

## Auto40

A robust flow cytometer from  
Apogee Flow Systems Ltd



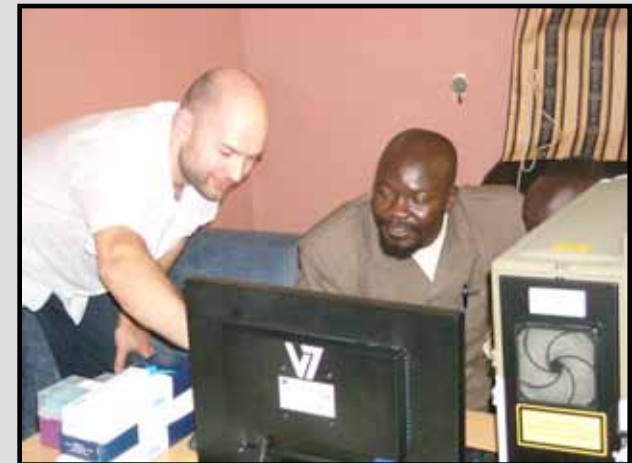
- o With tens of millions of HIV infected people across the world, there is an urgent need for high quality, reliable and easy to use CD4 counters.
- o CD4+ T-Lymphocytes are the primary target of HIV, and as such are depleted in number as the disease progresses. Flow cytometry is the standard method for monitoring patients' "CD4 count" and thereby determining the course of treatment.
- o The Auto40 is a 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).
- o The instrument can be configured for many different applications. Options are available for monitoring cells expressing other antigens.  
In addition to the side scatter detector, up to 3 fluorescence detectors can be installed, allowing for future changes in your testing requirements.



- o Designed for monitoring patients' lymphocyte subpopulations, e.g.:
  - CD4 and CD8 absolute count
  - CD4:CD8 ratio
  - CD4 & CD8 % of total lymphocytes (CD45).
- o Automatic cluster detection algorithm (automated elliptical gating) to minimise operator training and variability
- o Option to manually analyse difficult / damaged samples
- o Easy to use & maintenance free
- o Configured to meet your requirements:
  - ✓ choice of up to 3 fluorescence &
  - ✓ 1 light scatter detector
  - ✓ choice of laser (blue or green) to match your budget and needs.



- o Rugged design developed for and proven in military environments
- o Alignment free, self cleaning and self calibrating.
- o A single platform solution with full flow cytometer functionality
- o Full access to complete flow cytometry data sets (cytogram data)
- o Easy sample preparation: Add blood, incubate, add buffer then run!
- o Absolute counting fluidic system (no need to add calibration beads)
- o Built in LAN compatible PC running Windows XP
- o Remote technical support & instrument operation available from Apogee via the internet



Apogee offers a choice of reagents:



### **CD4 or CD8 Thermostable Reagents**

Reagents for either CD4 (Cat#1430) or CD8 (Cat#1441) with reagent pre-dispensed into test tubes for maximum convenience. Three year shelf life (refrigerated) and stable for 4 weeks at 45 degrees Centigrade!

**Stabilized Reagent:** CD4 absolute count reagent or CD4-CD45 dual reagent for CD4%. Shelf-life 12 months at room temperature – no need to refrigerate!

### **CD4, CD8, CD45 (liquid form)**

Low cost reagents: CD4-FITC (Cat#1438), CD8-FITC (Cat#1431) and CD45-RPE (Cat#1435)

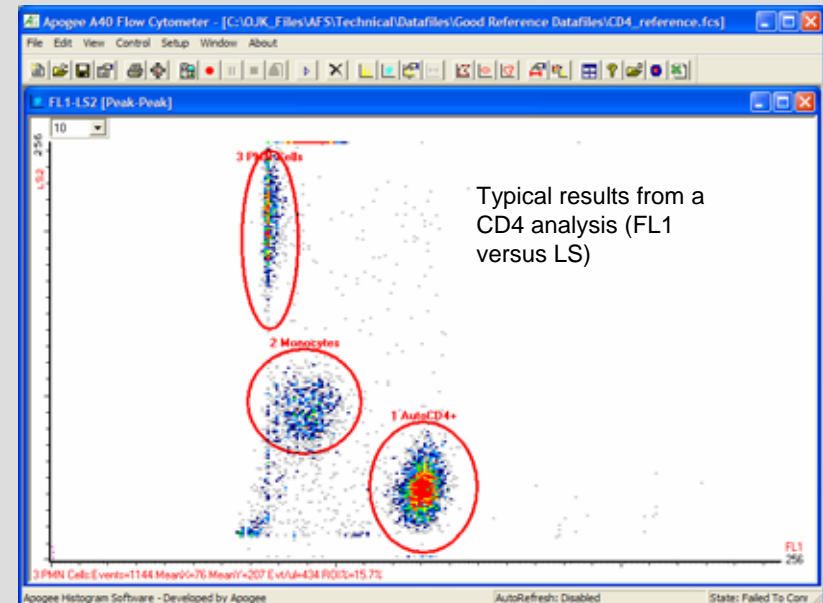
The CD45 allows the total lymphocytes to be counted by a second fluorescence detector (optional Cat#9901). When used simultaneously with, for example, a CD4 reagent, the percentage of total lymphocytes is given (CD4%). This is necessary for pediatric samples.

Your choice of laser and fluorescence detectors will determine reagent compatibility. Consult Apogee for further advice.

# Operating Procedure

It is desirable, but not essential, for the operator to understand the histogram graph. The software automatically verifies the data and displays the CD4 count. However, most users like to see the full histogram data as shown here.

1. Turn on the machine
2. Run a 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 15 minutes
  - d. Add 450µl of buffer
  - e. Vortex
4. Choose the appropriate protocol from the File menu (e.g. 'CD4' or 'CD4% of Lymphocytes')
5. Run your samples, allowing the Auto-Lymphocyte software to analyse each data set, display the result and automatically save the data.
6. If you want, click the 'Excel Export' button to add the data to a spreadsheet report.



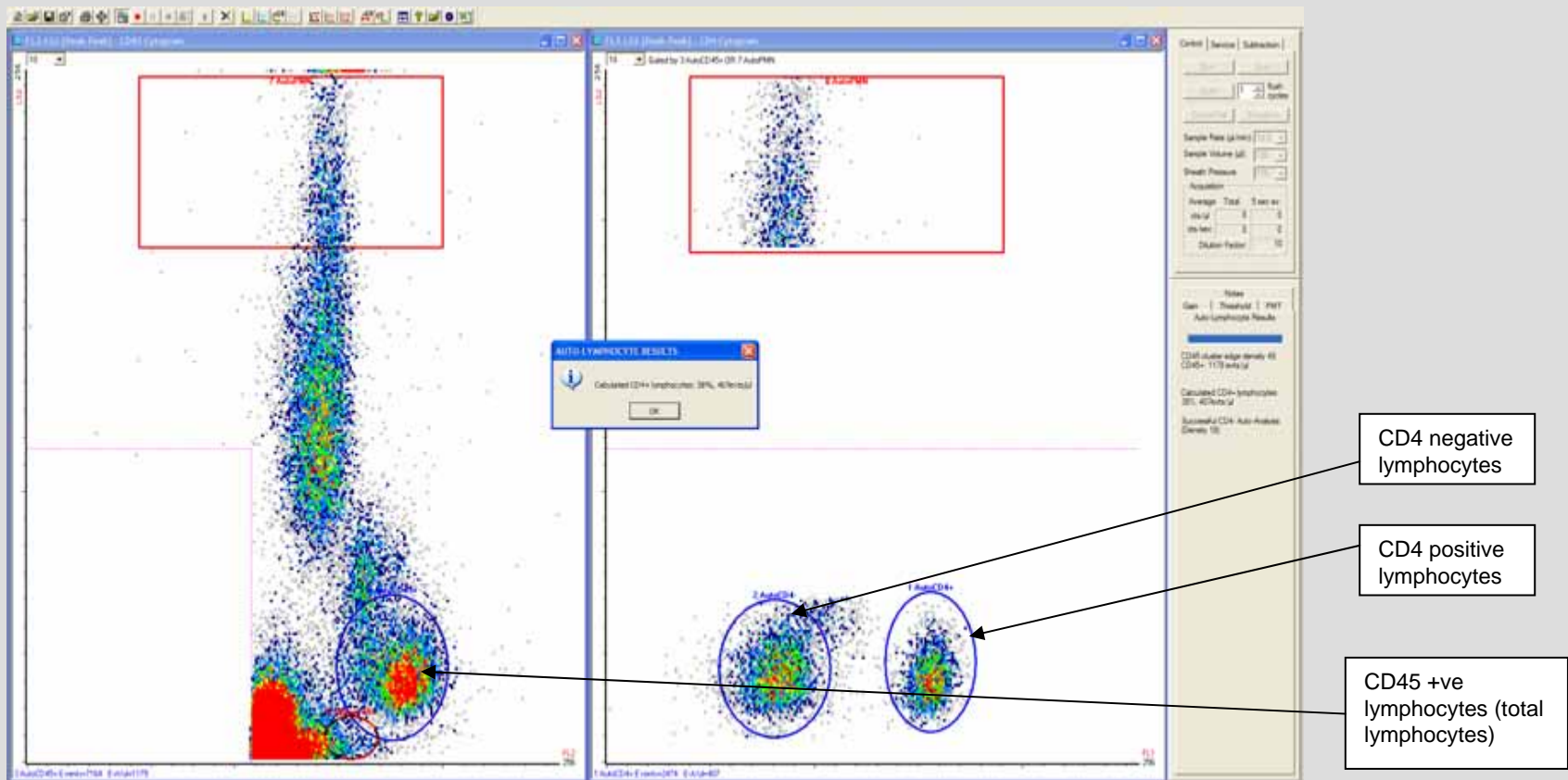
## Notes:

- 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 100 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,



# Results

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
<b>AIDS-defining illness or severe symptoms* (AI)</b>	Any value	Any value	Treat
<b>Asymptomatic** (AI)</b>	CD4+ T cells < 200/mm <sup>3</sup>	Any value	Treat
<b>Asymptomatic (BII)</b>	CD4+ T cells > 200/mm <sup>3</sup> but ≤ 350/mm <sup>3</sup>	Any value	Treatment should be offered following full discussion of pros and cons with each patient (See full text.)
<b>Asymptomatic (CII)</b>	CD4+ T cells > 350/mm <sup>3</sup>	≥ 100,000	Most clinicians recommend deferring therapy, but some clinicians will treat. (See full text.)
<b>Asymptomatic (DII)</b>	CD4+ T cells > 350/mm <sup>3</sup>	<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<sup>3</sup>. 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<sup>3</sup>, however, the majority of clinicians would offer therapy at a CD4+ T cell threshold < 350/mm<sup>3</sup>. 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<sup>3</sup>, but risk after initiation of therapy does not vary considerably at > 200 cells/mm<sup>3</sup>.

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.)

Immune Category	< 12 months		1-5 years		6-12 years	
	CD4+/ul	% of total lymphocytes	CD4+/ul	% of total lymphocytes	CD4+/ul	% of total lymphocytes
<b>Cat 1: No suppression</b>	≥1500	≥25%	≥1000	≥25%	≥500	≥25%
<b>Cat 2: Moderate suppression</b>	750-1499	15%-24%	500-999	15%-24%	200-499	15%-24%
<b>Cat 3: Severe suppression</b>	<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-MiniFCM ("parent" model), have demonstrated the instrument's reliability.

Testing has included:

- o 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+/ $\mu$ l). No significant difference in the performance was observed between the different analyzed subsets (male, female, children).

- o Another important study was performed at the Le Dantec Hospital (Laboratoire de Bactériologie - Virologie) in Dakar, Senegal. In this study the AutoA40 was evaluated against the Becton Dickinson FACSCCount.

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.



- o Apogee operates under a Quality Management System based upon BS EN ISO 13485:2003 Medical devices.
- o The AutoCD4 system is UK registered as a CE-IVD product and complies with European requirements.



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/](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>