PointCare NOW™ Technical Note

Introduction to our Technology

And

Summary of Evaluation and

Performance data

Introduction

The PointCare NOW™ is a portable, and very fast, diagnostic instrument specifically designed for HIV/AIDS point-of-care use in decentralized settings. The PointCare NOW produces test results in 8 minutes eliminating delays and patient attrition that undermine care and treatment in every setting. While there are 12 diagnostic parameters reported by the PointCare NOW™, this technical note is focused on CD4 lymphocyte absolute counting, a key parameter in HIV/AIDS patient management.

New Reagent Technology

The PointCare NOW™ is based on new chemical reagent technology that offers key breakthroughs in CD4 counting.

First, the new reagent technology chemically marks blood CD4 lymphocytes in a reaction that takes less than 30 seconds. Normally, with traditional flow cytometers, this marking step requires times of the order of 30 minutes or more. By having such a fast reaction, the PointCare NOW™ reports a printed CD4 count in minutes, while the patient is in the clinic and available for counselling. Other systems report results hours or even days later. This PointCare NOW™ feature is one key to enabling point-of-care CD4 counting.

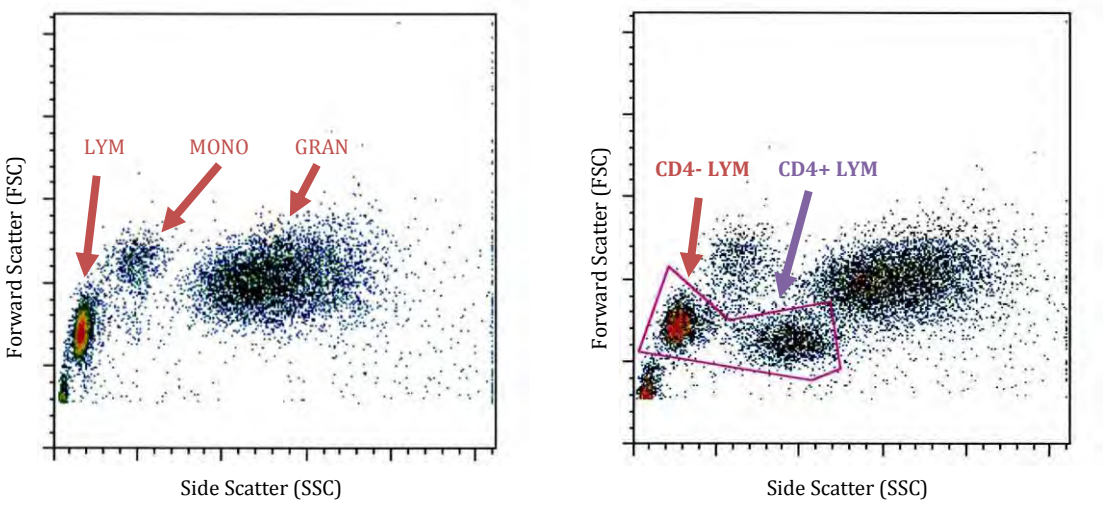


Figure 1: Data from a Becton Dickenson LSR II research flow cytometer. The plot on the left shows a clear WBC differential obtained without the presence of any immunolabelling reagents. The plot above right shows a 30 second CD4 reaction using the PointCare colloidal gold -reagent.

Second, the new reagent technology uses a monoclonal antibody marker for CD4 lymphocytes that can be shipped without a cold chain and is stable even in high temperature storage. PointCare was the first to achieve this breakthrough, and did so by developing a manufacturing process that lyophilizes (freeze-dries) the monoclonal antibody marker. Some manufacturers "dry" their reagents but do not "freeze-dry" them. Drying enhances storage to a certain extent, but does not achieve the ultra-stability of the PointCare lyophilized marker which can be stored for more than a year at 30C. This fact has been proven in real time studies covering a year or more, and is not based on projections or estimates.

New Engineering Technology

The PointCare NOW™ also features breakthroughs in engineering technology.

One is the extraordinary bio-safety of the system. It is of utmost importance that point-of-care clinical personnel be as well protected from open blood tube hazards as are their counterparts in central laboratories. By eliminating the open blood tube and using a one-of-a-kind cap piercing front end, complete liquid confinement, and automated waste de-contamination, ***the PointCare NOW™ is the only point-of-care CD4 counter that meets guideline standards for operation outside a containment hood.*** It is therefore the only system of its kind that offers bio-safety to operators in decentralized settings.

Another engineering accomplishment is the portability of the system. ***The instrument can be taken from its companion travel case and be running samples in less than 15 minutes. Normally it requires days to set up a flow cytometer for operation.*** The PointCare NOW™ operates on approximately 60 Watts of average power which translates to about 6 hours running time on its battery or 8 hours with its combination battery and photovoltaic solar panel.

This extraordinarily low power consumption and stability is made possible by the use of solid state photodetectors and LED's rather than out-dated and unstable photomultiplier tube and laser technology.

By reading on you will learn about the principle upon which CD4 counting in the PointCare NOW™ is based, how the internal and external quality control features work, and see CD4 performance data that has been produced independently in the field.

Measurement Principle

While the PointCare NOW™ offers new technology for CD4 counting, it is based on well-proven design principles found in high quality haematology analysers. In fact the PointCare NOW™ reports very useful haematology parameters such as a complete white cell count, a white cell differential count and haemoglobin with every CD4 count.

The PointCare NOW™ flows single cells through the focal point of a light emitting diode (LED) and detects light scatter and light blockage by the cells. Using the same principles as found in modern haematology analysers, the PointCare NOW™ distinguishes and counts all basic white cell classes (lymphocytes, monocytes, neutrophils, eosinophils and basophils) by light scatter. ***What is new, is that the PointCare NOW™ distinguishes and counts CD4 lymphocytes by light scatter.***

Counting CD4 lymphocytes by light scatter is accomplished with a CD4 monoclonal antibody that is labelled with colloidal gold rather than with older technology fluorescent molecules. When PointCare NOW™ CD4 monoclonal antibodies bind to the surface of CD4 lymphocytes, those lymphocytes scatter much more light than normal because they are now covered with gold nano-particles. The system easily identifies these CD4 lymphocytes and counts them.

More importantly, since the PointCare NOW™ is also a haematology analyser, it never confuses a monocyte with a lymphocyte (even in cases with abnormal monocyte counts such as in tuberculosis

patients) and consequently ***avoids the - pitfall of falsely counting monocytes as CD4 lymphocytes that is common to CD4 counters based on fluorescence.***

New Quality Control Technology

External quality control materials for CD4 counting are generally preserved human blood cells that are run as a blood sample at least once per day and the CD4 counts are compared to previous results (Levey-Jennings analysis) or to an "acceptance band" provided by the control material manufacturer. These materials are meant to indicate that samples are being prepared for analysis in a consistent fashion and that the instrument is consistently processing the samples over periods of days and months. External quality control materials are useful when multiple operators process samples and when sample processing involves manual steps that require close attention and training.

Despite their usefulness, traditional external quality control materials have limitations in decentralized clinics. External quality control materials are expensive and many decentralized clinics with low patient numbers fail to use them. External quality control materials have limited shelf life and generally need refrigeration. Lastly, with most flow cytometers there is generally no indication as to the location of the problem if the control material result is not consistent with previous data. The clinic could be faced with a malfunctioning instrument or a training problem. A service person therefore generally arrives "blind" to what may have gone wrong.

The PointCare NOW™ uses a modified approach to quality assurance that surmounts several of the above limitations. In the PointCare *NOW*™ there are three points of control.

The first point of control is ***Monocyte Check***. All monocytes carry the CD4 surface antigen, but at lower density than is the case for lymphocytes. The PointCare NOW™ automatically uses the dot plot or cytogram to verify that all monocytes in the patient sample have been labelled by the CD4 monoclonal antibody to a proper level on every sample run. Because any "mistake" in sample processing or any "interfering substance" in the patient sample will affect monocyte and lymphocyte CD4 labelling equally, the system infers that if all monocytes have been labelled properly, then all CD4 lymphocytes have been labelled properly as well. ***Monocyte Check operates on every patient sample and at no cost to the user.***

The second point of control is ***Daily Check.*** This is a polystyrene bead based control that is run once per day to determine that all automated pipetting, all light scatter detection and all flow rates are consistent. ***Daily Check currently has 24 months at 42C temperature stability and is provided at no cost to the user.***[1]

The third point of control is an extensive system of ***Internal Control Point Checks***. Over 50 internal control points are automatically checked on every sample run. The PointCare NOW™ reports any alerts from these control points on the printed patient result and stores the information for retrieval by service personnel. ***Most importantly these alerts tell the user where the problem lies so that service can be performed efficiently.***

[1] Real time stability testing is ongoing and is expected to extend this storage time at 42C.

Finally, the PointCare NOW™ also uses a standard, commercial, external quality control material from ***Streck Laboratories (CD-Chex***). ***This product is independently assayed by Streck and control bands are published with each lot.*** Streck CD4 control products have been used for proficiency testing in flow cytometry and CD4 counting for decades and were developed to accommodate both light scatter and fluorescence technologies.[2]

Daily Checks and CD-Chex are automatically tracked by Levey-Jennings software in the PointCare NOW™.

In summary, the PointCare NOW™ provides a comprehensive quality control system that:

1. Tells if the instrument is working on every sample and not just once a day.
2. Lowers the cost especially in clinics that run only a few samples per day.
3. Lifts some of the burden of short shelf life and need for refrigeration.
4. Provides a way to know exactly what had failed in the instrument or reagents.

[2] Some newer products do not preserve cell size, shape, and granularity, and cannot be used in hematology analyzers and light scatter flow cytometers. These products are not recommended for the PointCare *NOW*™.

Performance Data

A. FDA Submission Data

The PointCare NOW™ System is cleared by the United States Food and Drug Administration (FDA 510(k) clearance) and as part of that clearance received the lowest complexity rating awarded to any CD4 counting system under FDA regulation (CLIA rating of moderate complexity).

Early performance data comparing the PointCare NOW™ System to other FDA cleared systems such as those manufactured by Becton Dickenson and Beckman Coulter was collected for the 510(k) submission. The FDA 510(k) submission data (2007) compared the PointCare NOW™ System to the Beckman Coulter (BCI) EPICS-XL PLG Single Platform flow cytometer. The regression statistics are summarized below.[3]

Regression summary CD4 count (PointCare NOW™ *vs.* BCI EPICS-XL PLG)

- N = 160
- Data range: 2/microlitre < CD4 < 7,000/microlitre
- Regression line: y =0.933x + 26.0 (Slope = 0.933; Intercept = 26.0)
- Correlation: R = 0.975

Regression summary CD4% (PointCare NOW™ *vs.* BCI EPICS-XL PLG)

- N = 163
- Data range: 0.3% <CD4%< 70%
- Regression line: y =0.952x + 0.057 (Slope = 0.952; Intercept = 0.057)
- Correlation: R = 0.883

[3] Regression plots are included in Appendix A (FDA Submission Data).

Performance Data

B. Haiti Evaluation

The Haiti National Public Health Laboratory conducted an evaluation that compared the PointCare NOW™ System to both the Becton Dickenson FACSCount reference system and a Manual Count method using Cytospheres (Beckman Coulter). The samples were first analysed on the PointCare NOW™ and then transported to the GHESKIO main lab in Port-au-Prince to be analysed on the BD FACSCount. All samples were then counted twice using the manual method (with two different preps and two different technicians) and the average of the two counts were used for the comparison. The PointCare NOW™ had better calibration agreement (slope and intercepts of the regression lines) with the Manual Count method than the FACSCount had with either the PointCare NOW™ or the Manual Count method. The regression results are shown below.[4]

Regression Summary of Haiti Evaluation

CD4 count (PointCare NOW™ *vs.* Manual Count (Cytospheres))

- Regression line: y =0.867x + 3 (Slope = 0.867; Intercept = 3)
- Correlation: R = 0.882

CD4 count (PointCare NOW™ *vs.* FACSCount)

- Regression line: y =0.763x + 55 (Slope = 0.763; Intercept = 55)
- Correlation: R = 0.924

CD4 count (FACSCount *vs.* Manual Count (Cytospheres))

- Regression line: y =0.763x + 108 (Slope = 0.763; Intercept = 108)
- Correlation: R = 0.948

[4] Regression plots are included in Appendix B (Haiti Evaluation).

Performance Data

B. Haiti Evaluation

After the samples were analysed for CD4 counts, haematology results were collected from the Sysmex-KX-21N. Haemoglobin (Hgb), neutrophil count (Neut) and percent (Neut%), lymphocyte count (Lym) and percent (Lym%), and White blood count (WBC) results were compared to those reported on the PointCare NOW™. A summary of the regression results is shown below.

Regression Summary of Haiti Evaluation

PointCare NOW™ *vs.* Sysmex-KX-21N

Hgb

- Regression line: y =1.0x + 0.51 Correlation: R = 0.98

Neut%

- Regression line: y =0.84x + 6.1 Correlation: R = 0.96

Neut

- Regression line: y =1.0x + 0.15 Correlation: R = 0.98

Lym%

- Regression line: y =0.94x + 4.5 Correlation: R = 0.98

Lym

- Regression line: y =1.1x + 0.06 Correlation: R = 0.98

WBC

- Regression line: y =1.1x + 0.06 Correlation: R = 0.99

Performance Data

B. Haiti Evaluation

The Haiti evaluation also included a precision study with the following criteria having been established by the CDC and the Haiti National Public Health Laboratory: (1) a minimum of 20 repetitions for the same sample, and (2) a specimen must be in the CD4 range of ≤ 350. Therefore, a strategic decision was made to use the stabilized CD4 Low control material.

Precision Summary using CD4 Low Control

Unbiased data:

- Mean CD4 count: 205.5
- Standard deviation: 35.7
- Coefficient of variation: 17.4%

Adjusted for thermal drift*:

- Mean CD4 count: 203.4
- Standard deviation: 27.7
- Coefficient of variation: 13.5%

Performance Data

C. South African Study

A large private lab in South Africa conducted a study in 2008 comparing the PointCare NOW™ to the FACSCalibur Single Platform flow cytometry system. The correlation and comparison results are summarized below.[5]

Correlation summary CD4 count (PointCare NOW™ *vs.* FACSCalibur)

- Data range: 100/microliter < CD4 < 1,600/microliter
- Regression line: y =0.935x + 84.07 (Slope = 0.935; Intercept = 84.07)
- Correlation: R = 0.924

Correlation summary CD4% (PointCare NOW™ *vs.* FACSCalibur)

- Data range: 5% < CD4% < 45%
- Regression line: y =0.850x + 6.34 (Slope = 0.850; Intercept = 6.34)
- Correlation: R = 0.851

Bland-Altman per-cent difference summary CD4 count (PointCare NOW™ *vs.* FACSCalibur)

- Standard Deviation: 13.1%
- Bias: +6.7%

Bland-Altman per-cent difference summary CD4% (PointCare NOW™ *vs.* FACSCalibur)

- Standard Deviation: 4.2%
- Bias: +3.4%

[5] Data plots are included in Appendix C (South Africa Study).

Performance Data

D. CDC Data

Recently (2010) the United States Centers for Disease Control and Prevention (CDC) carried out an independent performance study in Atlanta using a Becton Dickenson FACSCalibur instrument with TriTEST reagents as the predicate device. These independently acquired data were shared with PointCare Technologies and are summarized below[6].

Correlation summary CD4 count (PointCare NOW™ *vs.* FACSCalibur)

- Data range: 300/microliter < CD4 < 1,600/microliter
- Regression line: y =0.932x +75 (Slope = 0.932; Intercept = +75)
- Correlation: R = 0.974

Bland-Altman per-cent difference summary CD4 count (PointCare NOW™ *vs.* FACSCalibur)

- Data range: 300/microliter <CD4< 1,600/microliter
- Standard Deviation: 6.9%
- Bias: +1.2%

The CDC study did not include low-end replicate precision samples but it did include precision in mid-range for whole blood, the results of which are summarized below.

Normal Range Precision summary (CV %)

- Replicates: 10
- Number of samples: 4
- Sample 1 (~ CD4=700) PointCare NOW™ 6.7%, FACSCalibur 5.4%
- Sample 2 (~ CD4 = 1,000) PointCare NOW™ 5.1%, FACSCalibur 3.7%
- Sample 3 (~ CD4 = 1,000) PointCare NOW™ 5.1%, FACSCalibur 4.5%
- Sample 4 (~ CD4 = 1,400) PointCare NOW™ 3.8%, FACSCalibur 4.6%

[6] Data plots are included in Appendix D (CDC Data).

Performance Data

D. CDC Data

To augment the precision data from the CDC study, we have included below results obtained at PointCare Technologies, using Streck Laboratories CD-Chex™ mid and low external control material samples.

Streck Laboratories CD-Chex Precision summary (CV %)

- Replicates: 10
- Number of samples: 2 (Low and Mid range control materials)
- Sample 1 (~ CD4=200) PointCare NOW™ 7.1%
- Sample 2 (~ CD4=700) PointCare NOW™ 3.8%

The PointCare NOW™ system compared favourably with the FACSCalibur system in correlation, Bland-Altman and precision testing. A direct low-range precision comparison was not done, and example low range precision data using external control material is given instead in this report for completeness and the convenience of the reader.

Summary

The PointCare NOW™ is a diagnostic instrument specifically designed for HIV/AIDS point-of-care use in decentralized settings. This technical note summarized CD4 lymphocyte absolute counting with the PointCare NOW™; future technical notes will explore the other parameters reported by PointCare NOW™. New reagent technology, new engineering technology, and a new approach to quality control have combined to specifically meet the needs of point-of-care CD4 testing. Independent data is shown indicating comparable performance with the Becton Dickenson FACSCalibur Tri-Test system.

Appendix A (FDA Submission Data)

FIGURE 12 - CD4 T-Lymphocyte Count

Single Platform Comparison

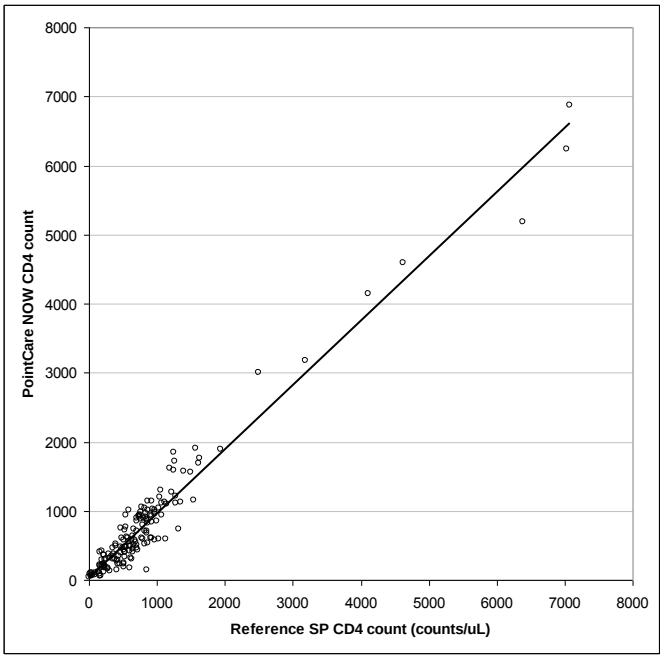
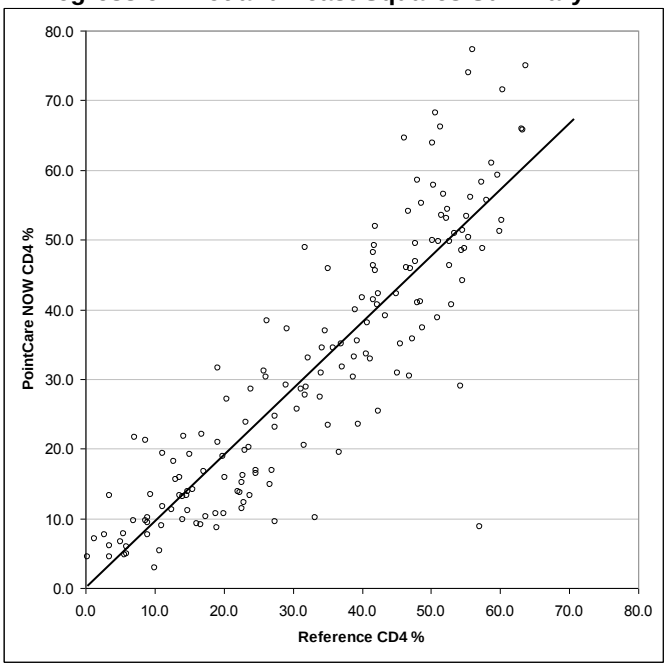
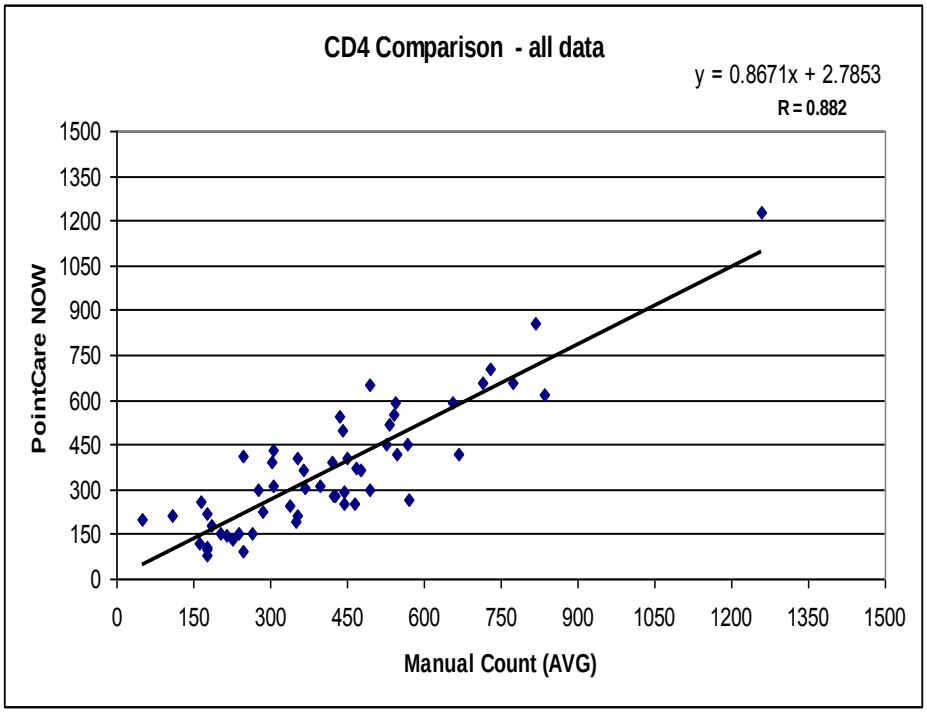


FIGURE 13 - CD4% of Total Lymphocytes

Regression Plot and Least Squares Summary



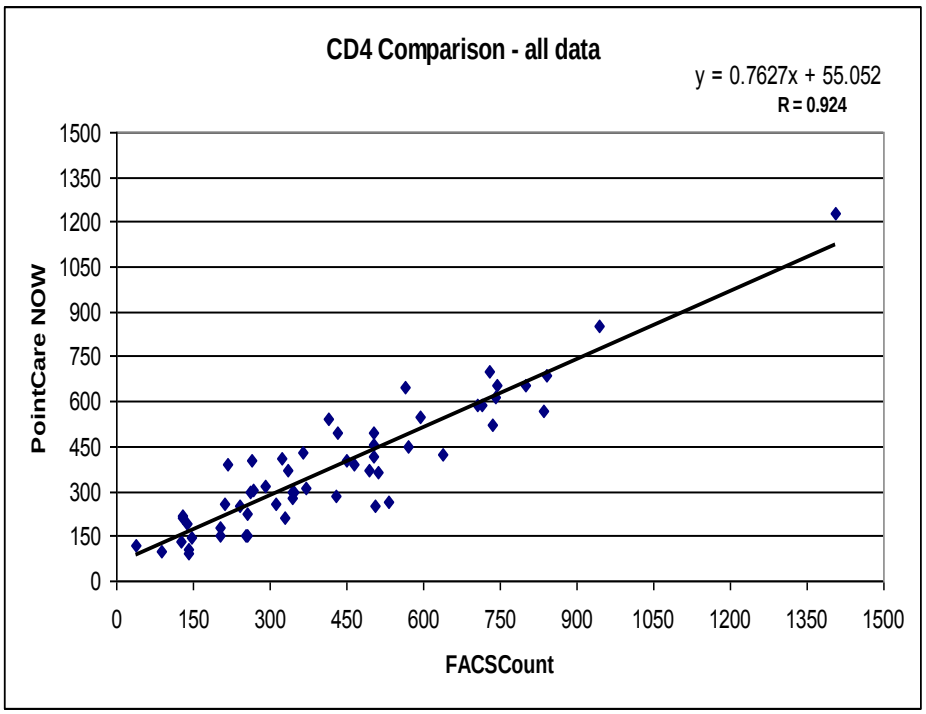
Appendix B (Haiti Evaluation)



PointCare NOW™

vs.

Manual Count
(Cytospheres)

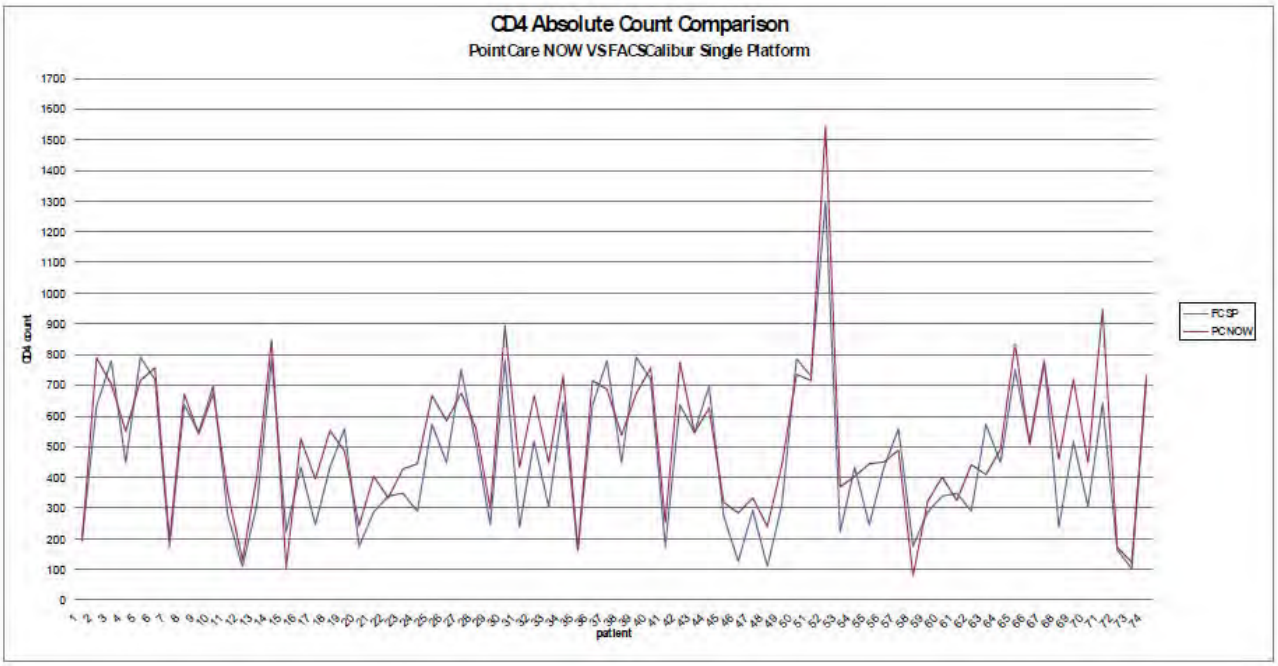


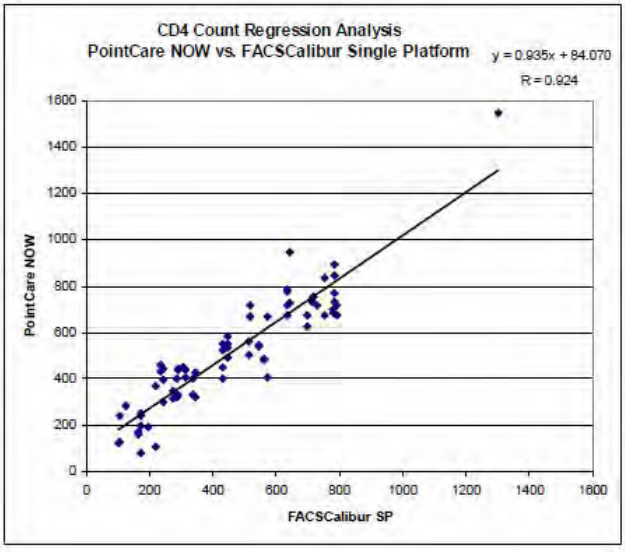
PointCare NOW™

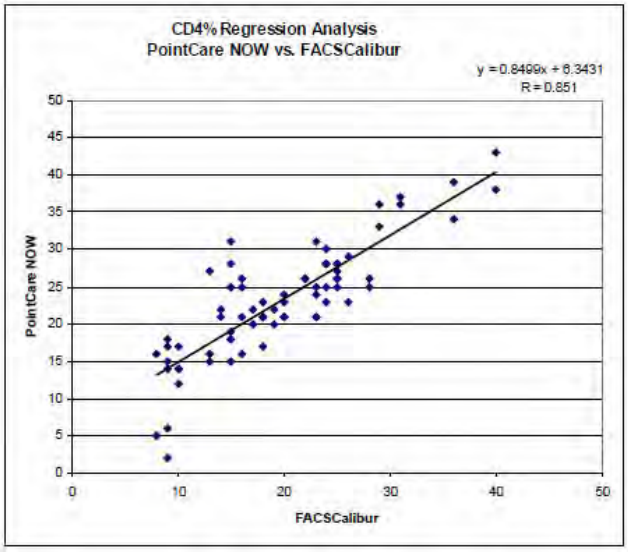
vs.

FACSCount

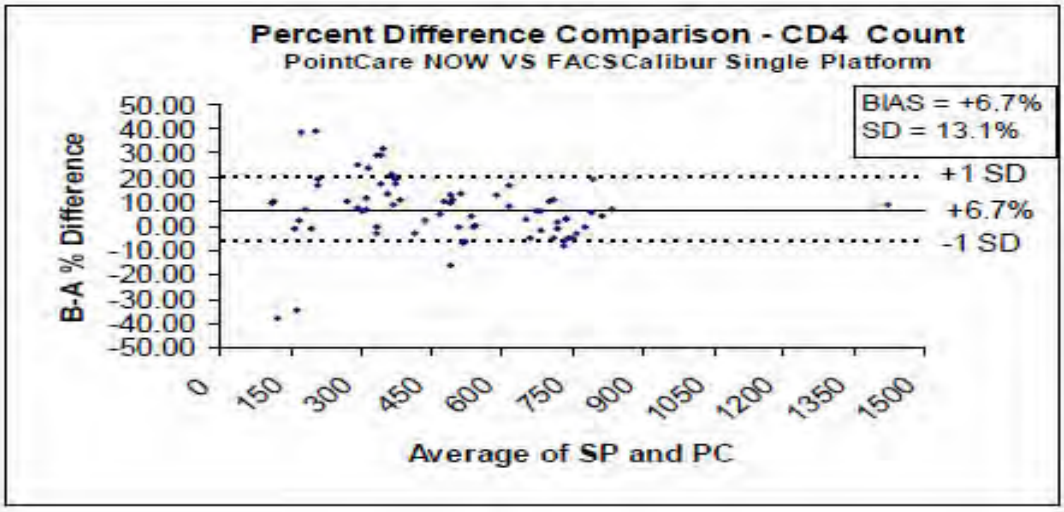
Appendix C (South Africa Study)

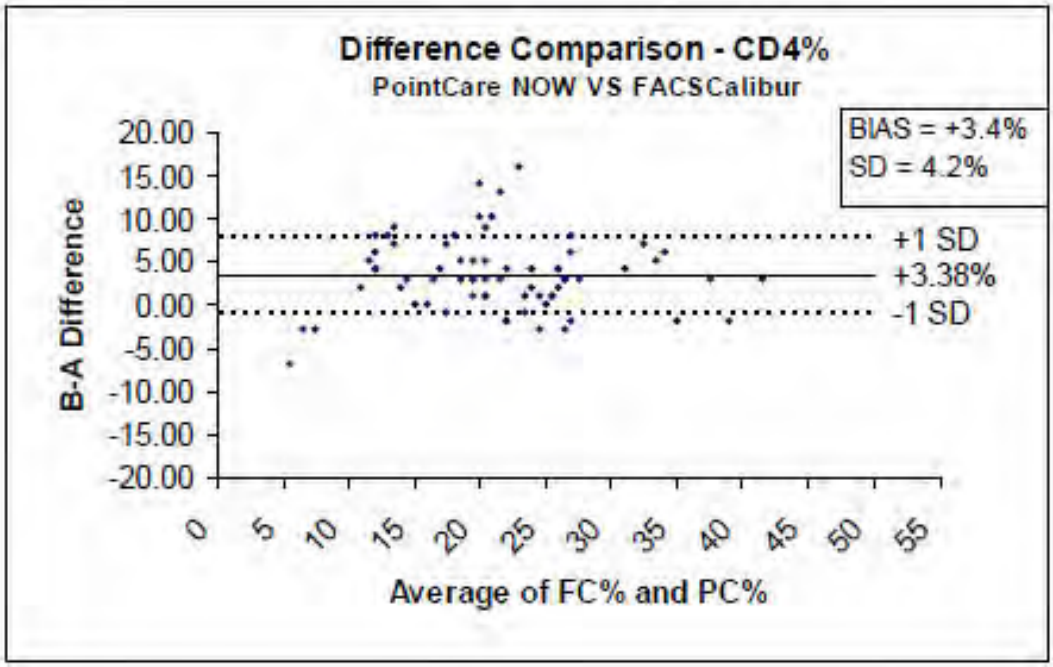




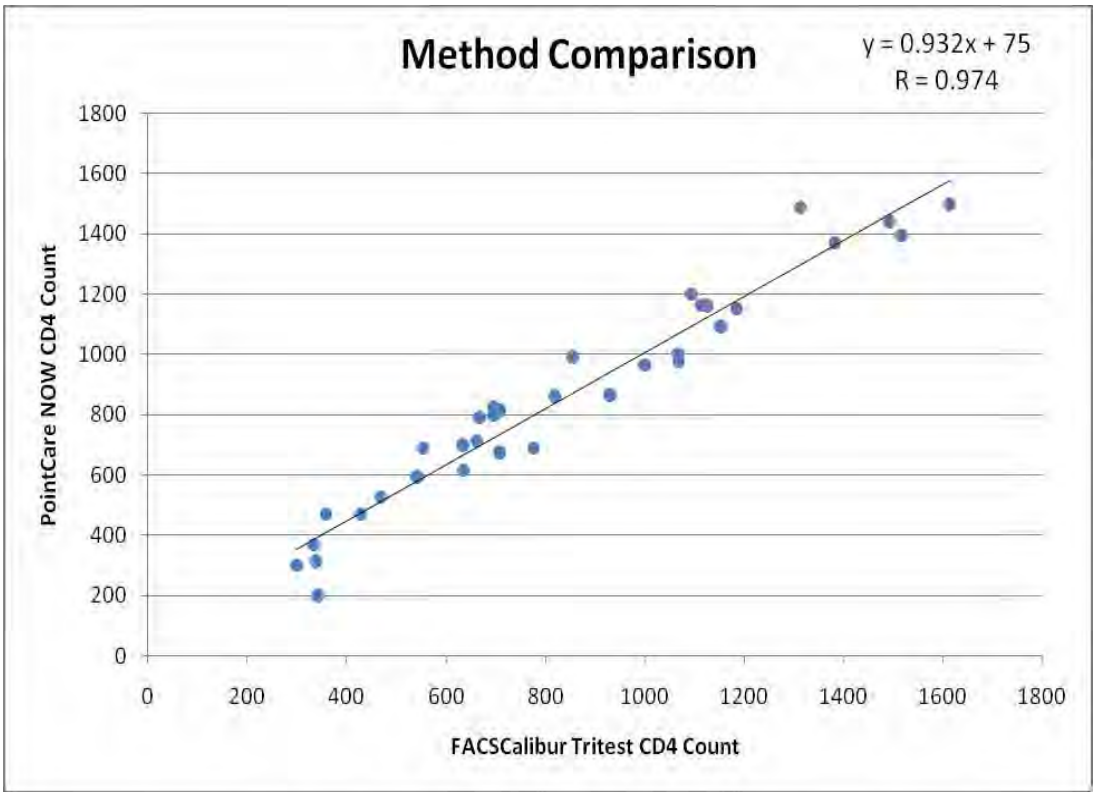


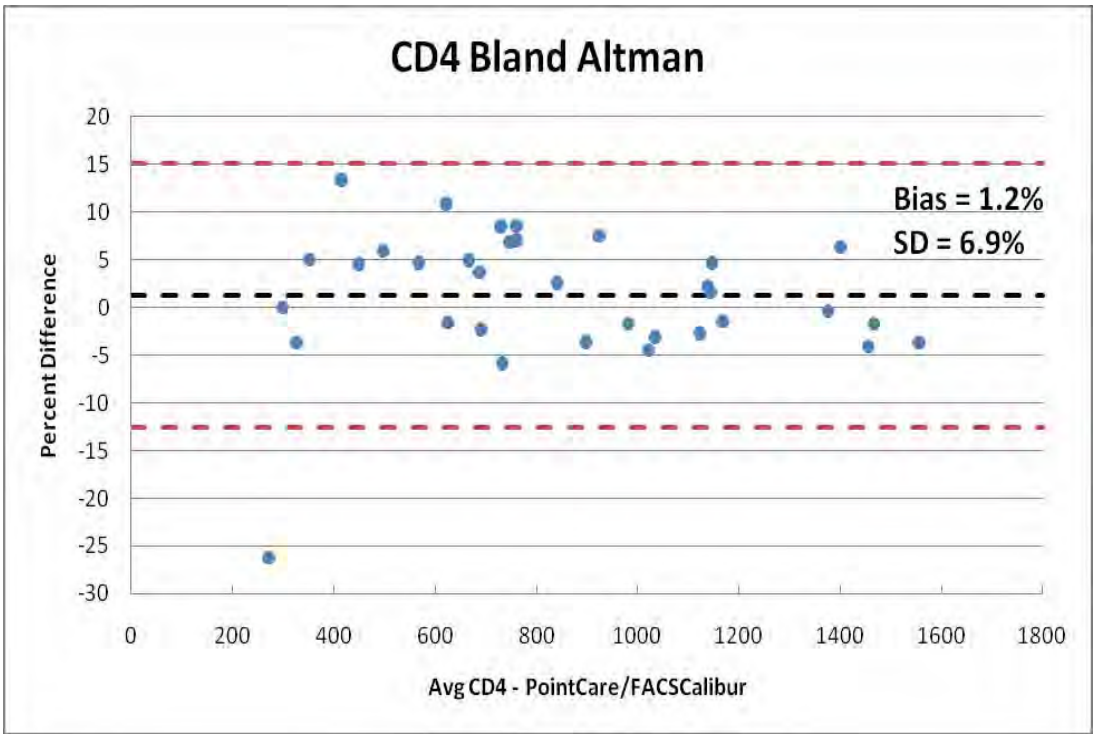
Appendix C (South Africa Study)





Appendix D (CDC Data)





Two outlier samples were -8SD and -6SD out of range in the Bland-Altman analysis. The cause was traced to out of range haematology parameters. They are not included in the above plots.