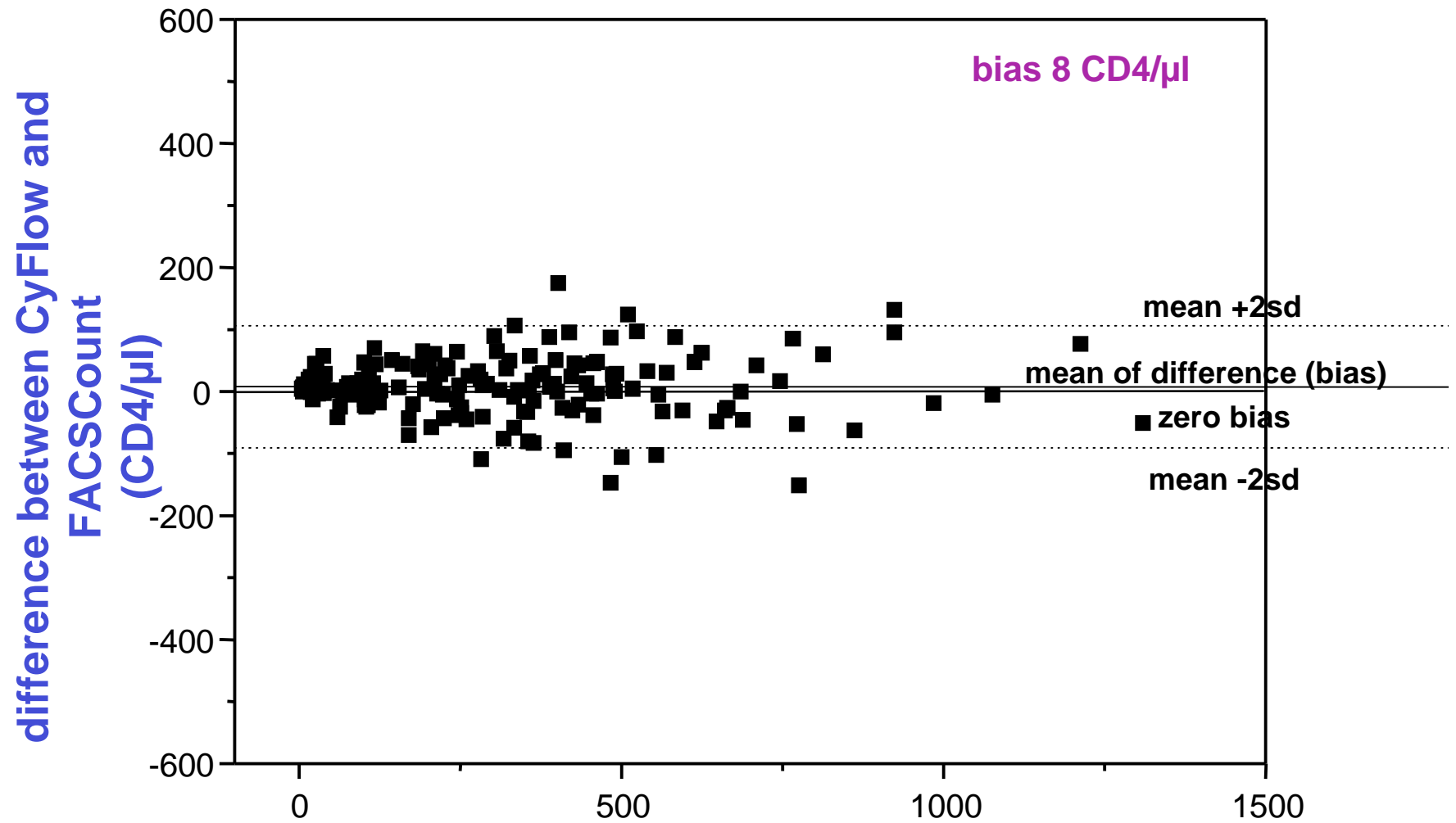
*Slides courtesy of Roland Gohde*

# Cameroon: CD4 Counting - CyFlow vs. FACSCCount



*Douala and Marua, Cameroon*

# Cameroon: CD4 - CyFlow vs. FACSCount Bland-Altman Plot



mean of methods CyFlow and FACSCount (CD4/μl)

*Douala and Marua, Cameroon*





# Summary Partec Cyflow

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- Simple and portable flow cytometric assay
- No formal comparisons with samples provided by external QA programs available at this time
- Reagent costs \$2-3/test
- Capital costs approx \$20,000
- Absolute CD4 only



THE GEORGE  
WASHINGTON  
UNIVERSITY  
MEDICAL CENTER  
WASHINGTON, DC

Center for Health Services  
Research and Policy  
Department of  
Health Policy  
School of Public Health  
and Health Services

**Forum for  
Collaborative  
HIV Research**

# PointCARE

*Slides courtesy of Cecil Sherrer*



# PointCARE System



%CD4 and Absolute CD4  
WBC, LY% and count  
Mobile; battery backup

Room temperature reagent storage and operation

# Closed- tube operation – biohazard containment via cap piercing



4. Lysing Reagent Tube or Cleaning Solution Tube

3. Rinse Tube

2. CD4 Reagent Tube

1. Patient Whole Blood Sample Tube

- Patient sample and reagents bar-code are tracked in the instrument.
- Ideal for low-volume, decentralized labs

# Automated Patient Results

HIV/AIDS Care Test Menu - Patient Results

Patient name: Jennifer Waite      Date: 2/23/2004  
Patient ID: 123456789      Clinic: pct  
Date of birth: 5/29/2001      Sample ID:  
Date of last visit: 2/23/2004      Lysing Reagent Tube ID:  
CD4 Reagent Tube ID:

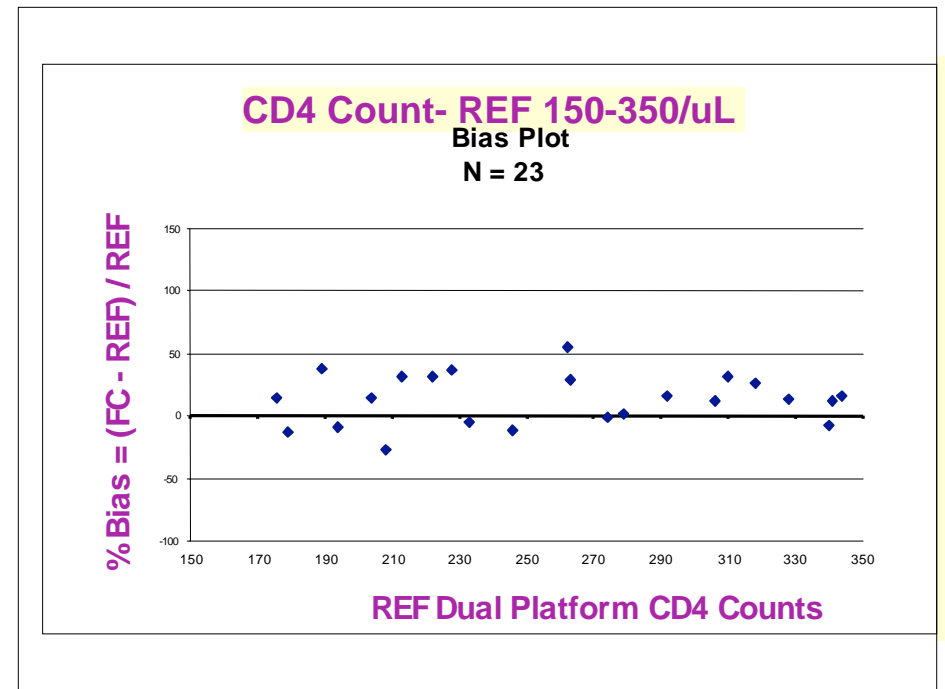
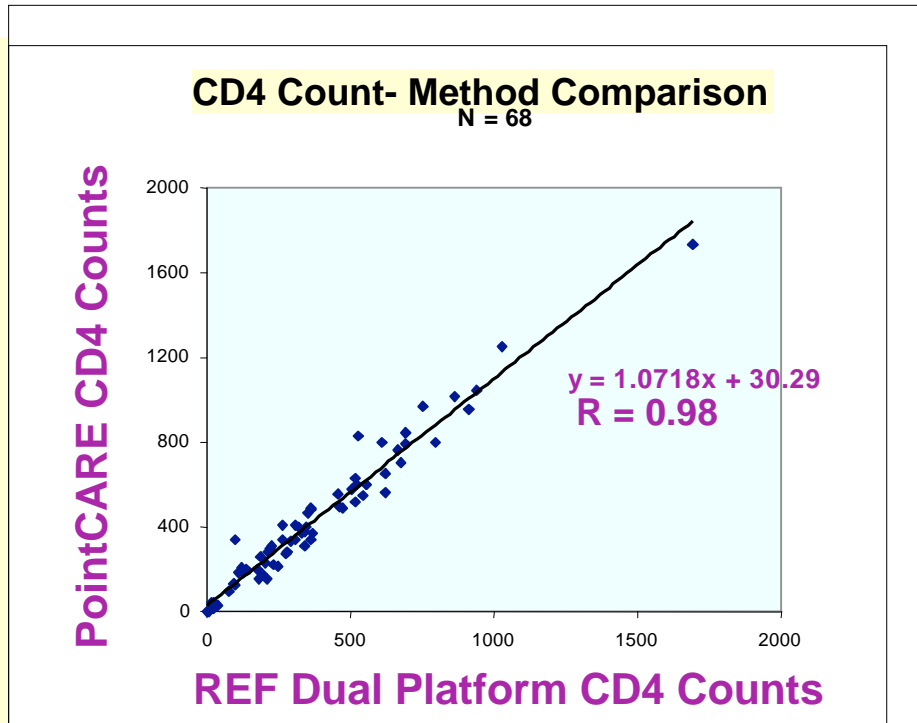
Parameter	Results	Units	Normal range
CD4 T Cell Counts	1124	/ CU MM	
CD4 %	50.0	%	
WBC Counts	6.9	10 <sup>3</sup> / CU MM	
Lymphocyte Counts	2.2	10 <sup>3</sup> / CU MM	
Lymphocyte %	31.9	%	

Print      Done

- Both CD4% & Absolute CD4
  - without beads
  - critical for pediatrics

- Depending on test volume, cost of patient result is under US\$10
- Cost of patient result includes:
  - All reagents and disposables
  - Operator time
  - CD4, CD4%, WBC, LY, LY%
  - Service

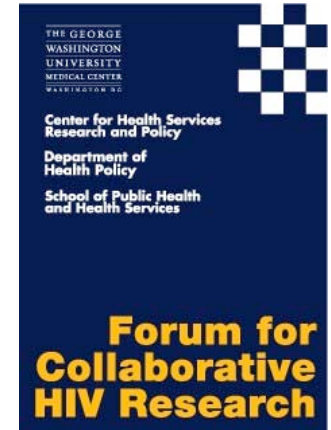
# PointCARE comparison with DP Flow



# Summary PointCARE

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- Recently received FDA approval
- %CD4 (as percentage of CD3+T cells)
- Cost approximately \$10/test
- Capital cost approx \$15,000-20,000



# Manual low cost assays for monitoring CD4

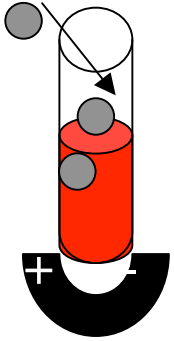
*Vidal, Omah Mooleedhar, Shahir Ali, CAREC Data from Crowe lab, Burnet Institute Melb and  
Dr Balakrishnan's lab, YRG Care Chennai  
Arlene Darmanie, Cecile Goddard*



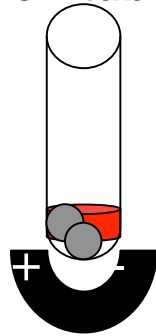
# CD4 manual methods

## Dynal assay

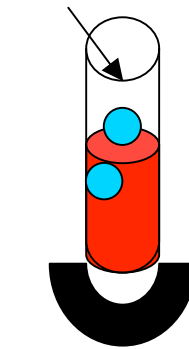
CD14  
Dynabeads®



Monocyte-depleted  
blood removed  
to new tube



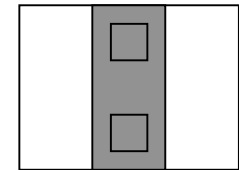
CD4  
Dynabeads®



Lysis and  
staining  
of nuclei

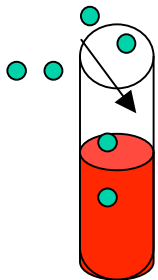


Count  
stained  
nuclei

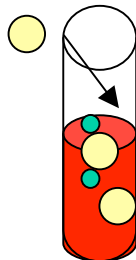


## Coulter assay

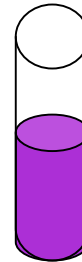
Monocyte  
blocking agent



CD4 cytospheres®



Add blood to  
staining solution



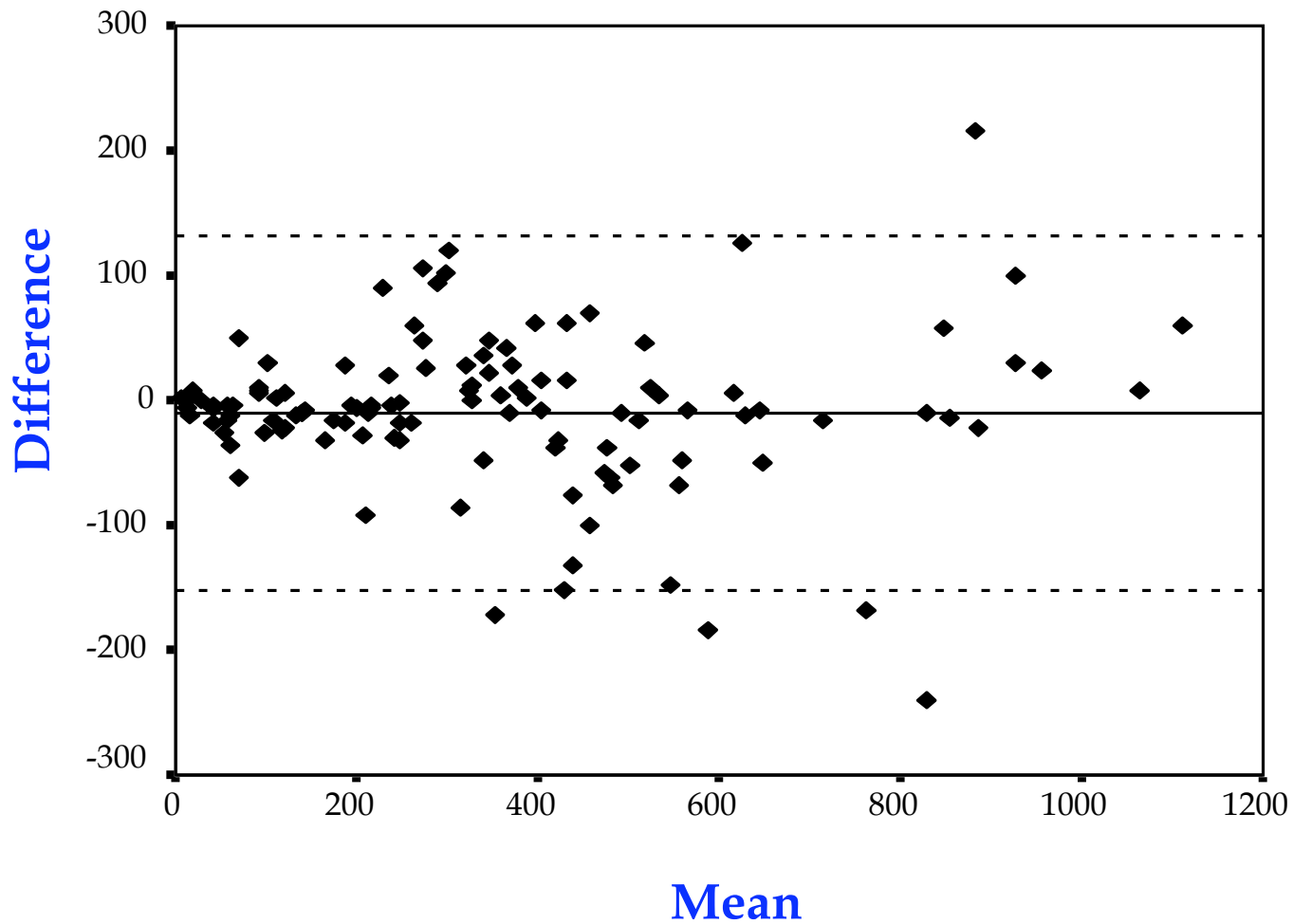
Count cells  
with beads  
attached

# What equipment is needed for these manual CD4 assays?

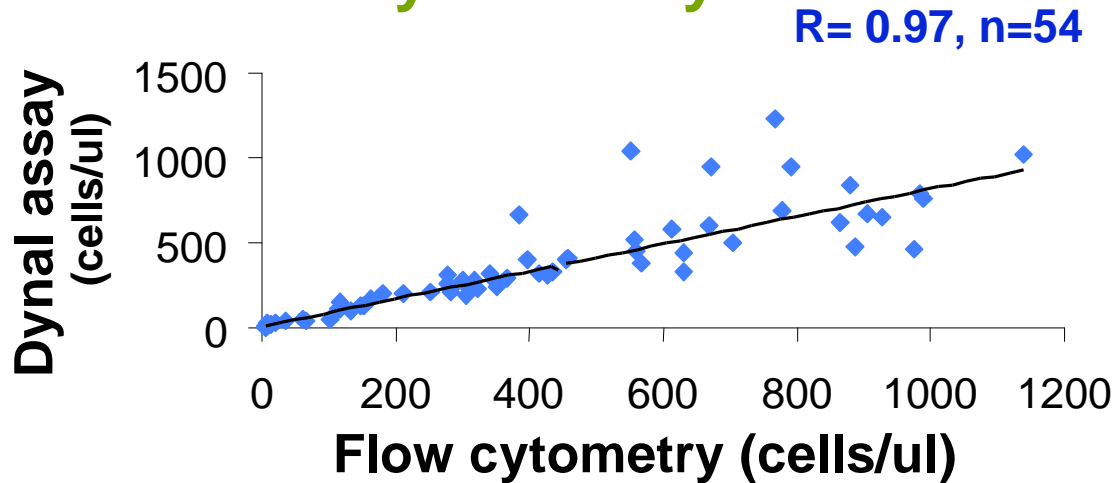
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- **Microscope with 40x objective**
  - **Hemocytometer 0.1 mm deep**
  - **Manual counter**
  - **Tubes**
  - **Pipettes**
- 
- **Plus rotating wheel and magnet for Dynal assay**

# Bland-Altman plot for difference against mean for CD4+ T-lymphocyte counts by flow cytometry and Coulter cyto-sphere assay

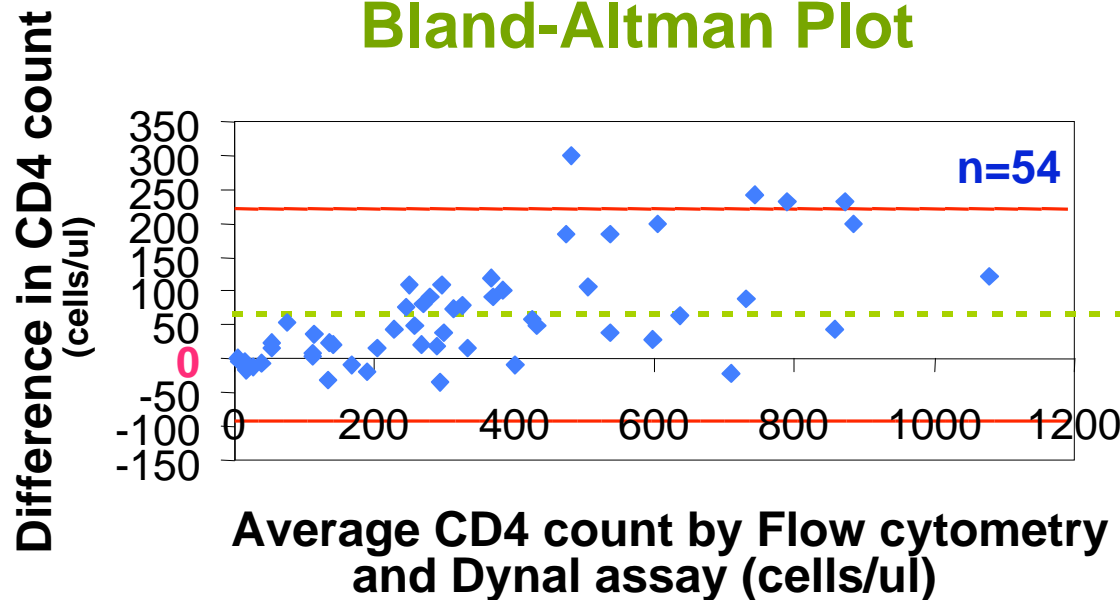


## Correlation of Flow SP and Dynal assay



**Dynal assay shows excellent association with flow cytometry**

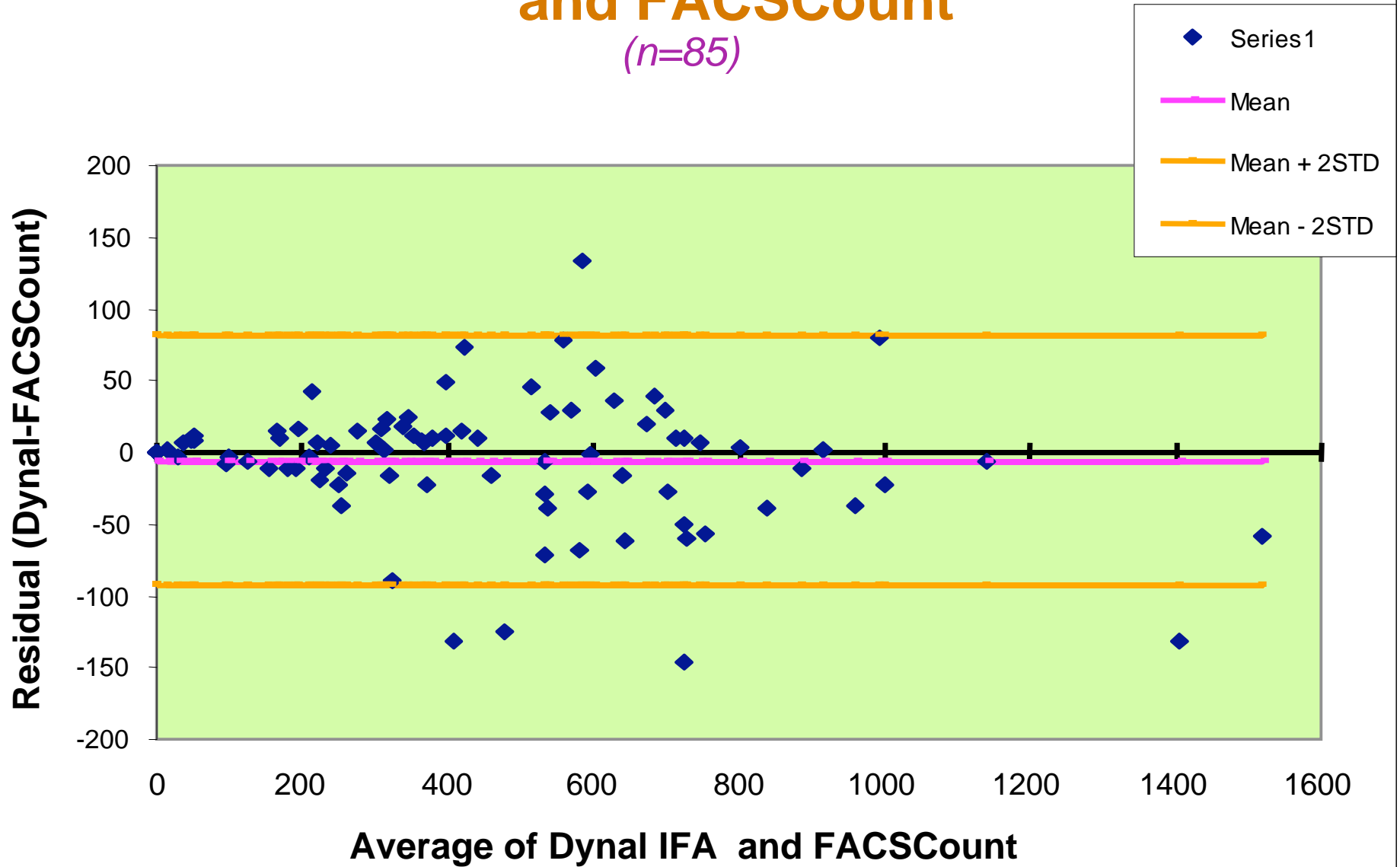
## Bland-Altman Plot



**Average Flow cytometry result is 65 cells/ $\mu$ l higher than Dynal assay**

# Comparison of CD4 Count between DYNAL and FACSCount

(n=85)



*Arlene Darmanie, et al CAREC*

# Coefficient of variation

## Dynal assay vs flow cytometry in West Africa

### DYNABEADS<sup>R</sup>

Sites	Site 1	Site 2	Site 3 Ref site	Site 4	Site 5	Site 6	All Sites
<b>CV</b>	<b>14.6</b>	<b>7.0</b>	<b>9.7</b>	<b>6.0</b>	<b>5.3</b>	<b>9.8</b>	<b>8.4</b>
<b>n</b>	<b>16</b>	<b>7</b>	<b>49</b>	<b>24</b>	<b>20</b>	<b>14</b>	<b>130</b>

### Flow Cytometry

	Reference site	Sites Distant from Reference site
<b>CV</b>	<b>8.3</b>	<b>19.7</b>
<b>n</b>	<b>20</b>	<b>45</b>

# Impact of the delay in sample handling on Dynabeads<sup>R</sup> Technique at room temperature

samples exhibiting  $\geq 20\%$  decrease in CD4 cell counts

<b>Time</b>	<b>n</b>	<b>% (95% CI)</b>
<b>Hour 4</b>	<b>0 / 28</b>	<b>0</b> (0, 12.3)
<b>Hour 8</b>	<b>3 / 28</b>	<b>10.7</b> (2.3, 28.2)
<b>Hour 12</b>	<b>5 / 28</b>	<b>17.9</b> (6.1, 36.9)
<b>Hour 24</b>	<b>14 / 28</b>	<b>50.0</b> (30.7, 69.4)



# Blood stabilizers

*Slides courtesy of Viv Granger and Dave Barnett, NEQAS UK and data from Amanda Lindholm Streck Laboratories*

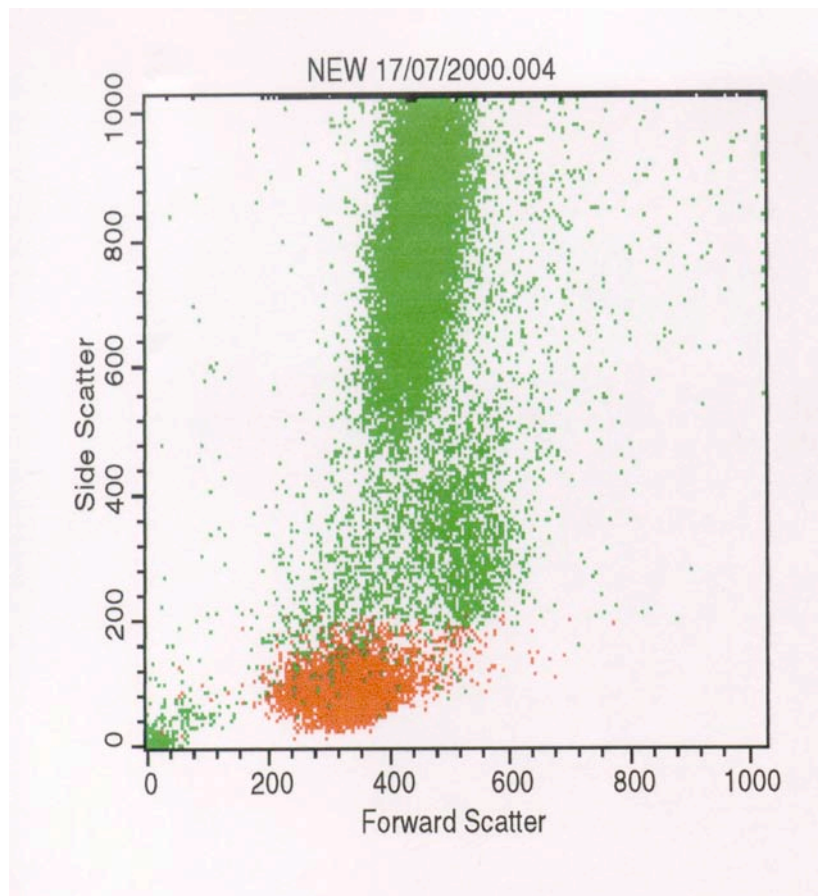


# Reagents for stabilizing blood samples

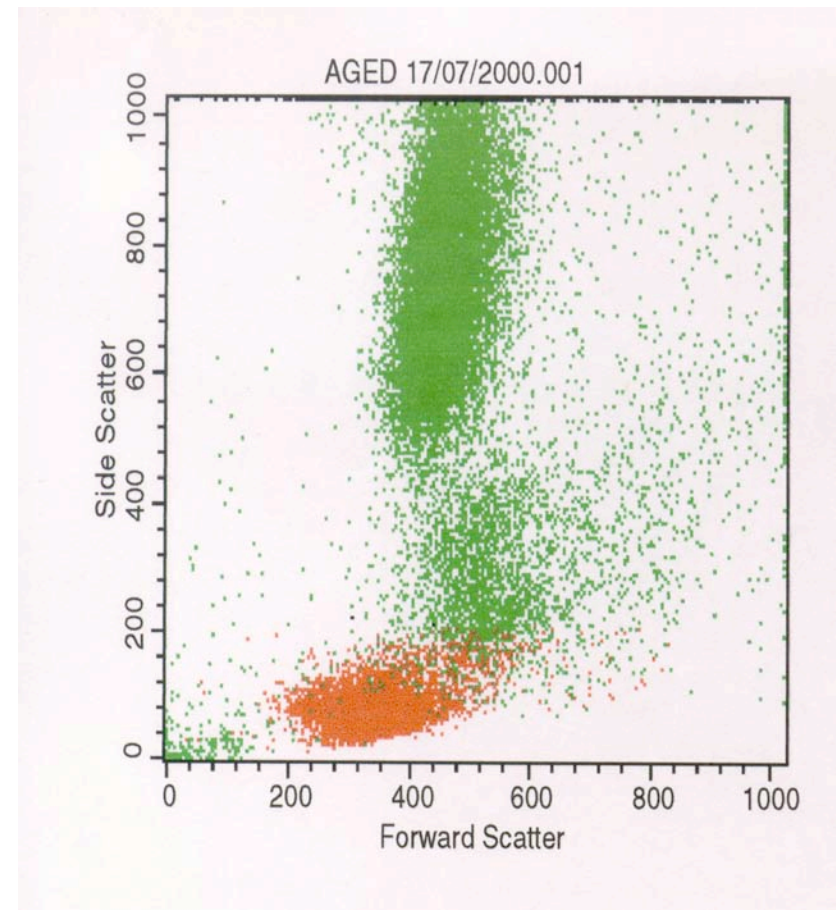
- ⚡ **Guidelines, CD4<sup>+</sup> T cell analysis: must be done within 18 hrs**
  - haematology analysers: difficulty producing a differential after 24 hrs
- ⚡ **CytoChex™ (Streck laboratories)**
  - Member of family of non cross-linking fixatives
  - Orig. designed to preserve WBCs in whole blood (1:1)
  - **Cyto-Chex BCT** contains 57ul preservative/anticoagulant
  - Samples stable 7 days at ambient temps
- ⚡ **NEQAS (UK)**
  - **TransFix™** lasts >10days, (1:10), <25<sup>0</sup> C
  - Transfix: allows transportation of fixed samples
- ⚡ **Both compatible with flow technology**
  - No data on stabilized blood and manual CD4 counts

# Flow Cytometric Analysis

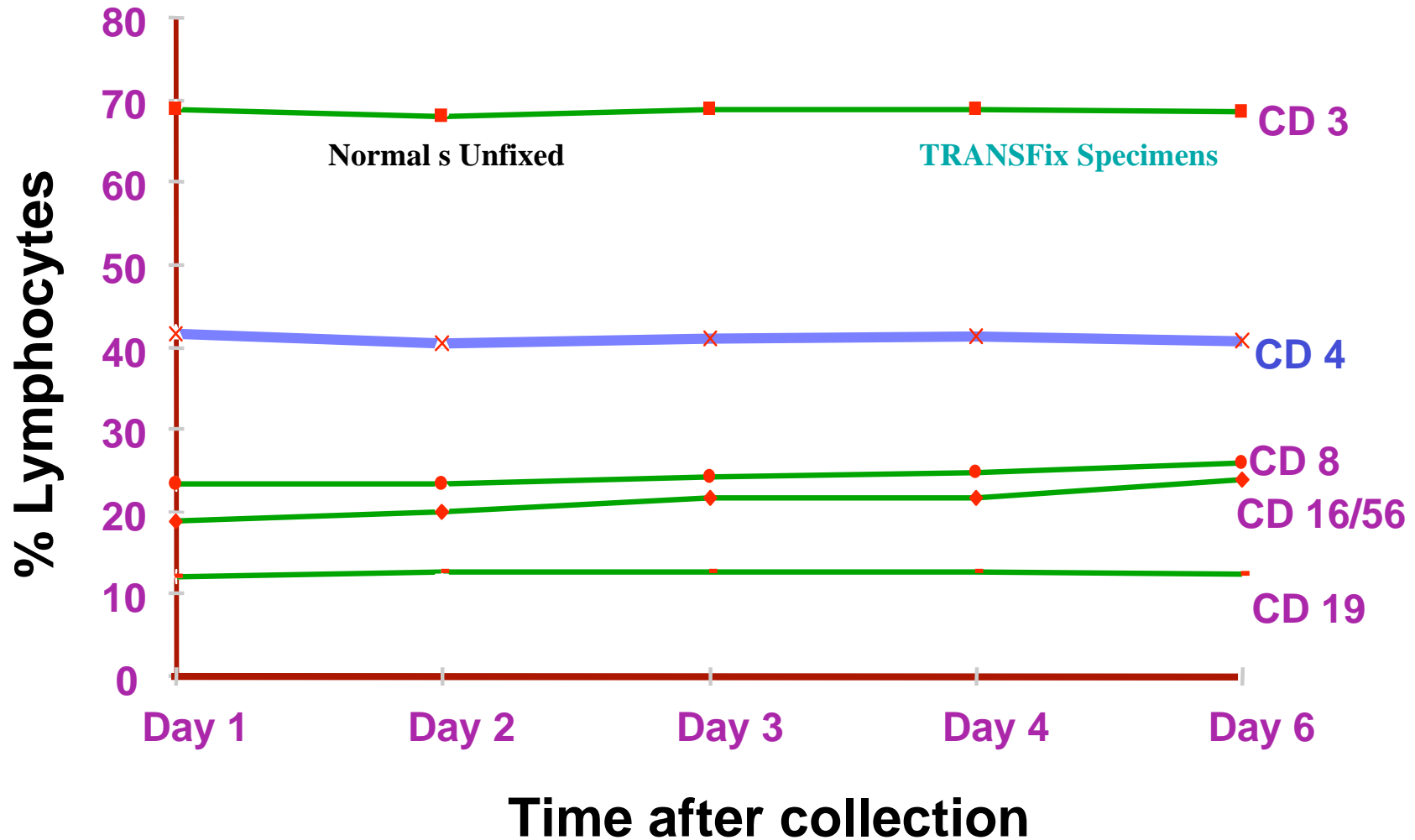
Fresh



Day 7

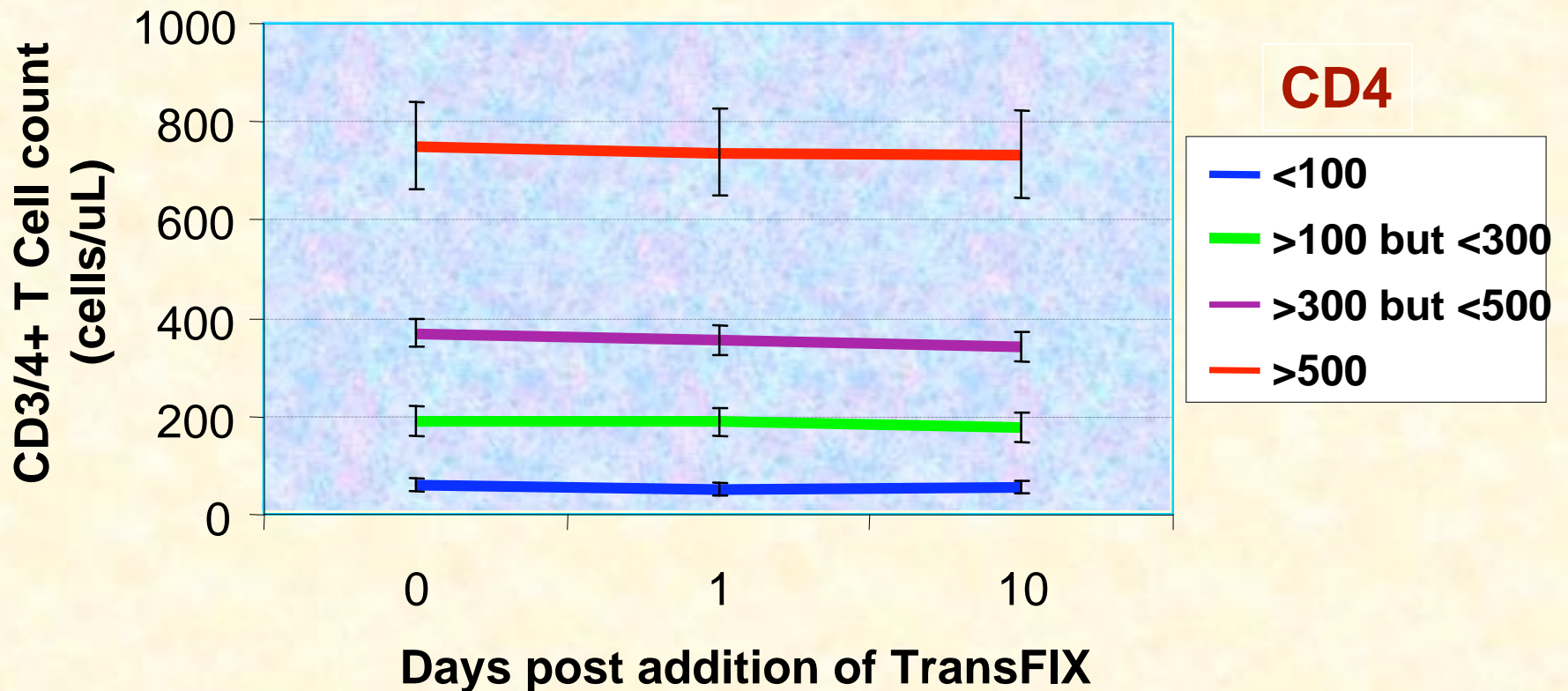


# Preservation of Lymphocyte Subsets with TransFIX



# Stability of CD3/4+ TCells post addition of TransFIX

## Stability of CD3/4+ T Cells post addition of TransFIX



# In the pipeline

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## ● LabNow

- Microchip, reader and digital camera
- Individual biochips
- Absolute CD4
- Tentatively planned for availability late 2005

## ● Semi-Bio manual slide technology

- Slide with an antiCD4 coated chamber that traps CD4+ cells during incubation
- Count CD4+T cells after staining

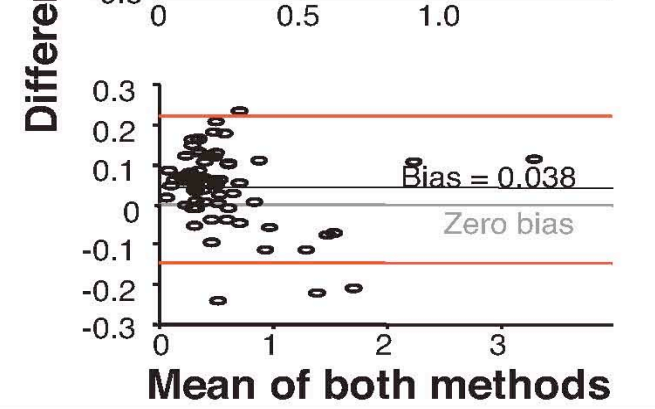
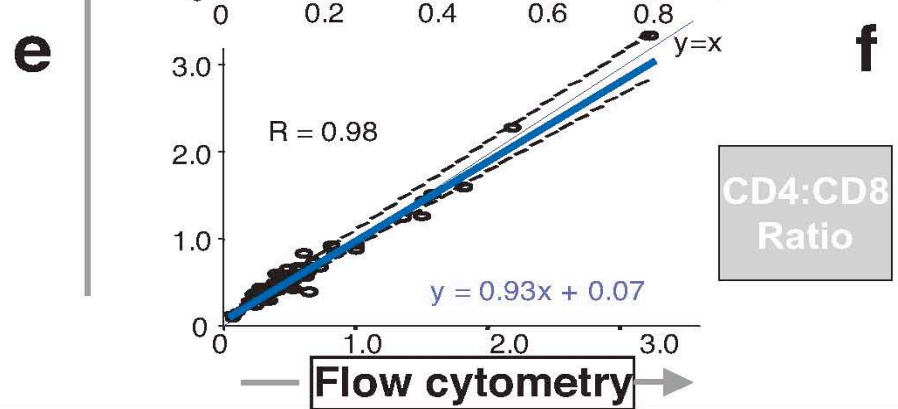
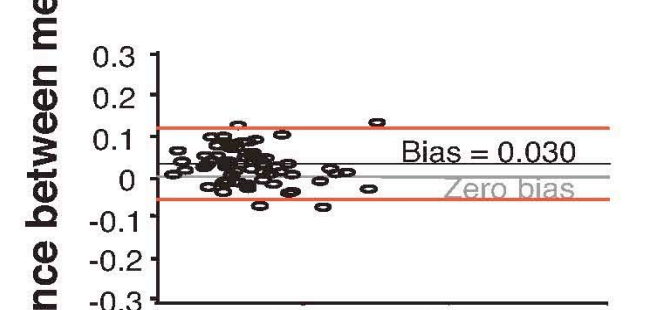
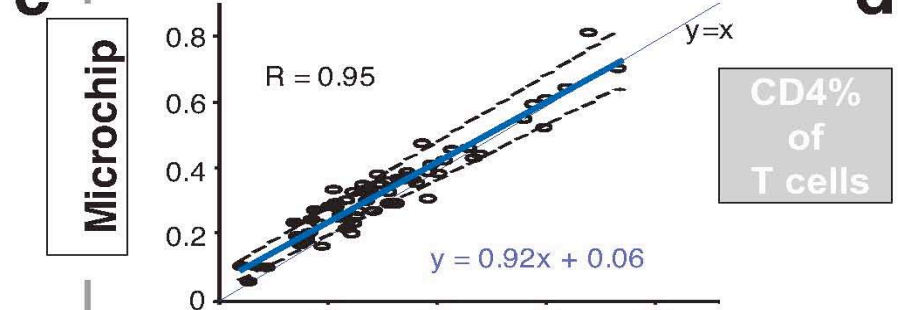
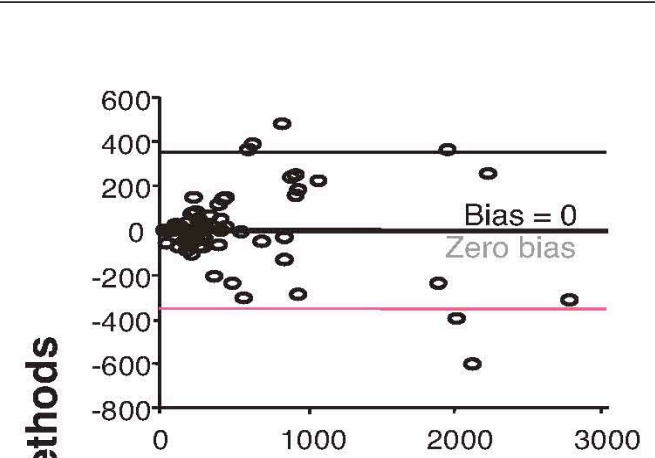
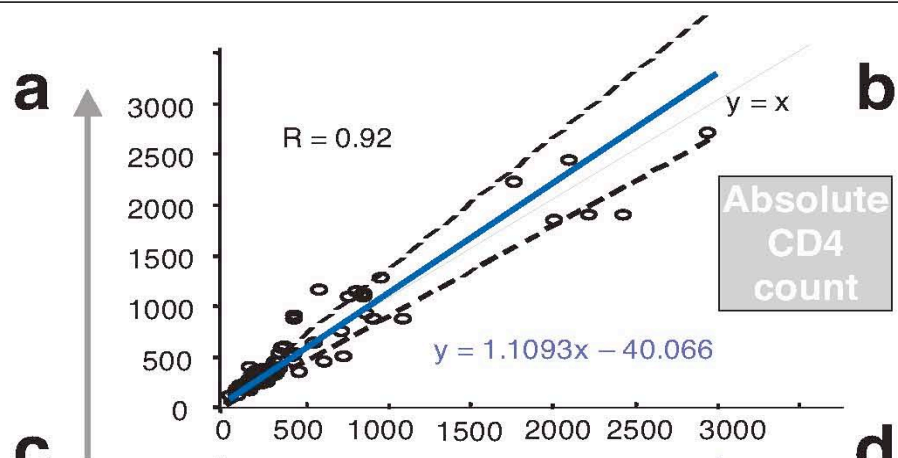
## ● Remote point-of-care technology

- No technical skill required
- For estimation of CD4+ T cells
- Early stages of development

## Commercialization – LabNow Corp (Austin, Teas)



**Microchip technology with a reader device and digital camera**





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**Forum for  
Collaborative  
HIV Research**

# Which low-cost CD4 assay to introduce?



## Depends on

### ■ Number of samples per day

- Low throughput, manual may be most cost-effective
- High throughput, flow method most cost effective (and definitely more practical)

### ■ Sophistication of lab

- Current methods require varying degree of technical skill
- Remote point of care methods under development

### ■ Availability of technical support & equipment maintenance training

- A key issue for flow cytometers
- Remote area, opt for manual or ship samples or ensure local engineers/technicians trained

### ■ Cost

# Word of caution when considering cost

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- **Hidden costs (ie non-kit costs) greatly influence the final cost to a testing laboratory in a resource-constrained country**
  - Labour (often less expensive)
  - Disposables (if available often more expensive)
  - Shipping costs
  - Importation costs
  - Infrastructure
  - Repeat assay runs
  - Instrument repair
- Pricing may be best negotiated at an international or country level with bulk procurement schemes

# Where are we up to?

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- All assays/methods continuing to undergo in-country analyses
- Rigorous independent evaluation, including clinical trial evaluation and independent statistical analysis, is absolutely essential
- Some technologies /assays recently licensed in USA (PLG, PointCARE)
- However all are still emerging technologies
- QA participation essential and establishing this should be part of the deal
- Countries should not purchase technologies that have not been inadequately validated



# Final thanks to

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