Manual Gating of Lymphocytes using Beckman Coulter AQUIOS Tetra Software

Prepared by Sylvester Hood for Immunology Quality Assessment

IQA Program Manager: Raul Louzao

In this presentation, you will learn how to adjust:

- FSC/SSC gating to exclude debris
- CD45/SSC gating
- EV/SSC gating to supplement CD45/SSC gating
- CD3 histograms
- CD4/CD8 gating

We will be using Beckman Coulter AQUIOS Tetra Software, but the same principles may be applied to other flow cytometry gating programs and platforms.

AQUIOS Review Screen

1. Instrument Designation
2. Auto Refresh
3. Completed Run List
4. Sample Identifiers and Run Information Area
5. Result Table Area
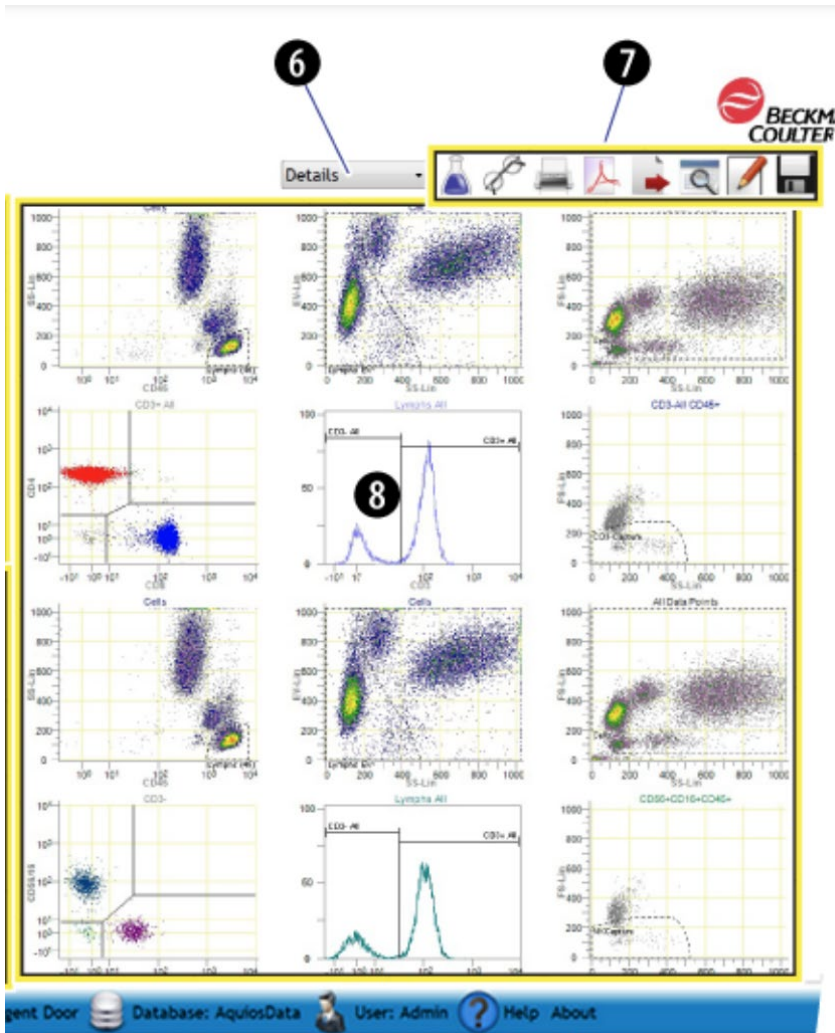
6. Graphic Options Selection (drop-down box)

7. Action Toolbar

8. Selected Graphic Display
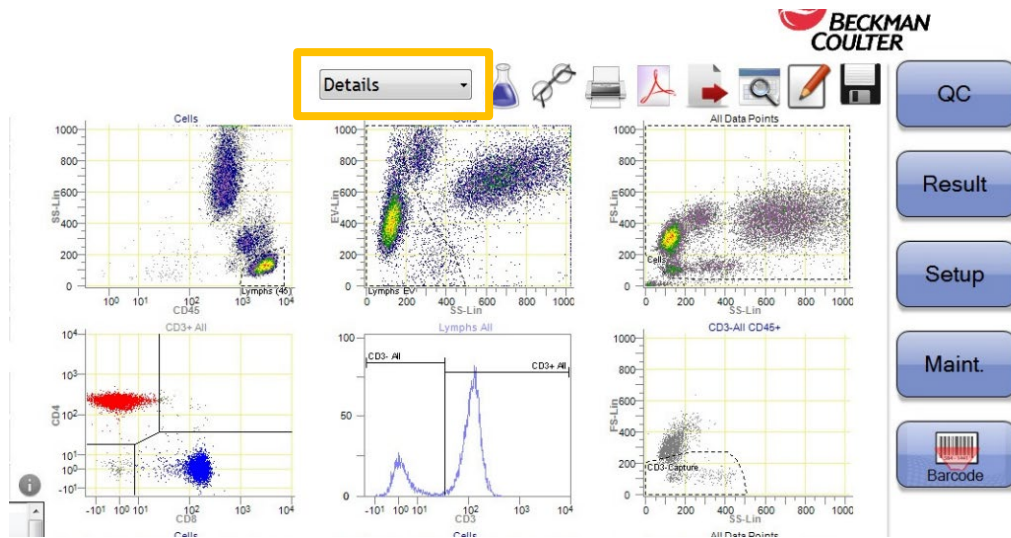
Graphic options are accessed by selecting the desired view from a drop-down menu.

The graphic display on the Review screen can be customized by selecting one of four predefined graph combinations:
- Histograms
- DotPlots
- Details
- Results

To edit select:

1. Review
2. Details
3. Pencil Icon

Then, double-click on the plot that you want to edit to enlarge it before making changes.
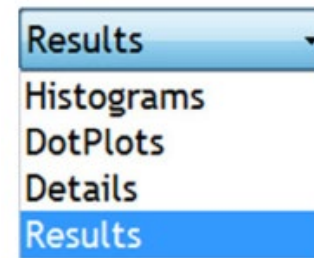
Double-click again to return to standard view.

Select the Green Check to refresh region statistics.

Editable Plots for a CD45/CD3/CD4/CD8 Test
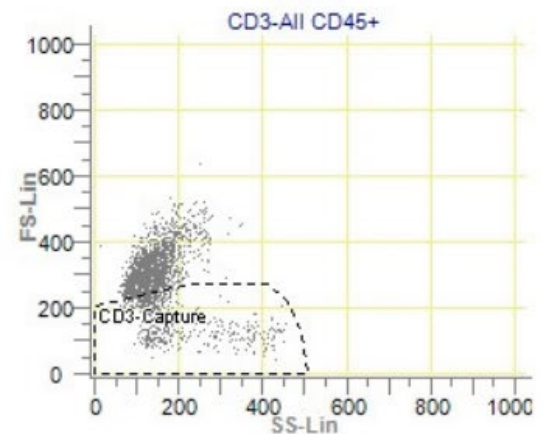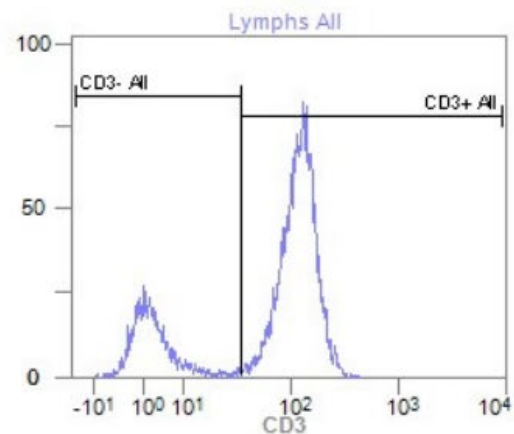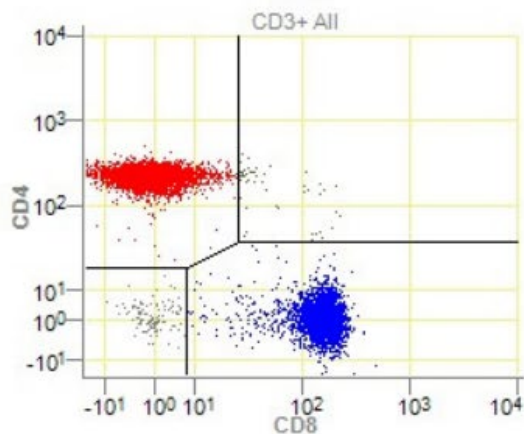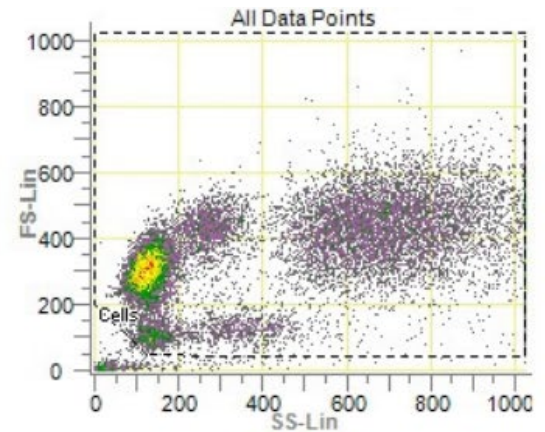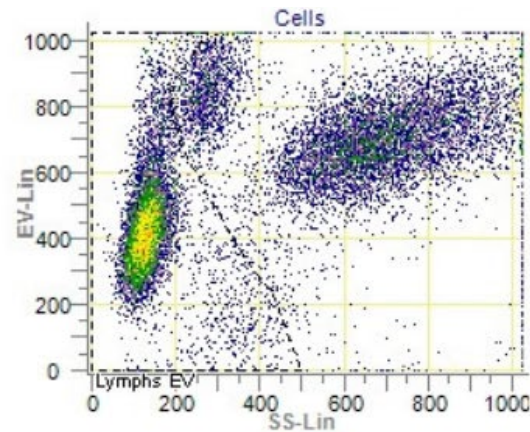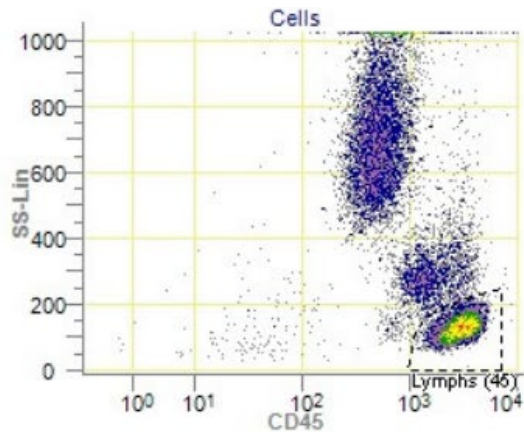
FSC vs SSC Plots

Unacceptable
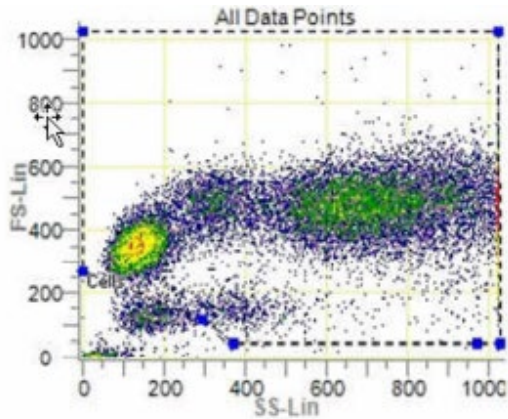
Please note that while they look similar, the FSC/SSC plot and the EV/SSC plot serve different functions in the analysis, so it is important to not confuse the two.

FSC/SSC Plot: Gated to include all Granulocytes, Monocytes, Lymphocytes, and Fallen Lymphocytes, Debris is excluded.

EV/SSC Plot: Gated to include Lymphocytes and Fallen Lymphocytes. This plot Supplements the CD45/SSC plot.

2. Granulocytes   3. Monocytes   5. Lymphocytes   6. Fallen Lymphocytes

Corrected
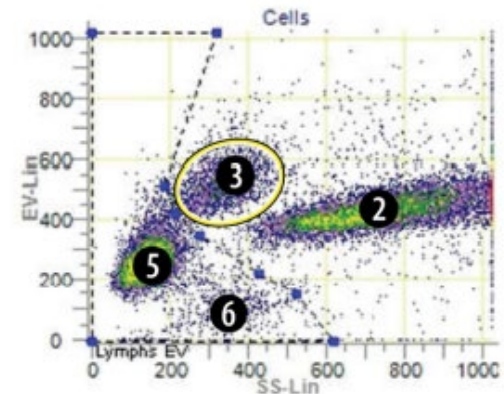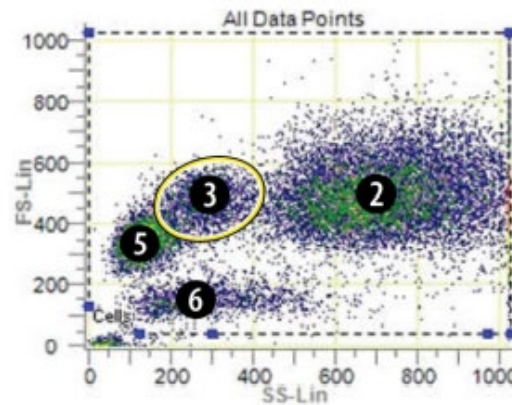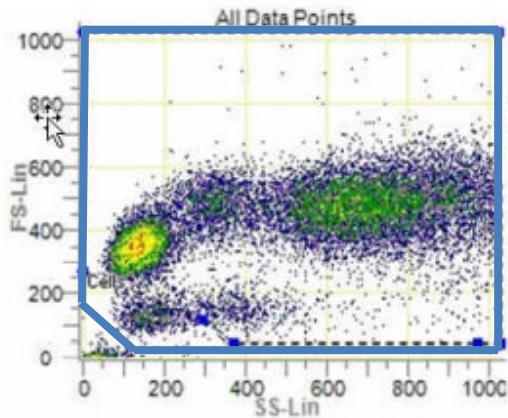
CD45/SSC and EV*/SSC Plots

Acceptable | **Unacceptable**

1. Debris / unlysed RBCs
2. Granulocytes
3. Monocytes
4. Basophils
5. Lymphocytes
6. Fallen Lymphocytes
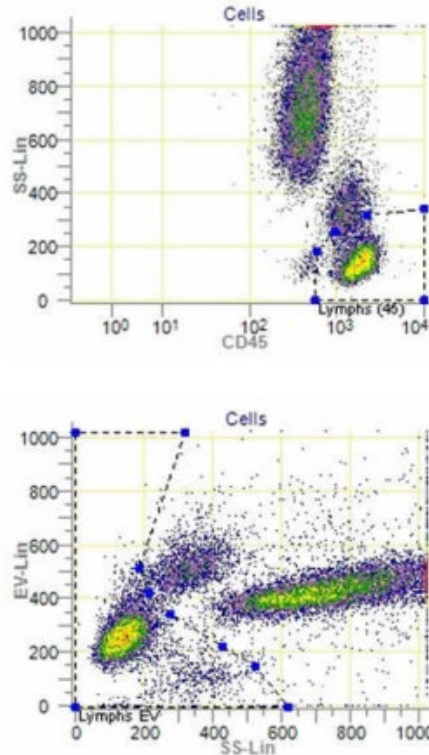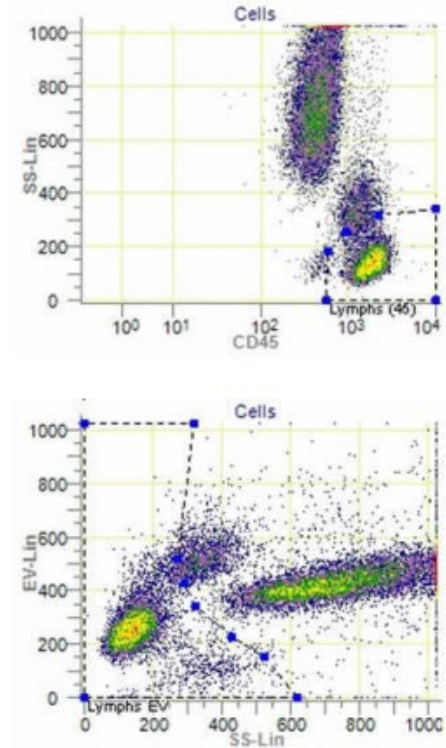
*EV stands for Extracellular Vesicle.

Monocyte Crashing in the CD45+/SSC Gate

As samples degrade, the monocytes crash into the lymphocyte population on the CD45 plots. Instead of presenting as a separate population, it ends up looking like a dimple on the upper left hand side of the lymphocyte population.
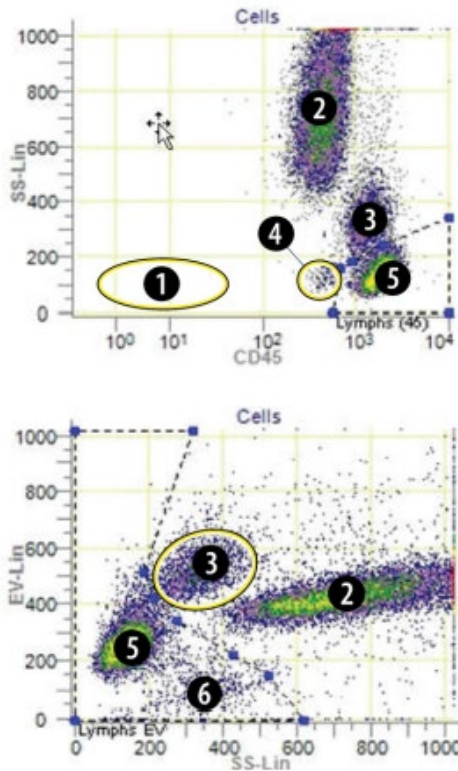
Normal Presentation   Degraded Sample

CD3 Histogram and CD4/CD8 Plot

Acceptable

Acceptable

Unacceptable

Unacceptable

Tips for UKNEQAS Samples:
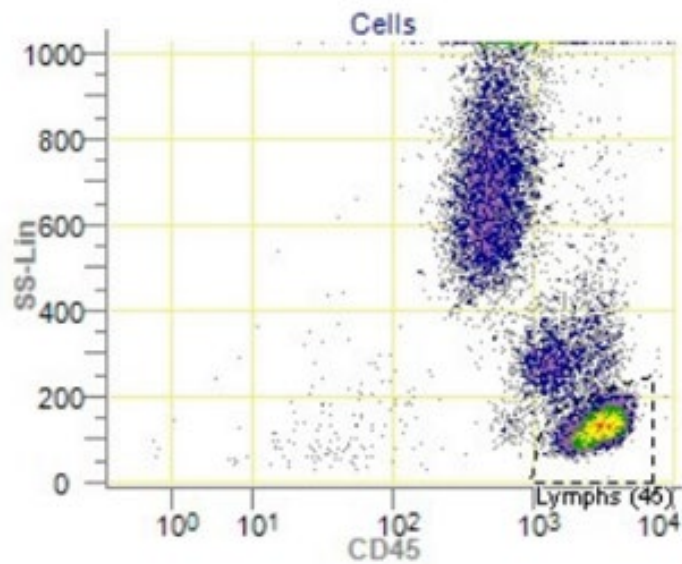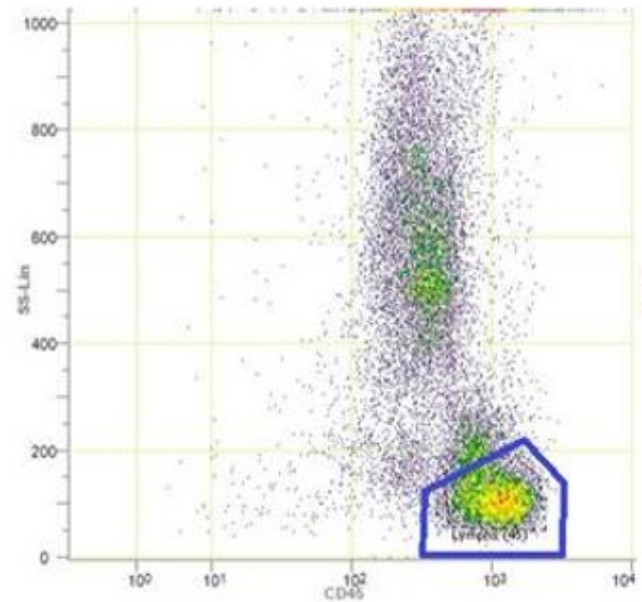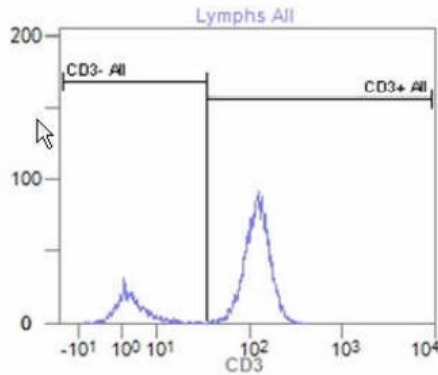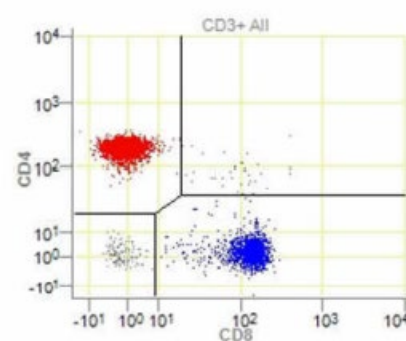
1. There will be more CD3 dim samples in your staining, so place your CD3- cut off a bit further to the left than for a fresh sample.
2. Both the CD4+ and CD8+ populations may also contain some dimly stained cells that trail behind (for CD8) or below (for CD4) the main population. Do not exclude these.
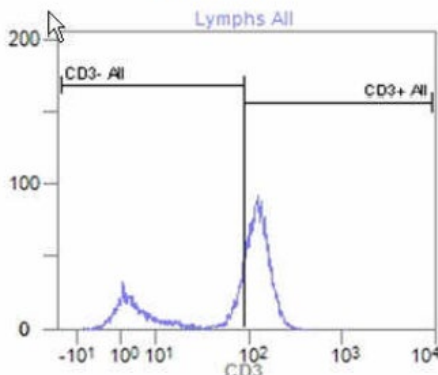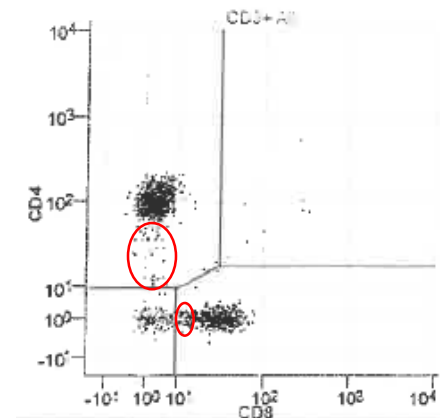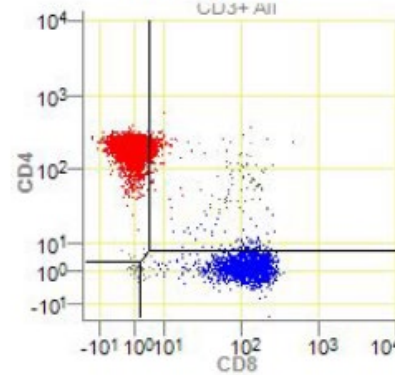
Reference: AQUIOS Tetra Software System Guide, PN B26364AB April 2015, Beckman Coulter

The staff at the IQA Center is available for technical consultation concerning laboratory techniques for these procedures. You may contact Raul Louzao at 919-684-5861 raul.louzao@duke.edu or Sylvester Hood at 919-613-4469 sfh7@duke.edu