



STEM CELL LABORATORY (STCL)



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Gate Adjustments in BD Multiset Software for CAP PT Samples and Process Control Cells JA3

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Author: MGREESE

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Gate Adjustments in BD Multiset Software for CAP PT Samples and Process Control Cells

This Job Aid should serve as a visual aid when performing lymphocyte gating in BD Multiset software for CAP proficiency test samples or Status Flow Process Controls. These test samples contain stabilized cells which may have altered staining characteristics when compared to fresh, non-stabilized test samples that we receive for patient testing. Dim, nonspecific staining may be present and must be handled by using the manual gate adjustment feature of Multiset.

Below are examples showing how CD8+ events were contaminated with events (nonspecific staining) causing a falsely elevated absolute CD8+ result. There may be other instances where this may occur with other markers in the lymphocyte analysis panel. Lab personnel should thoroughly review all results for control and proficiency testing samples paying particular attention to this issue.

Example 1 is an example of a reported CAP Proficiency Testing (PT) result that contained an outlier. In the plot showing CD8 vs CD3 (Middle upper plot), there were nonspecifically stained events that are counted as CD8+ T-cells. This occurs when the auto-gate feature in Multiset misidentifies these as CD8+ events, (lower right plot).

In order to correct this, The CD8 gate must be adjusted manually by using the manual adjustment feature in Multiset. See **Example 2** lower right plot.

Example 3 illustrates the auto-analysis with no manual gating to correct the misidentification of events.

NOTE: This result would always need manual gate adjustment as shown in Example 2.

Example 1

DUKE STCL
MultiSET™ Lab Report

EXAMPLE 1

ORIGINAL

RESULT

Director: JOANNE KURTZBERG MD
Operator: MELISSASoftware: MultiSET V3.0.2
Cytometer: (file-based analysis)Sample Name: CAP SURVEY FL-C 2018
Sample ID: CAPFL14101718
Case Number: LYMPHS
Panel Name: 4 Color TBNK + TruCDate Acquired: 17-Oct-18 12:00:03
Date Analyzed: Thu, Dec 6, 2018

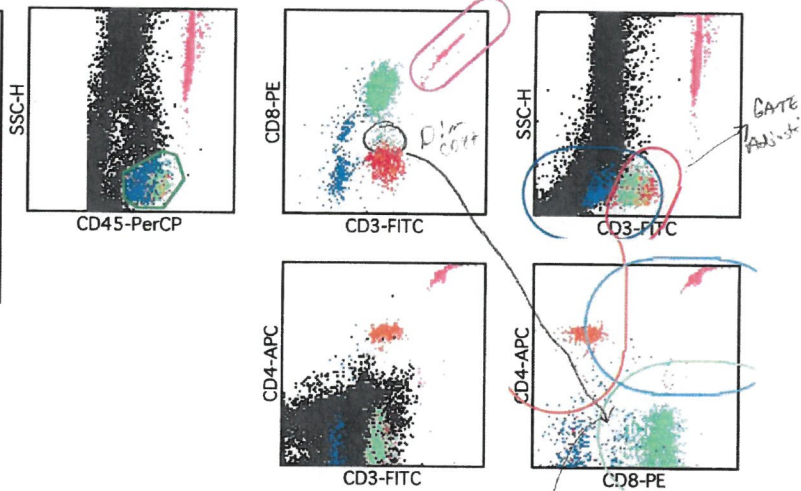
CD3/CD8/CD45/CD4 TruC

Data Set [1] Data File: CAPFL1410171802.01

Reagent Lot ID: 51606 Events Acquired: 27500 Abs Cnt Bd Lot ID: 18127 Attr Def File: 3/8/45/4 MLT/TruC v2.0

File ID: 20403C53-7F1C-4E93-BC41-DAF72FFD8B2B Beads Per Pellet: 49800

Lymph Events	5573
Bead Events	3930
CD3+ %Lymph	49
CD3+ Abs Cnt	693
CD3+CD8+ %Lymph	37
CD3+CD8+ Abs Cnt	522
CD3+CD4+ %Lymph	6
CD3+CD4+ Abs Cnt	79
CD3+CD4+CD8+ %Lymph	0
CD3+CD4+CD8+ Abs Cnt	1
CD45+ Abs Cnt	1412
T H/S Ratio	0.15 Lo



QC Messages:

Code 3: Sample integrity questionable. Visually inspect.
Code 7: Manual Gate is in effect.

Tube Name: CD3/CD16+56/CD45/CD19 TruC

Status: Not yet run

File Name:

Example 2

DUKE STCL
MultiSET™ Lab Report

EXAMPLE 2

Director: JOANNE KURTZBERG MD
Operator: MELISSASoftware: MultiSET V3.0.2
Cytometer: (file-based analysis)Sample Name: CAP SURVEY FL-C 2018
Sample ID: CAPFL14101718
Case Number: LYMPHS
Panel Name: 4 Color TBNK + TruCDate Acquired: 17-Oct-18 12:00:03
Date Analyzed: Thu, Dec 6, 2018

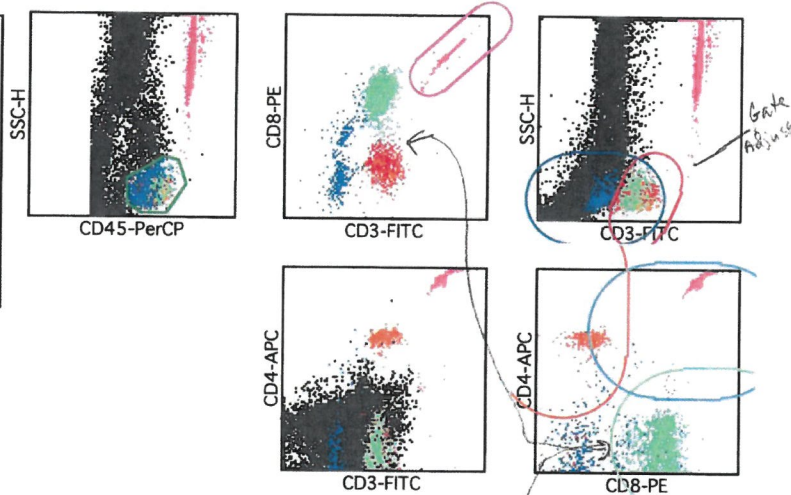
CD3/CD8/CD45/CD4 TruC

Data Set [1] Data File: CAPFL1410171802.01

Reagent Lot ID: 51606 Events Acquired: 27500 Abs Cnt Bd Lot ID: 18127 Attr Def File: 3/8/45/4 MLT/TruC v2.0

File ID: 20403C53-7F1C-4E93-BC41-DAF72FFD8B2B Beads Per Pellet: 49800

Lymph Events	5573
Bead Events	3930
CD3+ %Lymph	49
CD3+ Abs Cnt	693
CD3+CD8+ %Lymph	35
CD3+CD8+ Abs Cnt	500
CD3+CD4+ %Lymph	6
CD3+CD4+ Abs Cnt	79
CD3+CD4+CD8+ %Lymph	0
CD3+CD4+CD8+ Abs Cnt	1
CD45+ Abs Cnt	1412
T H/S Ratio	0.16 Lo



QC Messages:

Code 3: Sample integrity questionable. Visually inspect.
Code 7: Manual Gate is in effect.DIM EVENTS GATED OUT
↓ CD8+ / cd

Tube Name: CD3/CD16+56/CD45/CD19 TruC

Status: Not yet run

File Name:

Example 3

DUKE STCL
MultiSET™ Lab ReportDirector: JOANNE KURTZBERG MD
Operator: MELISSASoftware: MultiSET V3.0.2
Cytometer: (file-based analysis)Example 3 - NO manual
adjustment
Software
Auto gateSample Name: CAP SURVEY FL-C 2018
Sample ID: CAPFL14101718
Case Number: LYMPHS
Panel Name: 4 Color TBNK + TruCDate Acquired: 17-Oct-18 12:00:03
Date Analyzed: Thu, Dec 6, 2018

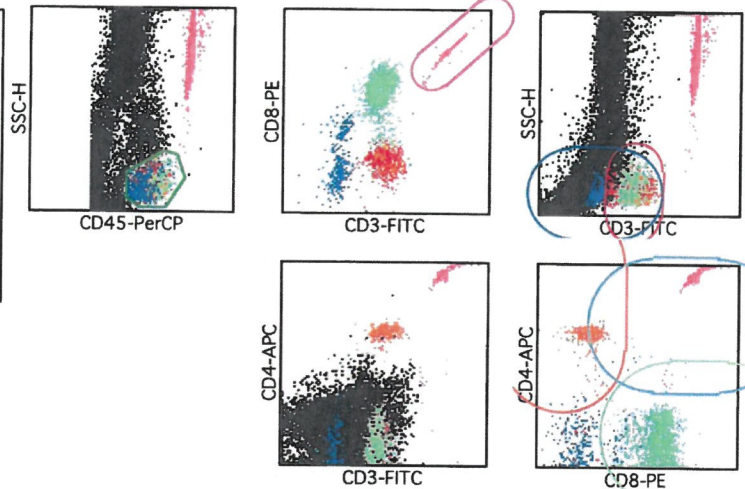
CD3/CD8/CD45/CD4 TruC

Data Set [1] Data File: CAPFL1410171802.01

Reagent Lot ID: 51606 Events Acquired: 27500 Abs Cnt Bd Lot ID: 18127 Attr Def File: 3/8/45/4 MLT/TruC v2.0

File ID: 20403C53-7F1C-4E93-
BC41-DAF72FFD8B2B Beads Per Pellet: 49800

Lymph Events	5573
Bead Events	3930
CD3+ %Lymph	54
CD3+ Abs Cnt	762
CD3+CD8+ %Lymph	38
CD3+CD8+ Abs Cnt	540
CD3+CD4+ %Lymph	6
CD3+CD4+ Abs Cnt	85
CD3+CD4+CD8+ %Lymph	0
CD3+CD4+CD8+ Abs Cnt	1
CD45+ Abs Cnt	1412
T H/S Ratio	0.16 Lo



QC Messages:

Code 3: Sample integrity questionable. Visually inspect.

Tube Name: CD3/CD16+56/CD45/CD19 TruC

Status: Not yet run

File Name:

Signature Manifest**Document Number:** FLOW-GEN-009 JA3**Revision:** 01**Title:** Gate Adjustments in BD Multiset Software for CAP PT Samples and Process Control Cells JA3

All dates and times are in Eastern Time.

FLOW-GEN-009 JA3 Gate Adjustments in BD Multiset Software for CAP PT Samples and Process Ctrl Cells**Author**

Name/Signature	Title	Date	Meaning/Reason
Melissa Reese (MGREESE)		12 Feb 2019, 12:36:14 PM	Approved

Management

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATE02)		13 Feb 2019, 07:12:01 PM	Approved

Medical Director

Name/Signature	Title	Date	Meaning/Reason
Joanne Kurtzberg (KURTZ001)		14 Feb 2019, 07:32:22 AM	Approved

Quality

Name/Signature	Title	Date	Meaning/Reason
Bing Shen (BS76)			
Lisa Eddinger (LE42)			
Taylor Orr (TSO4)			
Richard Bryant (RB232)		14 Feb 2019, 07:47:04 AM	Approved

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Name/Signature	Title	Date	Meaning/Reason
Sandy Mulligan (MULLI026)		12 Apr 2019, 06:59:21 PM	Approved