
Flow Systems
Apogee
Auto40 Flow Cytometers
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The Fight Against HIV/AIDS

With tens of millions of HIV infected people across the world, there is an urgent need for high quality, reliable and easy to use CD4 counters.

CD4+ T-Lymphocytes are the primary target of HIV, and as such are depleted in number as the disease progresses. Flow cytometry is the standard method for monitoring patients' CD4 count and thereby determining the course of treatment.

The Auto40 is a volumetric flow cytometer capable of all the common CD4 related tests: CD4 count & percentage (% of total lymphocytes), CD8 count and percentage, CD4:CD8 ratio and the total lymphocyte count (CD45).

...Performance where it counts

In addition to CD4, a wide range of other antigens (CD3, CD45, CD38, CD8) can be measured. Contact Apogee for details.

The standard system offers side scatter (LS2) and two fluorescence colours (FL1, FL2). An optional third fluorescence detector (FL3) can be installed to allow detection of 3 antigens simultaneously.

Auto40 Features

Designed for lymphocyte phenotyping, primarily CD4 count and CD4% of total lymphocytes, but expression of many other antigens can be measured

Automatic cluster detection algorithm facilitates operation

Option to manually analyse difficult / damaged samples

Easy to use (one day operator training) & maintenance free

Auto40 Features (continued)

Rugged design developed for and proven in military environments and developing countries: Alignment free, self cleaning and self calibrating.

A single platform solution with full flow cytometer functionality

Full access to complete flow cytometry data sets

Easy sample preparation: Add blood, incubate, add buffer, analyse.

Absolute counting fluidic system eliminates the need for beads mixed into the samples, and thus avoids errors due to loss of counting beads (overestimate of cell count)

Built in LAN compatible PC running Windows XP

Reagents for CD4/CD8/CD45 and more…

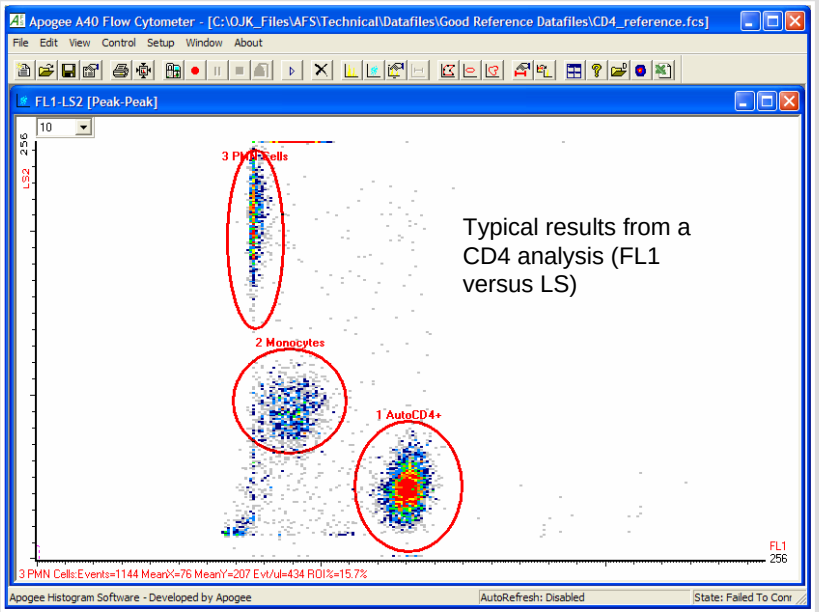
The Auto40 is a high precision flow cytometer with volumetric sample delivery system. The cost and stickiness problems of counting beads are avoided by the use of a precision syringe sampling system which delivers sample to the flow cell at a precisely controlled rate.

Flow cytometry based CD4 counting systems require fresh whole blood samples, antibody labelled (CD4 and CD45) and lysed.

Consult Apogee to discuss your best reagent options.

Software automatically verifies the data and displays the CD4 count. To verify the results the user has the histogram data to check at a glance that the Regions of Interest (ROIs) have been adjusted correctly.

1. Turn on machine
2. Run control sample of Apogee calibration beads
3. Prepare your samples
    a. Add 50µl of blood to the reagent tube
    b. Vortex
    c. Incubate in dark at room temperature 25 minutes
    d. Add 450µl of buffer
    e. Vortex
4. Choose the appropriate protocol from the File menu (e.g. 'CD4' or 'CD4%')
5. Run your samples
6. Save, print or export results to Excel
7. Run a final control sample of calibration beads then shut down

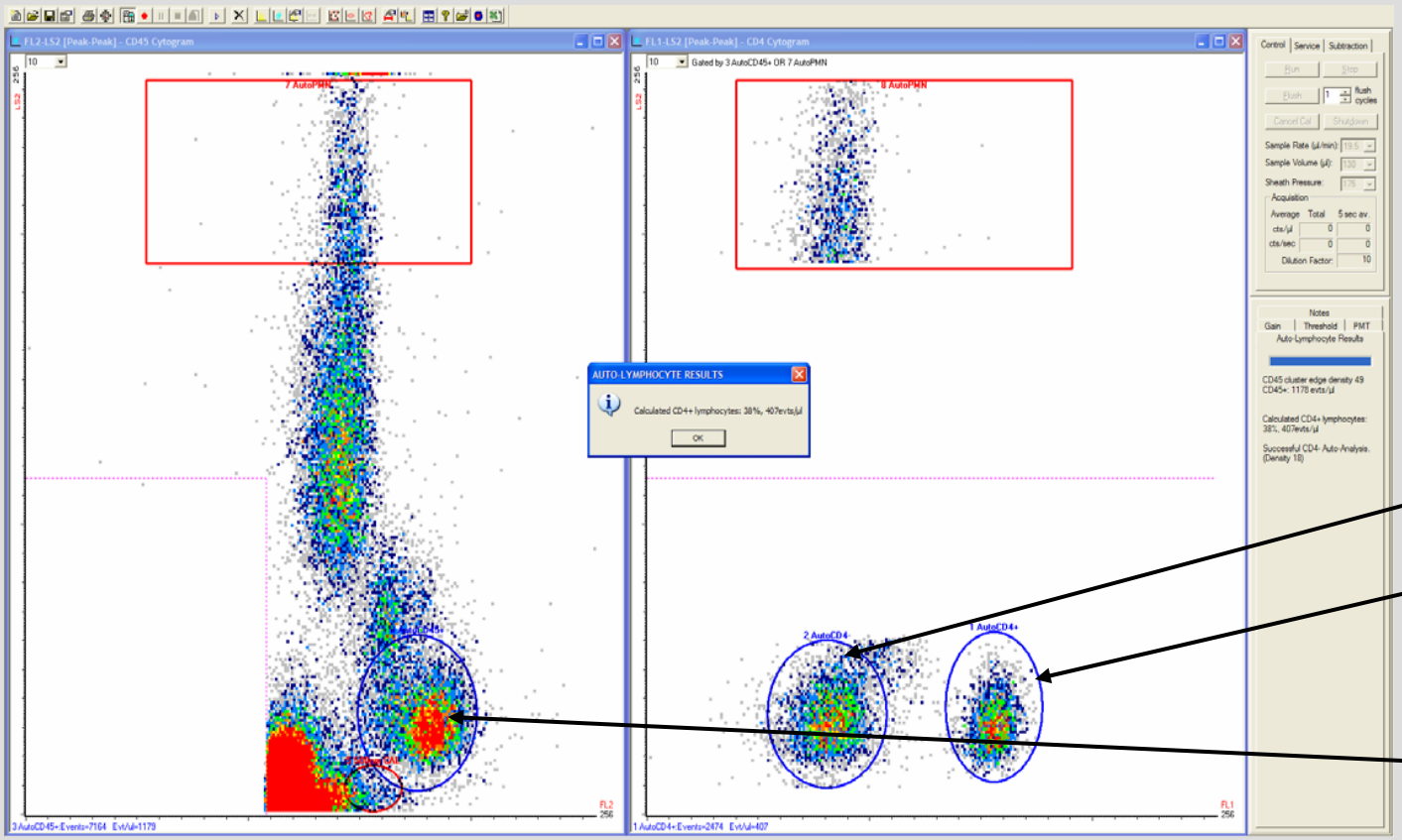


Typical results from a CD4 analysis (FL1 versus LS)

NOTES:

- Collect blood samples in EDTA according to standard procedures.
- In between each sample the machine automatically cleans itself and saves the data. (You can accept the automatic file name, or enter your own sample identifier.)
- The CD4 count is given immediately the sample finishes (typically 120 seconds, but samples with few CD4+ cells require a longer run time). Data can also be analysed at a later time because full histogram data are automatically stored for each sample on the machine's internal hard drive,

Typical Results: CD4% of total lymphocytes



Indications for Initiating Antiretroviral Therapy for the Chronically HIV-1 Infected Patient

(from http://www.aidsinfo.nih.gov/Guidelines)

Clinical Category	CD4+ Cell Count	Plasma HIV RNA	Recommendation
AIDS-defining illness or severe symptoms* (AI)	Any value	Any value	Treat
Asymptomatic (AI)**	CD4+ T cells < 200/mm^3	Any value	Treat
Asymptomatic (BII)	CD4+ T cells > 200/mm^3 but ≤ 350/mm3	Any value	Treatment should be offered following full discussion of pros and cons with each patient (See full text.)
Asymptomatic (CII)	CD4+ T cells > 350/mm^3	≥ 100,000	Most clinicians recommend deferring therapy, but some clinicians will treat. (See full text.)
Asymptomatic (DII)	CD4+ T cells > 350/mm^3	<100,000	Defer therapy

The optimal time to initiate therapy is unknown among persons with asymptomatic disease and CD4+ T cell count of >200 cells/mm^3. This table provides general guidance rather than absolute recommendations for an individual patient. All decisions regarding initiating therapy should be made on the basis of prognosis as determined by the CD4+ T cell count and level of plasma HIV RNA indicated in table 4, the potential benefits and risks of therapy, and the willingness of the patient to accept therapy.

*AIDS-defining illness per Centers for Disease Control, 1993. Severe symptoms include unexplained fever or diarrhea > 2-4 weeks, oral candidiasis, or >10% unexplained weight loss.

** Clinical benefit has been demonstrated in controlled trials only for patients with CD4+ T cells < 200/mm^3, however, the majority of clinicians would offer therapy at a CD4+ T cell threshold < 350/mm^3. A collaborative analysis of data from 13 cohort studies from Europe and North America found that lower CD4 count, higher HIV viral load, injection drug use, and age over 50 were all predictors of progression to AIDS or death in antiretroviral-naïve patients beginning combination antiretroviral therapy. These data indicate that the prognosis is better for patients who initiate therapy at > 200 cells/mm^3, but risk after initiation of therapy does not vary considerably at > 200 cells/mm^3.

For full details please refer to http://aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf

CD4 counts from pediatric samples require consideration under guidelines which differ from those for adult samples. The below table shows the 1994 Revised Human Immunodeficiency Virus Pediatric Classification System: Immune Categories Based on Age-Specific CD4+ T Cell Count and Percentage*.

(* Modified from: CDC. 1994 Revised classification system for human immunodeficiency virus infection in children less than 13 years of age. *MMWR,* 1994; 43 (No. RR-12): p. 1–10.)

	< 12 months		1-5 years		6-12 years	
Immune Category	**CD4+/ul**	**% of total lymphocytes**	**CD4+/ul**	**% of total lymphocytes**	**CD4+/ul**	**% of total lymphocytes**
Cat 1: No suppression	≥1500	≥25%	≥1000	≥25%	≥500	≥25%
Cat 2: Moderate suppression	750-1499	15%-24%	500-999	15%-24%	200-499	15%-24%
Cat 3: Severe suppression	<750	<15%	<500	<15%	<200	<15%

The AutoA40 system has been evaluated against competing systems in external studies by international reference laboratories. Reliability testing in several African countries in addition to military trials of the A40-Military model ("parent" model), have demonstrated the instrument's reliability.

Testing has included:

The Infectious Diseases Clinic, San Raffaele Hospital, Milano, Italy

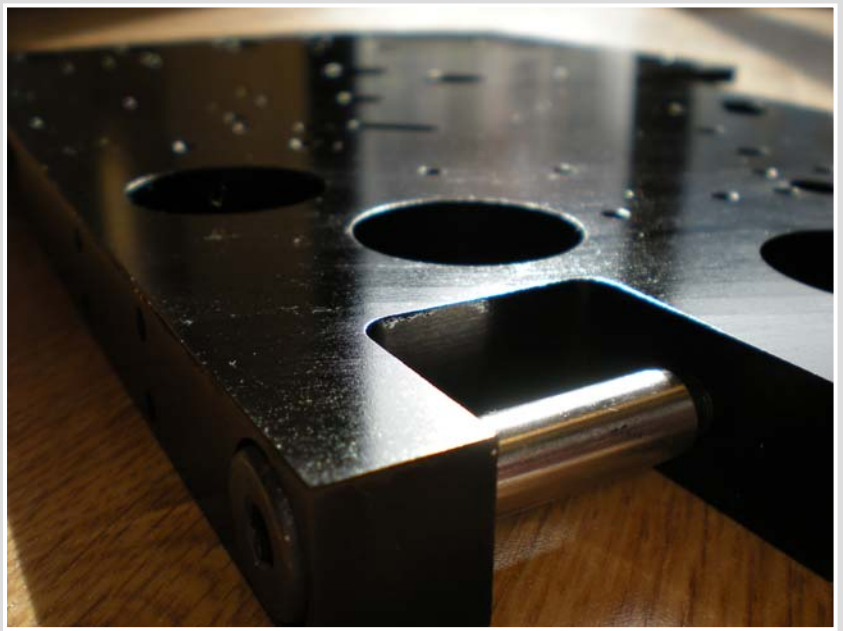
220 blood samples from HIV infected patients were run at the Laboratory of Diagnostic Immunology. Data was compared against results from the Beckman Coulter Epics XL + panLeuko double platform (4 colors). Both CD4 and CD8 antigens were measured and excellent correlation achieved across the whole measurement range (0-1800 CD4+/µl). No significant difference in the performance was observed between the different analyzed subsets (male, female, children).

Le Dantec Hospital (Laboratoire de Bactériologie - Virologie), Dakar, Senegal. In this study the AutoA40 was evaluated against the Becton Dickinson FACSCount.

160 blood samples (100 from HIV infected patients and 60 from normal blood donors) were evaluated for CD4 within 6 hours from blood collection. Samples from 84 females, 63 males and 13 children were analyzed.

Products manufactured under a Quality Management System based upon BS EN ISO 13485:2003 Medical devices.

The Auto40 system is UK registered as a CE-IVD product and complies with European regulations.



1. “A Simplified Flow Cytometry Method of CD4 and CD8 Cell Counting Based on Thermoresistant Reagents: Implications for Large Scale Monitoring of HIV-Infected Patients in Resource-Limited Settings”
Silvia Barbesti et al.
Cytometry Part B (Clinical Cytometry) 68B:43-51 (2005)

2. “Scaling up Antiretroviral Therapy in Resource-Limited Settings: Treatment Guidelines for a Public Health Approach”
WHO: http://www.who.int/3by5/publications/documents/arv_guidelines/en/

3. “Antiretroviral Therapy for HIV Infection in Adults and adolescents in Resource-Limited Settings: Towards Universal Access” 2006 revision
WHO: http://www.who.int/hiv/pub/guidelines/WHO%20Adult%20ART%20Guidelines.pdf

4. “BHIVA guidelines for the treatment of HIV-infected adults with antiretroviral therapy 2005”
British HIV Association: http://www.bhiva.org/guidelines/2005/BHIVA-guidelines/

5. “Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection” (November 3, 2005)
http://www.aidsinfo.nih.gov/Guidelines

...Performance where it counts

Installation Requirements

Size	Analyzer (W32 x H48 x D48cm) plus monitor, keyboard & mouse
Weight	Approx. 25kg (flow cytometer only)
Power	100-240VAC, 50/60Hz, 550W
	(UPS with battery backup included)
Operating conditions	5-35ºC room temperature, <90% humidity

Optics

Laser	Choice between 488nm or 532nm solid state lasers
Filters	Factory set. No operator alignment.
Scatter	Large angle, high sensitivity scatter optics
	Sensitivity 300nm, resolution 40nm (latex spheres)
Fluorescence	Up to 3 colors (channels)
Optical Detectors	Photomultiplier tubes with gain automatically set during Auto-Calibration

Fluidics

Sample	Volumetric sample injection using high precision syringe
	Adjustable sample aspiration volume, 130-300ul
	High frequency real time absolute count measurement.
	Selectable sample flow rate from 1.4 to 100ul/min
Sheath	Purified water sheath system. Use your own laboratory water
	Refillable sheath tank with level sensor
	Optional closed loop sheath fluid system
Sample concentration	Sample concentrations up to 10^9 per ml

Data Management

Hardware	Internal PC running Windows XP
File transfer & Printing	USB and LAN connections
Signal Processing	Data collection at up to 20,000 events per sec. Thresholds on any channel combination with ‘AND’ or ‘OR’ logic Pulse height and pulse area measurements on all channels Event time stamp for each particle 16 bit data acquisition (4.8 logarithmic decades) Histogram resolution software selectable from 32 to 65535 Display modes: linear and/or logarithmic gain selection for each datagram
Graphical User Interface	Apogee Histogram Software User configurable printed reports (via USB or LAN ports) Optional automatic file storage User configurable automated sample cycle (“Autocycle”) Export of statistics to Microsoft Excel Export of histogram data in .csv format Quick copy to clipboard feature for histograms Elliptical ROI cluster analysis for automated CD4 & CD4% Unlimited number of Histograms and cytograms Up to 10 amorphous gates on each histogram, 50 in total Gating using AND, OR and NOT logic RATIO parameter configurable for any 2 detectors FCS 2.0 compatible files Gain and subtraction settings adjustable during and after acquisition …plus much more!

Sensitivity & Accuracy

CD4+ Lymphocytes	From five CD4+ lymphocytes per micro-litre of blood (upper limit > 5000/µl)
	Precision: Coefficient of variation less than 10% for samples with > 200 CD4+/µl
	Coefficient of variation less than 20% for samples in range 100 - 200 CD4+/µl.
	Trueness: +/-10% for samples with > 200 CD4+/µl
	+/-40 CD4+/µl for samples with < 200 CD4+/µl

Speed

Max samples per hour	20 samples per hour
Min Analysis Time	90 seconds
Time between samples	90 seconds

Maintenance

Frequency	12 month service interval recommended

Consumables

Calibration beads	Apogee Cat # 1444 for Auto40-green (Brightness and concentration calibrated for automatic daily calibration)
Reagents	Consult Apogee to discuss your best options