

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

Requests (0) Running (0) Incomplete (0) Review (24)



Instrument: All

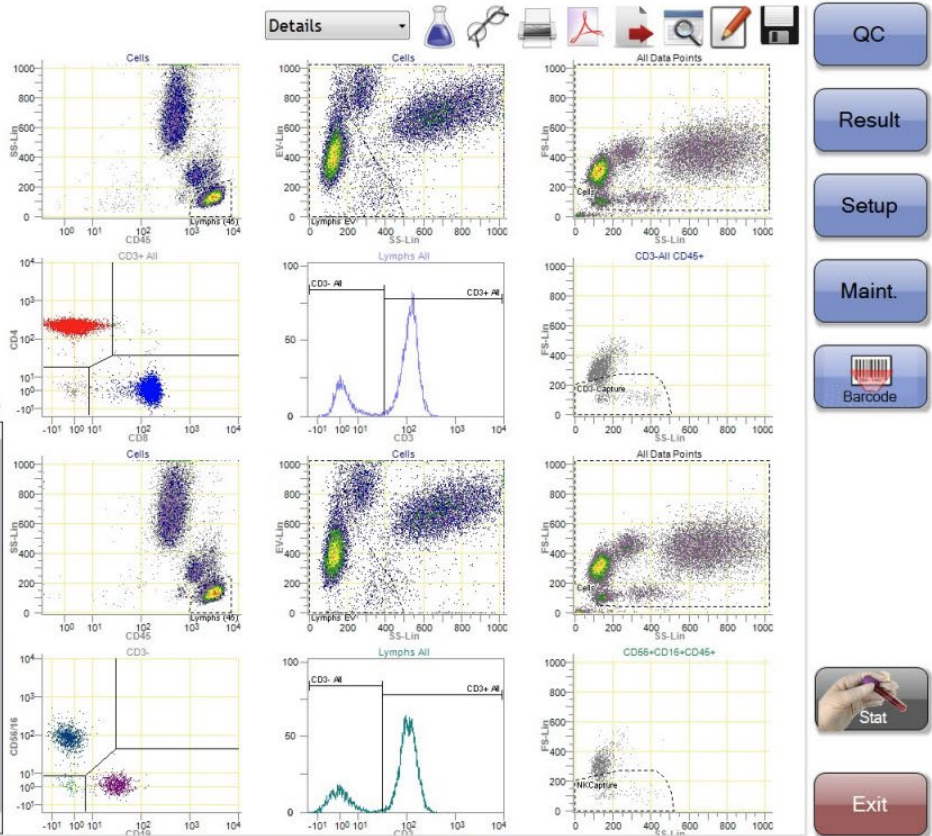
Auto Refresh

Sample ID	Patient Name	Test Name
89350141140	42226	Tetra 2+
89350141143	148217	Tetra 2+
89350141141	42226	Tetra 1
89350141144	148217	Tetra 1
89350141142	42226	Tetra Combo
89350141145	148217	Tetra Combo
6180004	AQUIOS IMMUN...	Tetra Combo
6170003	AQUIOS IMMUN...	Tetra Combo
89347465210	148217	Tetra 2+
89347465205	42226	Tetra 2+
89347465209	148217	Tetra 1
89347465206	42226	Tetra 1
89347465208	148217	Tetra Combo
89347465207	42226	Tetra Combo

Sample ID: 89350141145 Test: Tetra Combo
 Run Date: 02/03/2015 12:49 PM Collect Date: 02/03/2015
 Specimen Type: Whole Blood Inst. SN: AV039002
 Patient Name: 148217
 Patient ID: Gov. ID:
 Status: Location:
 Gender: Select Date of Birth: / /

Physician Name:
 Physician Code:
 Run Flags:
 Run Notifications:
 Comments:

Result	Value	Flag	Normal Range	Action Range
Average CD3+ (T Cells) Percent	78.02%			
Average CD3+ (T Cells) Count/uL	1.664			
CD3+/CD4+ (Helper T Cells) Percent	44.81%			
CD3+/CD4+ (Helper T Cells) Count/uL	989			
CD3+/CD8+ (Suppressor T Cells) Percent	32.58%			
CD3+/CD8+ (Suppressor T Cells) Count/uL	719			
CD3-/CD19+ (B Cells) Percent	9.04%			
CD3-/CD19+ (B Cells) Count/uL	186			
CD3-/CD56+CD16+ (NK Cells) Percent	11.99%			
CD3-/CD56+CD16+ (NK Cells) Count/uL	247			
CD4/CD8 Ratio	1.38			
*Total Lymphocytes (T+B+NK) Percent	99.15%			
*CD3+ Reliability Check	0.58%			
*CD3+ Intrapanel Check	-0.22%			
CD45+ Low SS Count/uL	2.208			
CD45+ Low SS Percent	49.51%			
CD45+ Count/uL	4.459			
CD3+ (T Cells) Percent Tetra 1	77.91%			
CD3+ (T Cells) Count/uL Tetra 1	1.720			
CD3+ (T Cells) Percent Tetra 2	78.13%			
CD3+ (T Cells) Count/uL Tetra 2	1.697			



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 Barcode
 Stat
 Exit

1 Requests (0) 2 Running (2) Incomplete (0) Review (22)

Instrument: All Auto Refresh

Sample ID	Test Name	Test Code
89350141141	42226	Tetra 1
89350141144	148217	Tetra 1
89350141142	42226	Tetra Combo
89350141145	148217	Tetra Combo
6180004	AGUIOS IMMUN...	Tetra Combo
6170003	AGUIOS IMMUN...	Tetra Combo
89347492318	148217	Tetra 2+
89347492305	42226	Tetra 2+
89347492308	148217	Tetra 1
89347492306	42226	Tetra 1
89347492308	42226	Tetra Combo
89347492307	42226	Tetra Combo
6180004	AGUIOS IMMUN...	Tetra Combo
6170003	AGUIOS IMMUN...	Tetra Combo
6180004	AGUIOS IMMUN...	Tetra Combo
6170003	AGUIOS IMMUN...	Tetra Combo
6180004	AGUIOS IMMUN...	Tetra Combo
6170003	AGUIOS IMMUN...	Tetra Combo
6180004	AGUIOS IMMUN...	Tetra Combo
6170003	AGUIOS IMMUN...	Tetra Combo

Sample ID: 89350141145 Test: Tetra Combo

Run Date: 02/03/2015 12:49 PM Collect Date: 02/03/2015

Specimen Type: Whole Blood Inst. SN: AV039002

Patient Name: 148217

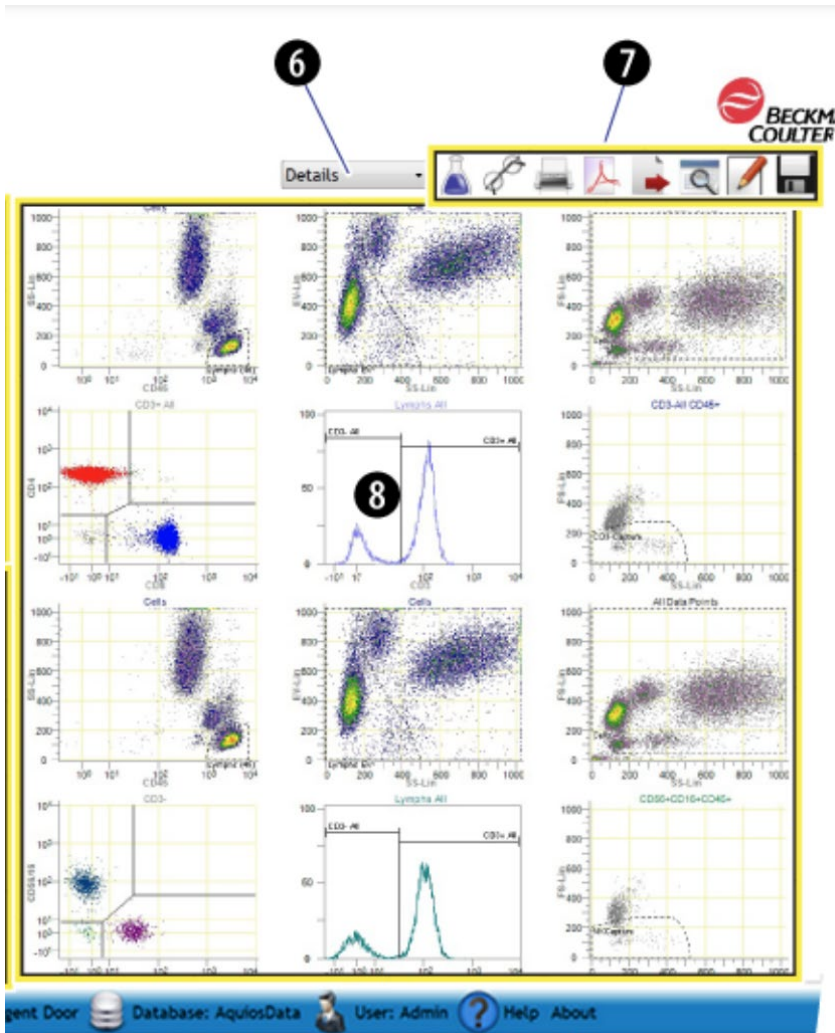
Patient ID: Gov. ID: Location: Date of Birth: / /

Physician Name: Physician Code: Gender: Select Date of Birth: / /

Run Flags: Run Notifications: Comments:

Result	Value	Flag	Normal Range	Action Range
Average CD3+ (T Cells) Percent	78.02%			
Average CD3+ (T Cells) Count/uL	1,064			
CD3+CD4+ (Helper T Cells) Percent	44.81%			
CD3+CD4+ (Helper T Cells) Count/uL	389			
CD3+CD8+ (Suppressor T Cells) Percent	32.58%			
CD3+CD8+ (Suppressor T Cells) Count/uL	719			
CD3+CD19+ (B Cells) Percent	9.04%			
CD3+CD19+ (B Cells) Count/uL	186			
CD3+CD56+CD16+ (NK Cells) Percent	11.98%			
CD3+CD56+CD16+ (NK Cells) Count/uL	247			
CD4/CD8 Ratio	1.38			
Total Lymphocytes (T+B+NK) Percent	99.15%			
CD3+ Reliability Check	6.58%			
CD3+ Integrity Check	-0.22%			
CD45+ Low SS Count/uL	2,298			
CD45+ Low SS Percent	49.51%			
CD45+ Count/uL	4,459			
CD3+ (T Cells) Percent Tetra 1	77.91%			
CD3+ (T Cells) Count/uL Tetra 1	1,720			
CD3+ (T Cells) Percent Tetra 2	78.12%			
CD3+ (T Cells) Count/uL Tetra 2	1,687			

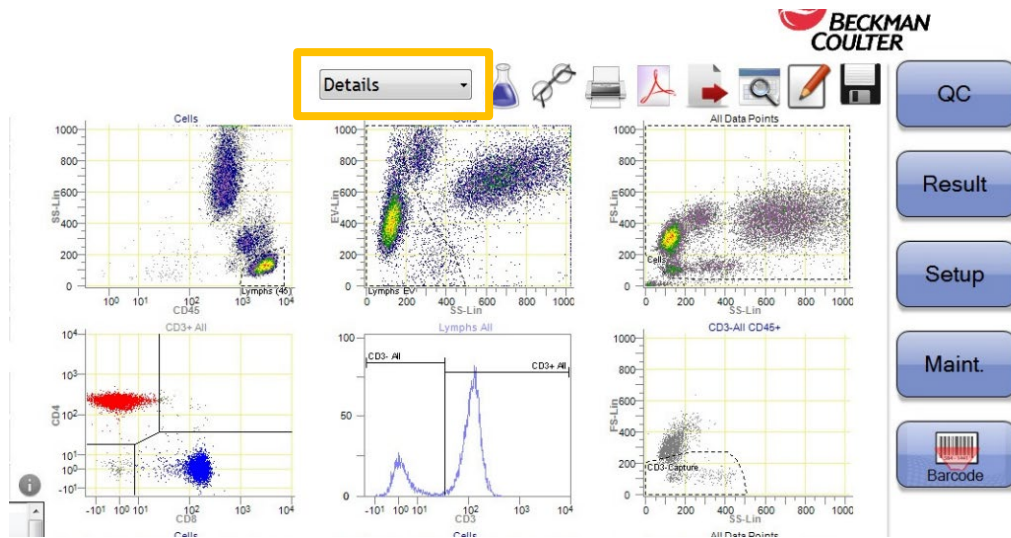
1. Instrument Designation
2. Auto Refresh
3. Completed Run List
4. Sample Identifiers and Run Information Area
5. Result Table Area



6. Graphic Options Selection (drop-down box)

7. Action Toolbar

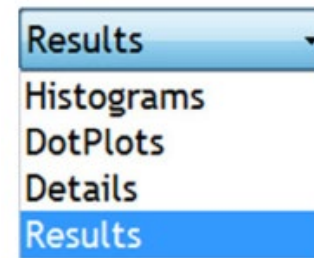
8. Selected Graphic Display



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



1

Requests (0) Running (0) Incomplete (0) **Review (24)**

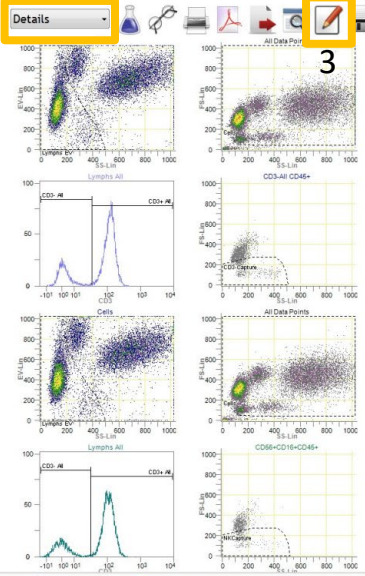
Instrument: All Auto Refresh

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60347460209	148217	Tetra 2+
60347460208	42226	Tetra 2+
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Average CD3+ (T Cells) Percent	78.02%			
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CD45+ Low SS Count/uL	2,289			
CD45+ Low SS Percent	49.51%			
CD45+ Count/uL	4,459			
CD3+ (T Cells) Percent Tetra 1	77.91%			
CD3+ (T Cells) Count/uL Tetra 1	1,720			
CD3+ (T Cells) Percent Tetra 2	78.13%			
CD3+ (T Cells) Count/uL Tetra 2	1,607			

2



QC
Result
Setup
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Barcode
Stat
Exit

To edit select:

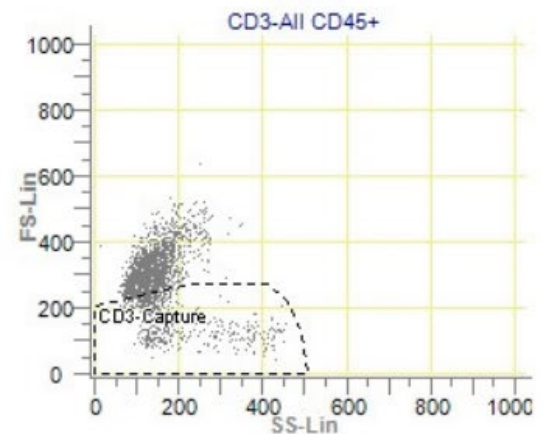
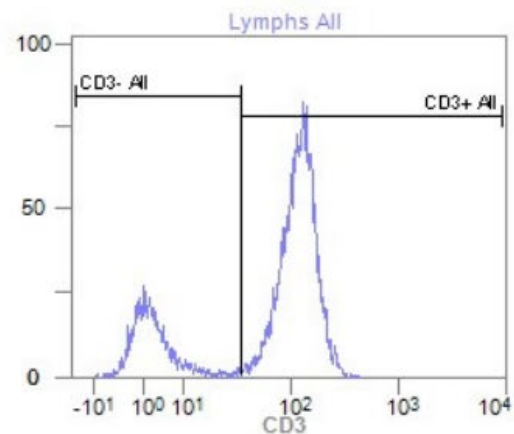
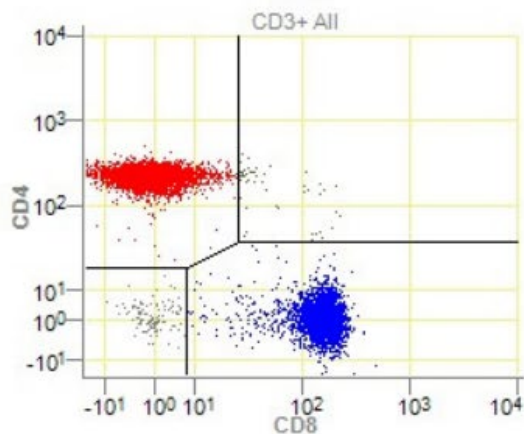
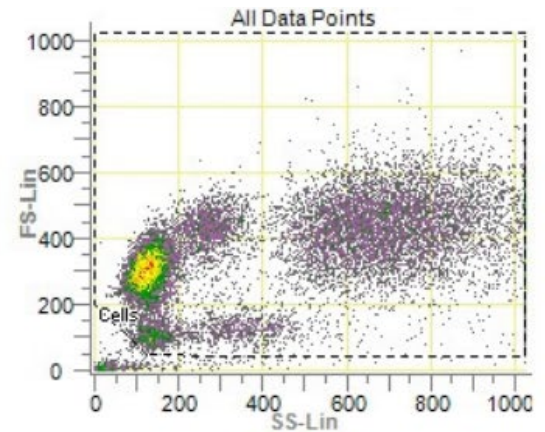
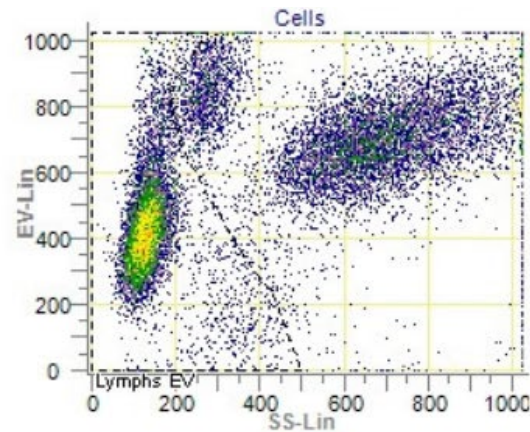
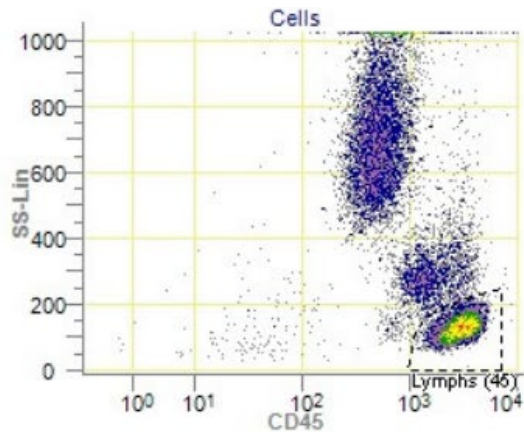
1. Review
2. Details
3. Pencil Icon

Then, double-click on the plot that you want to edit to enlarge it before making changes.

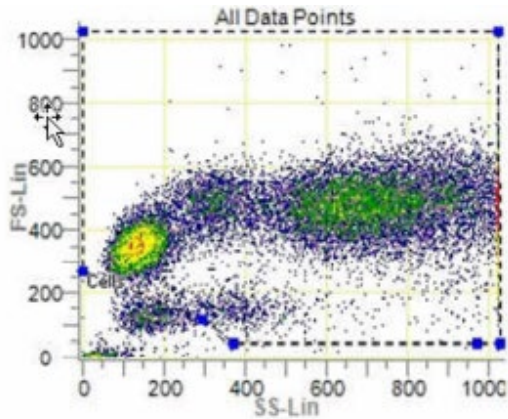
Double-click again to return to standard view.

Select the Green Check to refresh region statistics.

Editable Plots for a CD45/CD3/CD4/CD8 Test



Unacceptable



FSC vs SSC Plots

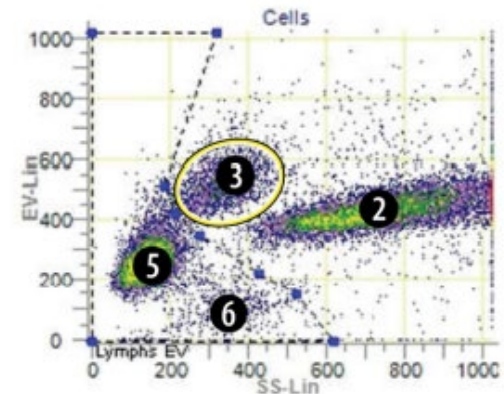
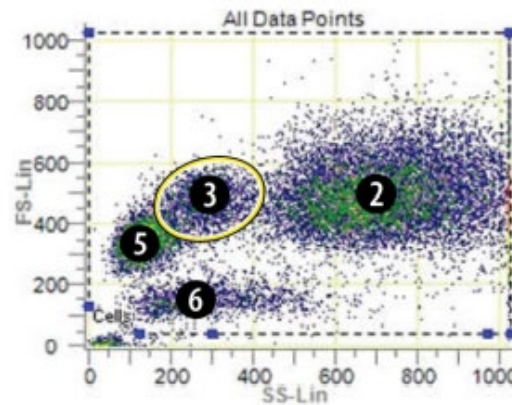
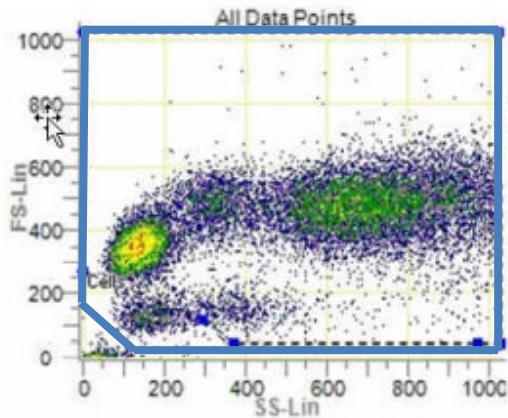
Please note that while they look similar, the FSC/SSC plot and the EV/SSC plot serve different functions in the analysis, so it is important to not confuse the two.

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

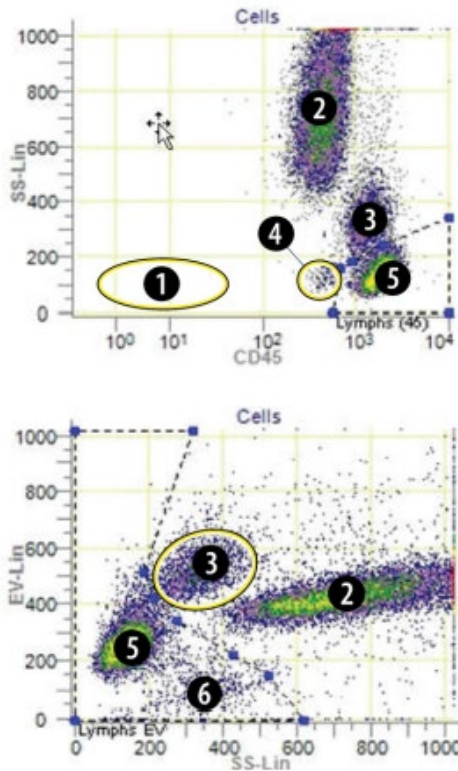
EV/SSC Plot: Gated to include Lymphocytes and Fallen Lymphocytes. This plot Supplements the CD45/SSC plot.

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

Corrected

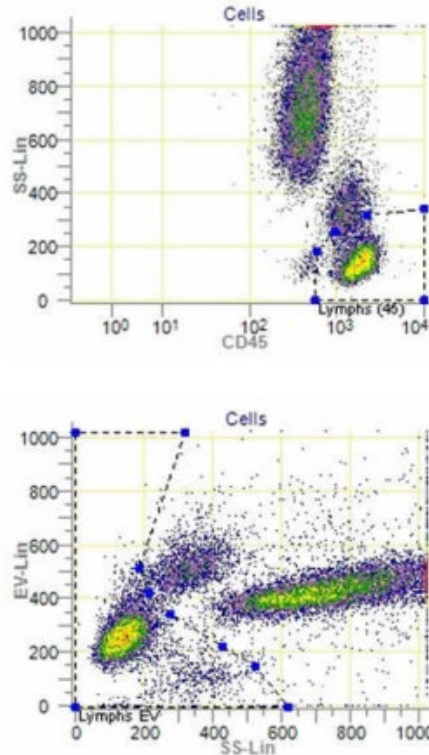


CD45/SSC and EV*/SSC Plots

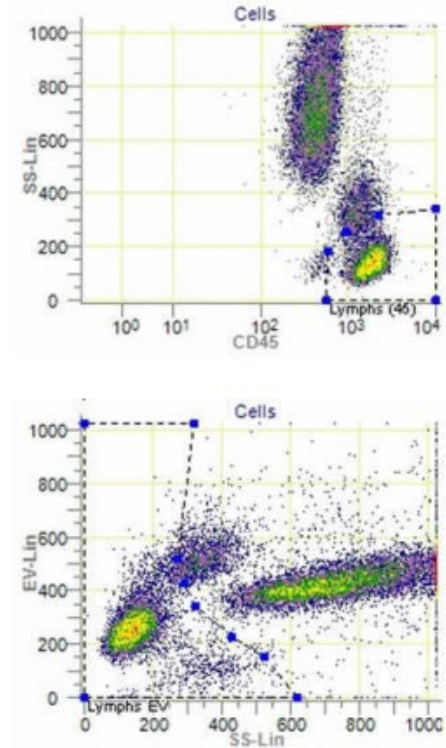


1. Debris / unlysed RBCs
2. Granulocytes
3. Monocytes
4. Basophils
5. Lymphocytes
6. Fallen Lymphocytes

Acceptable



Unacceptable

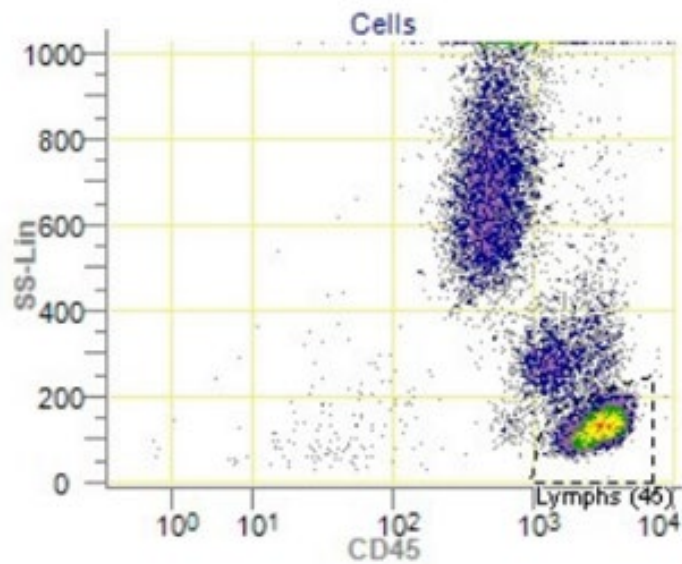


*EV stands for Extracellular Vesicle.

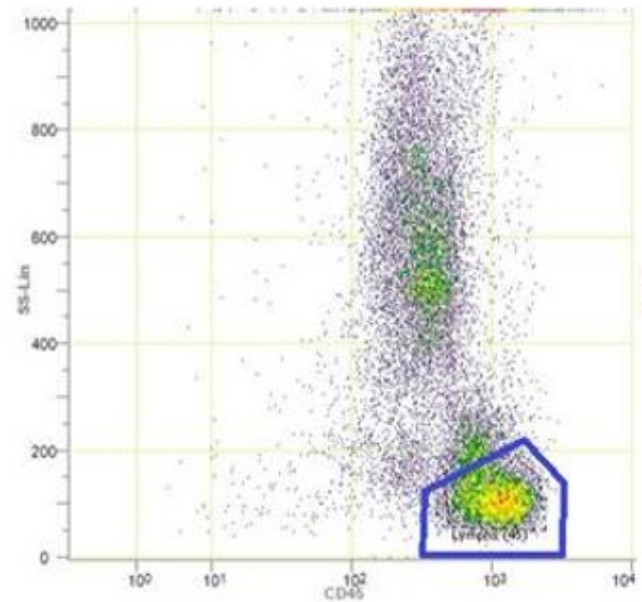
Monocyte Crashing in the CD45+/SSC Gate

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

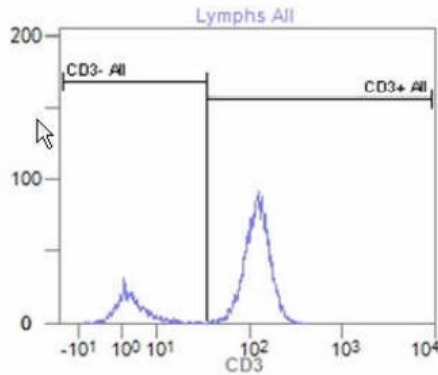


CD3 Histogram and CD4/CD8 Plot

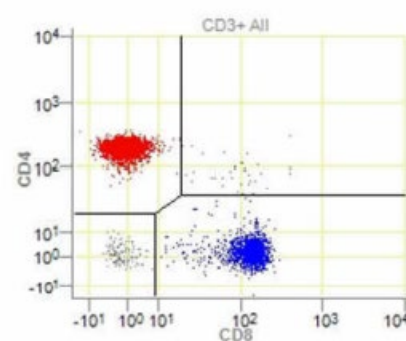
Tips for UKNEQAS Samples:

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.

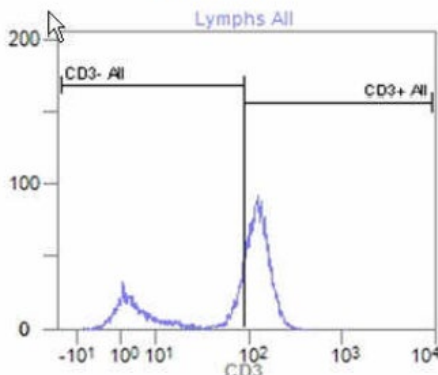
Acceptable



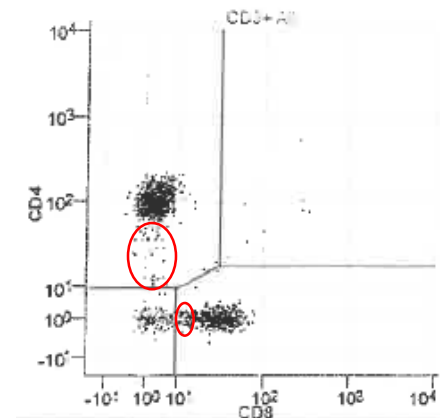
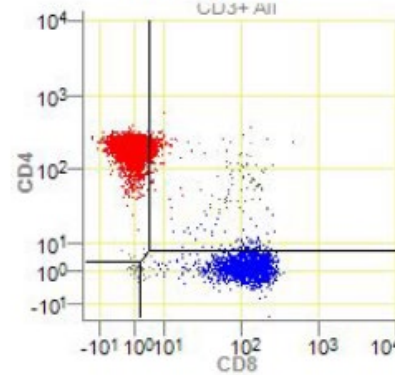
Acceptable



Unacceptable



Unacceptable



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

