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An Open Platform, High Speed CD4 T- Cell Monitoring Method Using Immunogold Conjugates: A Method Aimed at Reducing Loss to Follow-Up in Resource-Poor Settings

Peter Hansen[1], David Dombkowski[2], Michelle DeLelys[2], Amanda Restell[3], Dorothy Sylvia[3], Donald Barry[3], Sonia Kumar[3], Artem Zeygerman[3], Petra Krauledat[3], Frederic Preffer[2]

[1]Union Biometrica Tech, Inc., Canaan, New York, USA
[2]Massachusetts General Hospital, Dept. of Pathology, Boston, MA, USA
[3]PointCare Technologies, Inc., Marlborough, MA, USA

Background

Standard CD4 T-cell Test:

- Costly instrumentation using lengthy immunofluorescence labeling methods
- Requires cold-chain shipping (maintain 2-8℃ during shipping)
- Reagents need to be stored at 2-8℃
- Results are not usually available the same day

Resource-Poor Settings:

- Requires cost effective CD4 test method
- Unreliable power sources for refrigerated storage
- Patients distant from clinics often do not return for follow-up visits

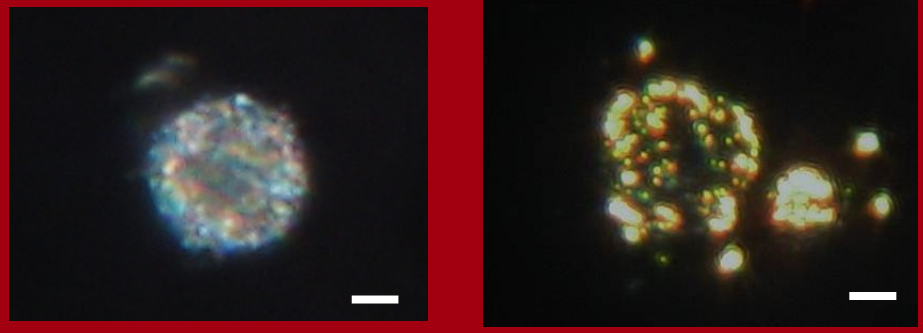
Methods

Immunogold CD4 T-Cell Assay

- Minimal sample preparation can be completed in < 1min
- Cationic surfactant neutralizes surface charge of immunogold resulting in ultra-fast labeling, in as little as 45 seconds
- Reagents are temperature stable and do not require cold chain shipping or refrigerated storage
- CD4 clusters can be detected with relatively simple, cost effective optics

CD4-Negative Lymphocyte

CD4-Positive Lymphocyte Labeled with Immunogold

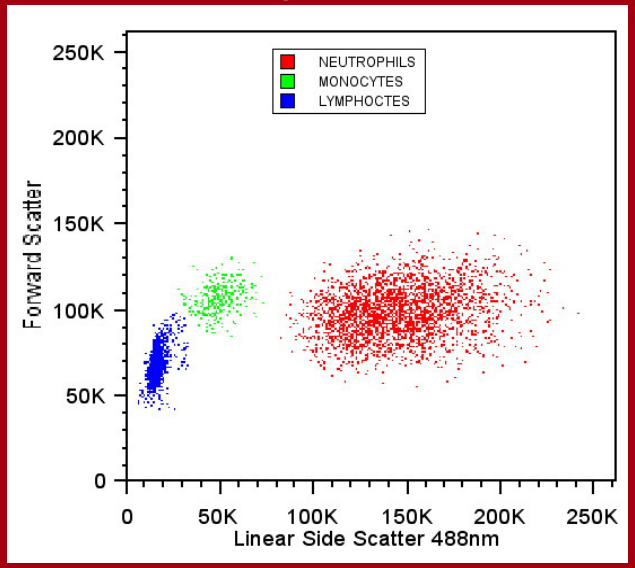


Darkfield reflectance images of lysed whole blood, scale bar is ~2µm

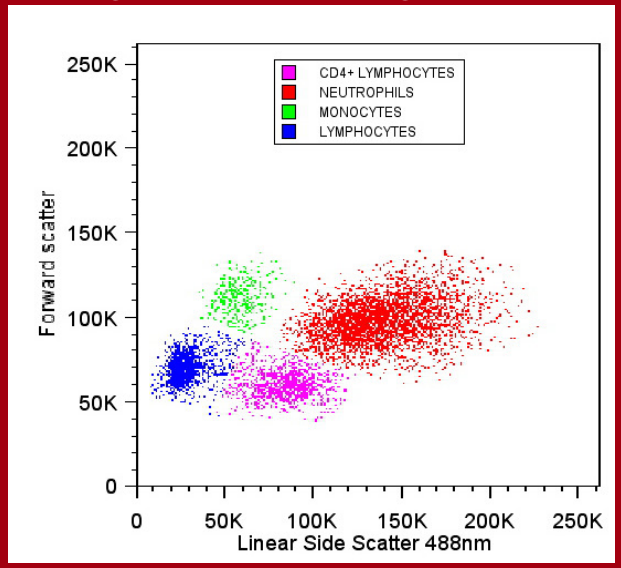
Reflectance images show dramatic increase in scattering for CD4+ cells labeled with targeted immunogold contrast agent

Results

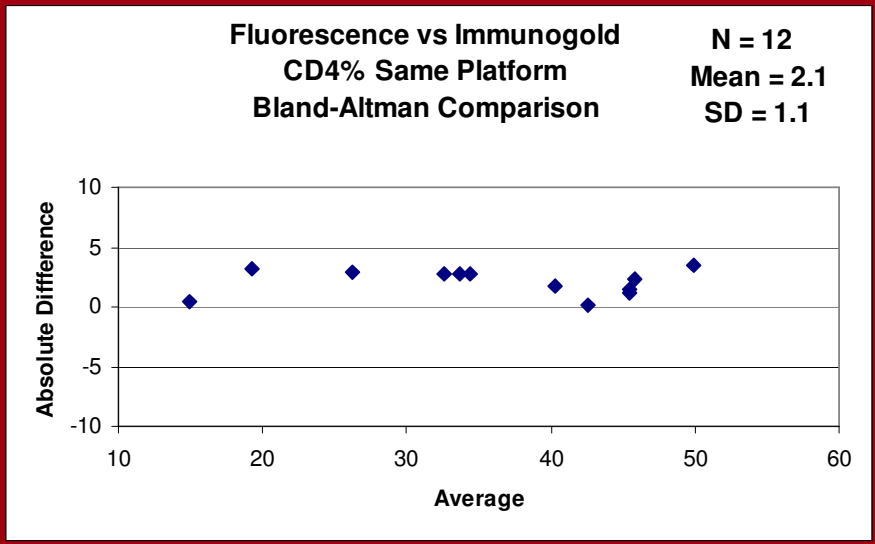
Unlabeled Light Scatter Plot



Immunogold Labeled Light Scatter Plot

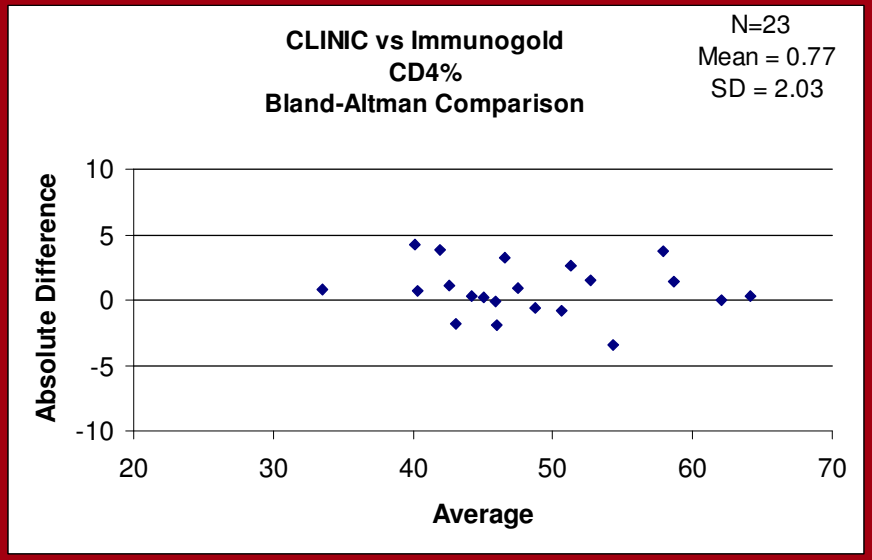


Bland-Altman comparison of 12 samples (6 clinical, 5 normal, and 1 manipulated to a low CD4% of 15) run on the same flow cytometer using the standard fluorescence method vs immunogold assay shows excellent agreement between the two methods.



Results

A Bland-Altman Comparison of 23 normal samples compares CD4% results from a flow cytometer using the immunogold assay to CD4% results obtained from a reference laboratory using the standard fluorescence analysis.



Conclusions

- Bland-Altman shows good agreement between the standard fluorescence method and the immunogold light scatter method
- Currently deployed flow cytometers can use immunogold to identify CD4 T-cells
- Immunogold delivers immediate CD4 results
- Immunogold is unusually heat stable (42C continuously for one year) eliminating cold-chain shipping and refrigeration