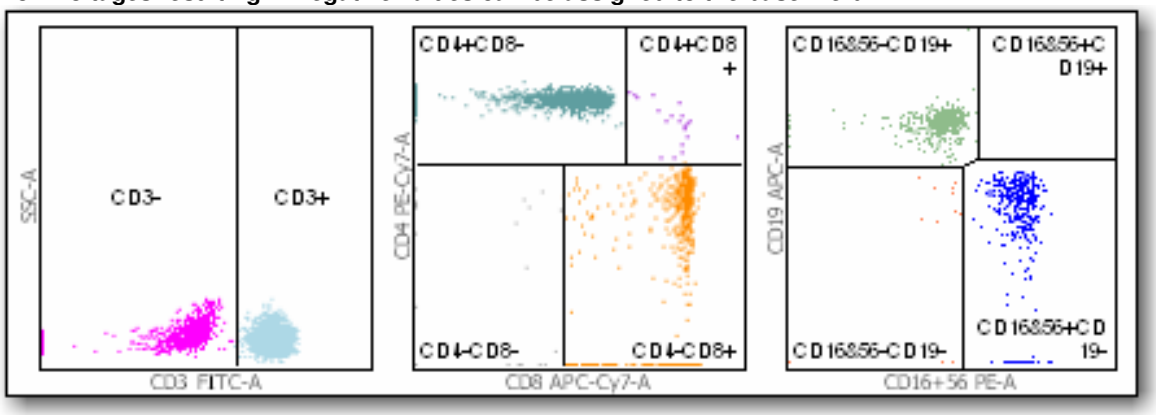
Troubleshooting Results with BD FACSCanto™ Clinical Software

Interpreting plots with dots on or near the axes

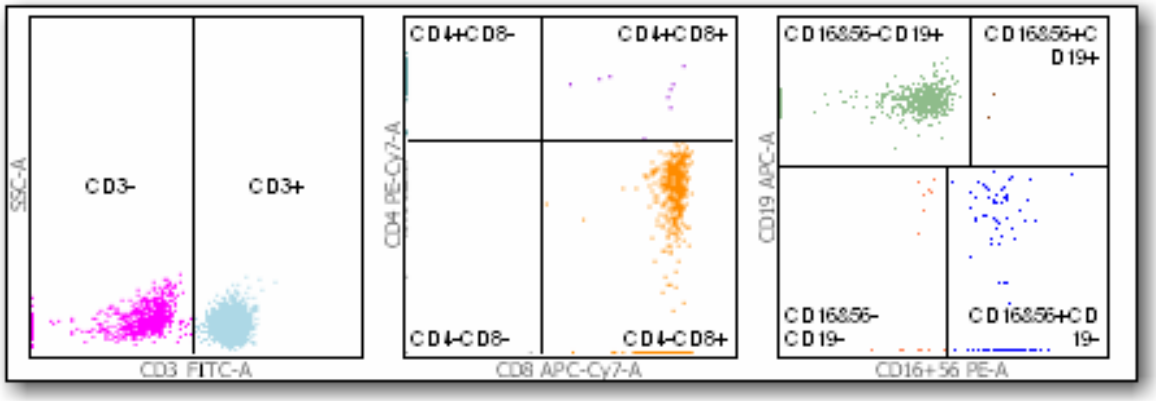
Immunofluorescence measurements in flow cytometry might result in signals that measure at or very near zero (mV). Since Log(0) is undefined, these events are assigned to the baseline (axis) bin of the log display scale. Similarly, logarithm values with low signal intensities that result in negative values are also assigned to this baseline bin. See the following figure (top row). Under rare conditions, events from a given patient sample may be predominantly assigned to this baseline bin due to very low signals. See the following figure (bottom row).

However, the higher signal intensities from the population will still be on scale. All of the binned events, including the baseline and above, are part of the original lymphocyte and gate classification. Therefore, the baseline bin events are correctly included in the numerical results shown on the Laboratory Report. This is expected behavior and does not impact the numerical results or the performance of the assay.

Low voltages resulting in negative values can be assigned to the baseline bin



Predominantly low signals for a given patient, assigned to the baseline bin



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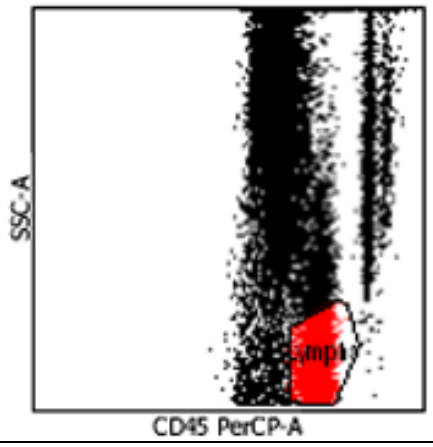
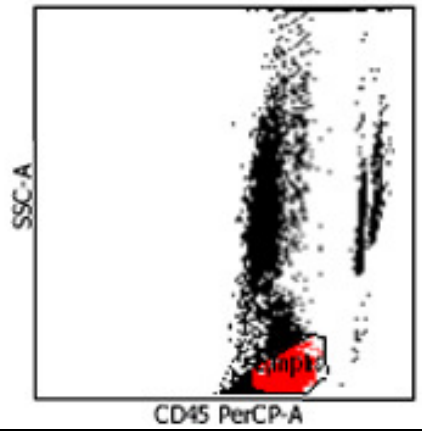
How do we analyze and gate commercial process controls with BD FACSCanto clinical software?

Observation	Possible Causes	Recommended Solutions
Cell populations in the CD45 vs SSC plot extend upward	Inadequate lysing of the sample	Prepare the sample again, and ensure that it is completely lysed.
		
Granulocytes with low side scatter in the CD45 vs SSC plot, no distinct monocyte population	Aged blood or stained cells	See the reagent package insert for stability limitations.
		

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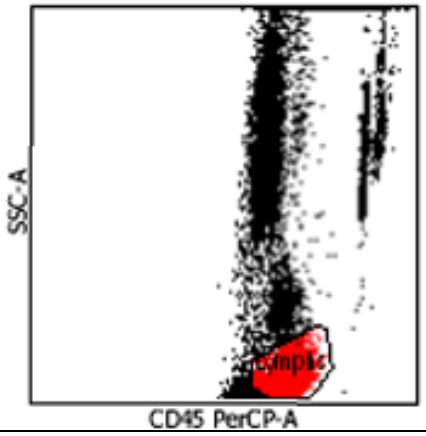
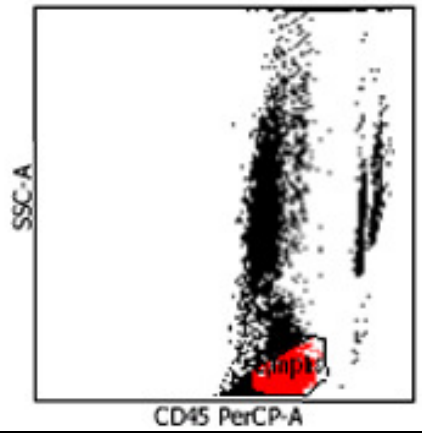
Debris encroaching on populations in the CD45 vs SSC plot	• Excessive mixing • Aged blood or stained cells	Prepare the sample again.
		
Observation	**Possible Causes**	**Recommended Solutions**
Vertically compressed populations	Side scatter too low	Reacquire the sample. Set SSC so granulocytes reach to top of the CD45 vs SSC plot.
	Lipemic sample	Check the reagent package insert for instructions.
		

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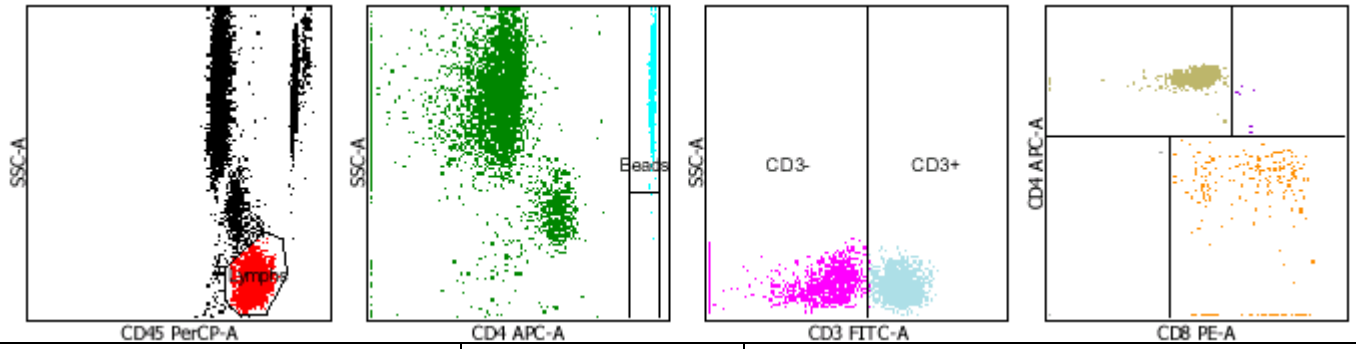
Indistinct populations; events sparse or missing from one population; lack of separation between the $CD3^-$ and $CD3^+$ cluster	Incorrect spectral overlap	Re-run the setup, optimizing for the application. Re-run the sample.



Granulocytes cut off at the top of the plot; stretched monocyte population	High SSC	Re-run the setup, optimizing for the application. Re-run the sample, lowering the SSC.