



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 R (0)		Rur	2) (0) Review (22)	Review (22)	
Instrume	ent: All		Auto Refresh		
Sangee ku	Fallent name	Teles Name			
622200 Per Per	44220	1020 1	Sample D: 89390141145 Test: Tetra Combo		
89350141144	148217	Tetra 1	Run Date: 02/03/2015 12:49 PM Collect Date: 02/03/	2015	
89350141142	42226	Tetra Cambo	Specimen Type: Whole Blood Inst. SN: AV0390	102	
89350141145	148217	Tetra Canbo	Patient Name: 148217		
6130004	AQUIOS IMMUN.	Tetra Cambo	Patient ID: Gov. ID:		
6170003	AQUIOS IMMUN	Tetra Canbo	Status: Location:		
85347465218	148217	Tero 2-	Gender: Soloct - Date of Birth- 1.1		
BOLL TADE DO		Terry De	denka. Select - Gode of birdi. 77		
03.34.149.200	-	100120	Physician Name:		
85347495209	148217	Tetra 1	Physician Code:		
89347465206	6	Tetra 1	Run Flags:		
89347465208	3	Tetra Canto			
05347465207	47276	Tetra Canton	Run Notifications:		
\$100004	AQUIOS IMMUN.	Tetra Canbo	Commente		
6130004	AGUIOS IMMUN	Tetra Contro	Comments:		
6130004 6170003	AGUIOS MINUN	Tetra Canbo Terra Canto	Comments:	Actor	
6130004 6170063 6120004	AGUIOS MEVUR AGUIOS MEVUR AGUIOS MEVUR	Tetra Contro Terra Contro Terra Contro	Comments: Result Value Flag Normal Range Range	Actor Rang	
6100004 6170003 6180004 6170003	AGUIOS IMMUN AGUIOS IMMUN. AGUIOS IMMUN AGUIOS IMMUN	Tetra Conto Tetra Conto Tetra Conto Tetra Conto	Comments: Result Value Flag Normal Range Verage 003+ (7 Cells) Percent 78.82% Kverage 003+ (7 Cells) Count VL 1,554	Actor Rang	
6130004 6170003 6130004 6130004	AGUIOS BIMUN. AGUIOS BIMUN. AGUIOS BIMUN. AGUIOS BIMUN.	Tetra Conto Tetra Conto Tetra Conto Tetra Conto Tetra Conto	Comments: Value Flag Normal Range Vessor 003+ (7 Cells) Percent 78.02% Flag Normal Range Vessor 003+ (7 Cells) Percent 78.02% 1.054 1.054 203+ (7 Cells) Percent 44.81% 1.054 1.054	Actor Rang	
6100004 6100004 6100004 6100003 6100004 61700003	AGUICS IMMUN AGUICS IMMUN AGUICS IMMUN AGUICS IMMUN AGUICS IMMUN	Tetra Contro Tetra Contro Tetra Contro Tetra Contro Tetra Contro Tetra Contro	Comments: Value Flag Normal Range Vesspr 003+ (T Cels) Percent 78.02% Flag Normal Range Vesspr 003+ (T Cels) Percent 78.02% Flag Normal Range Z03+ (T Cels) Percent 1.054 1.054 Z03+ (T Cels) Percent 44.81% 1.054 Z03+ (T Cels) Percent 44.81% 1.054 Z03+ (T Cels) (Resent V Cels) Resent 1.054 1.054	Actor Rang	
6130004 6130004 6130004 6130004 6130004 6130004 6130004	AGUIDS BIMUNU. AGUIDS BIMUNU AGUIDS BIMUNU. AGUIDS BIMUNU. AGUIDS BIMUNU. AGUIDS BIMUNU.	Terra Canbo Terra Canbo Terra Canbo Terra Canbo Terra Canbo Terra Canbo Terra Canbo	Comments: Value Flag Normal Range Vesspr C03+ (T Cels) Percent 78.02% Flag Normal Range Vesspr C03+ (T Cels) Percent 78.02% Flag Normal Range Z03+ (CD4+ (Higher T Cels) Percent 44.81% Flag SFL Z03+ (CD4+ (Higher T Cels) Percent 58.95 Flag SFL Z03+ (CD4+ (Suppersor T Cels) Percent 52.55% Flag SFL	Actor	
6130004 6130004 6130004 6130004 6130004 6130004 6130004 6130004	AGUICS BIMUNU. ACUDS BIMUN AGUIDS BIMUN AGUICS BIMUNU. AGUICS BIMUNU. AGUICS BIMUNU. AGUICS BIMUNU. AGUICS BIMUNU.	Tetra Canbo Terra Canbo Tetra Canbo Tetra Canbo Tetra Canbo Tetra Canbo Tetra Canbo	Comments: Value Flag Normal Range Vesspr C03+ (T Onlo) Percent 78.02% Range Vesspr C03+ (T Onlo) Percent 78.02% 1.054 203+ (T Onlo) Percent 44.81% 203+ (T Onlo) Percent 44.81% 203+ (T Onlo) Percent 64.81% 989 203+ (C04+ Supersont T Collo) Percent 32.56% 203+ (C04+ Supersont T Collo) Count/ul. 979 203+ (C019+ Bit Onlo) Count/ul. 9.04%	Actor	
6130054 6130053 6130054 6130054 6130054 6130054 6130054 6130054 6130055 6130054	AGUICS IMMUN AGUICS IMMUN. AGUICS IMMUN. AGUICS IMMUN. AGUICS IMMUN. AGUICS IMMUN. AGUICS IMMUN.	Tetra Canbo Tetra Canbo Tetra Canbo Tetra Canbo Tetra Canbo Tetra Canbo Tetra Canbo Tetra Canbo	Result Value Flag Normal Range Vesspr C03+ (T Onlo) Percent 78.02% 1054 Rvesspr C02+ (T Onlo) Percent 1.054 1.054 203+ (CD4+ Helper T Celle) Count (JL 989 99 203+ (CD4+ Helper T Celle) Percent 32.95% 99 203+ (CD4+ Supersont T Celle) Count (JL 989 99 203+ (CD4+ Supersont T Celle) Count (JL 99 99 203+ (CD4+ Supersont T Celle) Count (JL 99 90 203+ (CD4+ Supersont T Celle) Count (JL 99 944 203+ (CD4+ Supersont T Celle) Count (JL 98 944 203+ (CD4+ Supersont T Celle) Count (JL 98 944 203+ (CD4+ Supersont T Celle) Count (JL 98 944 203+ (CD4+ Supersont T Celle) Count (JL 98 944	Actor	
6130064 6130063 6130064 6130064 6130064 6130064 6130064 6130064 6130064 6130064	ADUIDS IMPURI. ADUIDS IMPURI. ADUIDS IMPURI ADUIDS IMPURI. ADUIDS IMPURI. ADUIDS IMPURI. ADUIDS IMPURI. ADUIDS IMPURI. ADUIDS IMPURI.	Tetra Canbo Terra Cantos Terra Cantos Tetra Canbo Tetra Canbo Tetra Canbo Tetra Canbo Tetra Canbo	Comments: Value Flag Normal Range Neege C03+ (T Cells) Percent 78.623, Neege C03+ (T Cells) Percent 78.623, Neege C03+ (T Cells) Count Vul. 1.654, 203+ (CD4+ Helper T Cells) Percent 4.4.313, 203+ (CD4+ Helper T Cells) Count Vul. 989, 203+ (CD4+ Helper T Cells) Count Vul. 989, 203+ (CD4+ Helper T Cells) Count Vul. 719, 203+ (CD4+ Helper T Cells) Count Vul. 719, 203+ (CD19+ (B Cells) Count Vul. 941, 203- (CD19+ (B Cells) Flowert 545, 203- (CD19+ (B Cells) Flowert 118, 203- (CD19+ (B Cells) Flowert 222,	Actor	
6130064 6130064 6130064 6130064 6130064 6130064 6130064 6130064 6130064 6130064	ACUICS INFUR. ACUICS INFUR ACUICS INFUR ACUICS INFUR. ACUICS INFUR. ACUICS INFUR. ACUICS INFUR. ACUICS INFUR. ACUICS INFUR. ACUICS INFUR.	Tetra Canbo Terra Canto Tetra Canbo Tetra Canbo Tetra Canbo Tetra Canbo Tetra Canbo Tetra Canbo Tetra Canbo	Comments: Value Flag Normal Range Neege CD3+ (T Cells) Percent 78.623, Neege CD3+ (T Cells) Percent 1.864 D3+ (D5+ Helper T Cells) Percent 44.813, D3+ (D5+ Helper T Cells) Count/U, 989 D3+ (D5+ Helper T Cells) Count/U, 989 D3+ (D5+ Helper T Cells) Percent 32.553, D3+ (D5+ Helper T Cells) Percent 32.553, D3+ (D5+ Helper T Cells) Percent 186 D3+ (D5+ (B Cells) Count/U, 188 D3+ (D5+ (B Cells) Percent 11.88, D3+ (D5+ (B Cells) Count/U, 24.7 D3+ (D5+ Re Cells) Count/U, 24.7 D3+ (D5+ Re Cells) Count/U, 24.7	Actor	
6130004 6130 6130 6130 6130 6130 6130 6130 613	ACUICS IMPURI. ACUICS IMPURI ACUICS IMPURI ACUICS IMPURI. ACUICS IMPURI. ACUICS IMPURI. ACUICS IMPURI. ACUICS IMPURI. ACUICS IMPURI.	Tens Canbo Tens Canbo Tens Canbo Tens Canbo Tens Canbo Tens Canbo Tens Canbo Tens Canbo	Comments: Value Flag Normal Range Neege C03+ (T Cells) Percent 78.62% Range Neege C03+ (T Cells) Count 64 1.864 200-(C04+ (Higher T Cells) Percent 44.81% D3+/C04+ (Higher T Cells) Count 64 989 203+(C04+ (Higher T Cells) Count 64 989 D3+/C04+ (Higher T Cells) Count 64 989 203+(C04+ (Higher T Cells) Count 64 779 D3+/C04+ (Higher T Cells) Count 64 984 188 203+(C019+ (B Cells) Count 64 D3+/C04+ (Higher T Cells) Count 64 98.94% 1188 203+(C019+ (B Cells) Count 64 D3+/C04+ (Higher T Cells) Count 64 247 1195% 204+(C019+ (Higher Cells) Count 64 D3+/C04+ (D14+ (Higher Cells) Count 64 247 241 247 D3+/C04+ (D14+ (Higher Cells) Count 64 247 138 138 Total Lynchrocytes (T-8+/Higher Cent 58.95% 138 138	Actor	
6130004 6170003 6130004 6170003 6130004 6170003 6130004 6170003 6130004 6170003	AGUICS MINUR. AGUICS MINUR AGUICS MINUR AGUICS MINUR. AGUICS MINUR. AGUICS MINUR. AGUICS MINUR. AGUICS MINUR.	Tess Canbo Tess Canbo Tess Canbo Tess Canbo Tess Canbo Tess Canbo Tess Canbo	Comments: Value Flag Normal Range Neege C03+ (T Cells) Percent 78.825, Range Neege C03+ (T Cells) Percent 78.825, Range Normal Range 1,864 200-(T Cells) Percent 44.815, D3+ (Col+ Heiger T Cells) Percent 44.815, 388 203+(Col+ Kepenser T Cells) Percent 388 D3+(Col+ Suppenser T Cells) Percent 39.845, 203+(Col+ Suppenser T Cells) Percent 39.84, D3+(Col+ Suppenser T Cells) Percent 59.84, 203+(Col+ Suppenser T Cells) Percent 188 D3+(Col+ Suppenser T Cells) Count/UL 59.84, 198, 203+(Col+ Suppenser T Cells) Percent 198, D3+(Col+ Col+ F)/K Cells (Caunt/UL 59.15, 198, 203+(Col+ Suppenser T Cells) Percent 287, D3+(Col+ Col+ F)/K Cells (Caunt/UL 59.15, 198, 203+(Col+ Suppenser T Cells) Percent 287, D3+(Col+ Suppenser T Cells) Count/UL 387, 198, 198, 203+(Col+ Suppenser T Cells) Percent 287, D3+(Col+ Suppenser T Cells) Count/UL 387, 198, 203+(Col+ Suppenser T Cells) Percent 287, D3+(Col+ Supene	Actor	
6130004 6170003 6130004 6170003 6130004 6170003 6130004 6170003 6130004 6170003	AGUICS MINUR. AGUICS MINUR AGUICS MINUR AGUICS MINUR. AGUICS MINUR. AGUICS MINUR. AGUICS MINUR. AGUICS MINUR.	Tetra Cantos Tetra Cantos Tetra Cantos Tetra Cantos Tetra Cantos Tetra Cantos Tetra Cantos Tetra Cantos Tetra Cantos Tetra Cantos	Comments: Value Filing Normal Range Neege CD3+ (T Cells) Percent 78.825, Range Neege CD3+ (T Cells) Percent 78.825, Range CD3+ (CD4+ (Higher T Cells) Percent 44.815, 10.84 CD3+ (CD4+ (Higher T Cells) Percent 44.815, 20.95, CD3+ (CD4+ (Higher T Cells) Percent 38.94, 20.95, CD3+ (CD4+ (Higher T Cells) Percent 39.84, 20.95, CD3+ (CD6+ (Suppease T Cells) Percent 39.84, 20.95, CD3+ (CD6+ (Suppease T Cells) Percent 39.84, 11.88, CD3+ (CD6+ (Suppease T Cells) Count-(J. 718, 20.95, CD3+ (CD6+ (Suppease T Cells) Percent 39.84, 11.88, CD3+ (CD6+ (Suppease T Cells) Count-(J. 287, 11.88, CD3+ (CD6+ CD16+ ///K Cells) Percent 287, 11.88, CD4+ (CD8 Page 1.38, 11.38, CD3+ (CD8 Page Cl3+ (Cl4+ (D4+ Cells)) Percent 25.95, 11.38, CD3+ (CD8 Page Cl3+ (Cl4+ (D4+ Cells)) Percent 25.95, 11.38, CD3+ (CD8 Page Cl3+ (Cl4+ (D4+ Cells)) Percent 25.95, 11.38,	Actor Rang	
6130004 6130004 6130004 6130004 6130004 6130004 6130004 6130004 6130004 6130004 6130004	AGUICS MINUR. AGUICS MINUR AGUICS MINUR AGUICS MINUR. AGUICS MINUR. AGUICS MINUR. AGUICS MINUR. AGUICS MINUR.	Tess Canbo Tess Canbo Tess Canbo Tess Canbo Tess Canbo Tess Canbo Tess Canbo	Comments: Value Flag Normal Range Neege CD3+ (T Cells) Percent 78.82%, Average CD3+ (T Cells) Percent 78.82%, Average CD3+ (T Cells) Percent 78.82%, Average CD3+ (T Cells) Count-UL 1,064 DD+/CD4+ (Higher T Cells) Count-UL 1,064 889 203+/CD4+ (Higher T Cells) Count-UL, BB3 889 CD3+/CD4+ (Higher T Cells) Count-UL, DD3+/CD4+ (Suppersont T Cells) Percent 35.95%, DD3+/CD4+ (N/Cells) Percent 96.44, DD3+/CD4+ (N/Cells) Percent DD3+/CD4+ (N/Cells) Count-UL, DD3+/CD4+ (N/Cells) Percent 188 DD3+/CD4+ (N/Cells) Percent DD3+/CD4+ (N/Cells) Count-UL, DD3+/CD4+ (N/Cells) Count-UL, DD3+/CD4+ (N/Cells) Percent 188 11.95%, DD3+/CD4+ (N/Cells) Percent DD3+/CD4+ (N/Cells) Count-UL, DD3+/CD8+Rate 1.38 11.95%, DD3+/CD4+ (N/Cells) Percent 138 CD3+/CD4+ (N/Cells) Percent 55.15%, DD3+/CD4+ (N/Cells) Percent 55.15%, DD3+/CD4+ (N/Cells) Percent 55.15%, DD3+/CD4+ (N/Cells) Percent CD3+/CD4+ (N/Cells) Percent 6.58%, CD3+/C04+ (N/Cells) Percent 6.58%, DD3+/C04+ (N/Cells) Percent 6.58%, DD3+/C04+ (N/Cells) Percent DD4+/CD3 Pirent 2.298 20.55%, Percent 6.58%, DD3+/C04+ (N/Cells) Percent DD3+/CD3+/CD3+/CD4+/C04+ (N/Cells) Percent 6.58%, DD3+/C04+ (N/Cells) Percent <td>Actor Rang</td>	Actor Rang	
6130004 6170003 6130064 6170003 6130064 6170003 6130064 6170003 6130066 6170003	ACUICS INVUR. ACUICS INVUR ACUICS INVUR ACUICS INVUR. ACUICS INVUR. ACUICS INVUR. ACUICS INVUR. ACUICS INVUR. ACUICS INVUR.	Tess Canbo Tess Canbo Tess Canbo Tess Canbo Tess Canbo Tess Canbo Tess Canbo	Comments: Value Flag Normal Range Neexpr CD3+ (T Cala) Parcent 78.82% Range Neexpr CD3+ (T Cala) Parcent 78.82% Range D3+/CD4+ (Higher T Cala) Parcent 1.664 1.664 D3+/CD4+ (Higher T Cala) Parcent 32.95% 1.064 D3+/CD4+ (Higher T Cala) Parcent 32.95% 1.070+/CD4+/RAC Range D3+/CD4+ (Higher T Cala) Parcent 32.95% 1.070+/CD4+/RAC Range D3+/CD14+ (Higher T Cala) Parcent 32.95% 1.070+/CD4+/RAC Range D3+/CD14+ (B Cala) Parcent 32.95% 1.070+/CD4+/RAC Range D3+/CD14+ (B Cala) Count /ul. 50 1.18 D3+/CD14+ (B Cala) Parcent 1.08 1.18 D3+/CD14+ (B Cala) Parcent 1.18 1	Actor	
6130004 6170003 6130064 6170003 6130064 6170003 6130004 6170003	ACUICS MINUR., ACUICS MINUR, ACUICS MINUR., ACUICS MINUR., ACUICS MINUR., ACUICS MINUR., ACUICS MINUR., ACUICS MINUR., ACUICS MINUR., ACUICS MINUR.,	Tets Carbo Tets Carbo Tets Carbo Tets Carbo Tets Carbo Tets Carbo Tets Carbo	Comments: Value Flag Normal Range Neespe CD3+ (T Cells) Percent 78.62% Range Neespe CD3+ (T Cells) Percent 78.62% Range ZD3+ (CD4+ (Heijer T Cells) Count VL 1,064 100 ZD3+ (CD4+ (Heijer T Cells) Count VL 808 100 ZD3+ (CD4+ (Heijer T Cells) Count VL 808 100 ZD3+ (CD4+ (Heijer T Cells) Count VL 808 100 ZD3+ (CD4+ (Heijer T Cells) Count VL 808 100 ZD3+ (CD4+ (Heijer T Cells) Count VL 804 100 ZD3+ (CD4+ Suppears T Cells) Count VL 804 108 ZD3- (CD19+ (B) Cells) Percent 100 118 100 ZD3- (CD19+ (B) Cells) Percent 118 100 118 ZD3- (CD19+ (B) Cells) Percent 95 118 100 ZD3- (CD19+ (B) Cells) Percent 95 118 100 ZD3- (CD19+ (B) Cells) Percent 95 118 100 ZD3- (CD19+ Recent Cells) Percent 95 118 100 ZD3- (CD19+ Recent Cells) Percent 128 118	Actor	
6130004 6170003 6170003 6170003 6170003 6170003 6170003 6170003	ACUICS IMPURI. ACUICS IMPURI ACUICS IMPURI ACUICS IMPURI. ACUICS IMPURI. ACUICS IMPURI. ACUICS IMPURI. ACUICS IMPURI. ACUICS IMPURI.	Tets Carbo Tets Carbo Tets Carbo Tets Carbo Tets Carbo Tets Carbo Tets Carbo	Comments: Value Flag Normal Range Neege C03+ (T Cells) Percent 78.623, Neege C03+ (T Cells) Percent 78.623, Neege C03+ (T Cells) Count/UL 1.664, 203+ (CD4+Helper T Cells) Percent 44.813, 203+ (CD4+Helper T Cells) Count/UL 989, 203+ (CD4+Helper Cells) Count/UL 989, 204+ (CD4+Helper Cells) Count/UL 989, 204+ (CD4+Helper Cells) Count/UL 2208, 204+ (CD4+Helper Cells) 223, 204+ (CD4+Helper Cells) 223, 204+ (CD4+Helper Cells) 223, 20	Actor	

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



6. Graphic OptionsSelection (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



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.

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

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

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







Corrected



- 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







Unacceptable





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.
- 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 <u>raul.louzao@duke.edu</u> or Sylvester Hood at 919-613-4469 <u>sfh7@duke.edu</u>



