Commercial Process Controls and Re-Gating Samples after a Run with BD Multiset™ Software

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

Commercial process controls frequently have dimmer than normal staining. For this reason, College of American Pathologists (CAP) specimens might need to be adjusted more frequently, since these cells are fixed, as are the process controls. In the product insert supplied with the CAP samples, visual inspection and manual gating are recommended when running the proficiency-testing specimens.

It is permissible to adjust the SSC and threshold on the samples to give the best separation between lymphs and monos. It is important to optimize the acquisition to give the software the best opportunity to analyze the data correctly.

Since the attractors are defined based on staining patterns observed with fresh whole human blood, the $CD3^+$ attractor might need to be adjusted on all tubes of a multitube panel. Verify that each subset is properly classified. See Figures 1a, 1b, and 1c. Note the inadequate resolution between the $CD3^+$ and $CD3^-$ lymphocytes in Figure 1b.

The first step that the software takes is to identify the bead population using the CD3/8/45/4 (if BD Trucount™ tubes are used). It is important not to include any cells from the $CD3^+$ and $CD8^+$ double positives, which can encroach on this population. It is also important to make sure that all of the beads are included within the bead gate. This is a critical gate because if the number of beads is falsely elevated it will make the calculation for absolute counts incorrect. Each entire distinct population should be represented by one unique color.

The second step that the software takes is to place the CD3 attractors, both $CD3^+$ and $CD3^-$. Once an event is classified by a primary attractor (bead or $CD3^+$ or $CD3^-$), it cannot be classified by another, even if another attractor overlaps it.

Review the Expert Gate. If events are included that should be eliminated, or if events were missed that should be included, then regate. Sometimes regating is necessary to get the cleanest lymphocyte gate. The operator can always return to the automatic expert gate.

Review the $CD3^+$ gate (CD3 vs SSC). Remember that the $CD3^+$ population can be dimmer with fixed samples, but do not adjust the gate to encroach into the $CD3^-$ population as the gate is shown doing in Fig. 1b.

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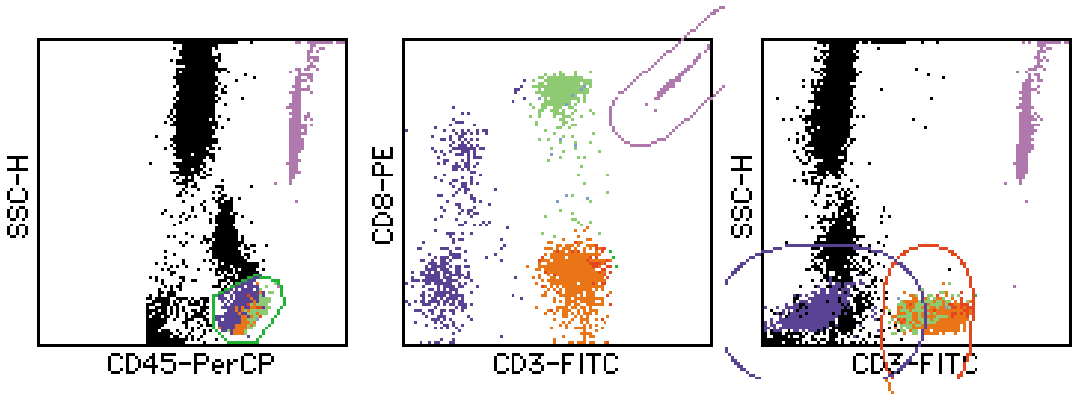

Figure 1a. Fresh whole blood sample showing adequate resolution between the $CD3^+$ and $CD3^-$ lymphocytes

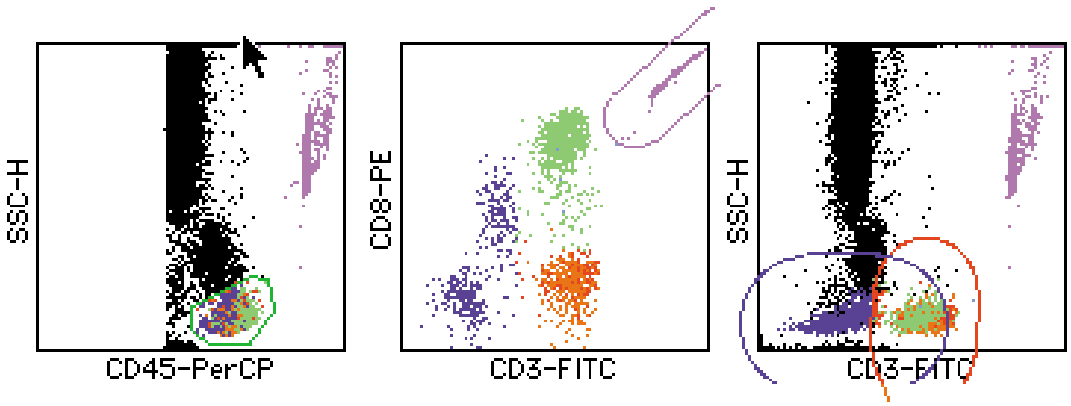

Figure 1b. Preserved process control *before* the $CD3^+$ attractor correction

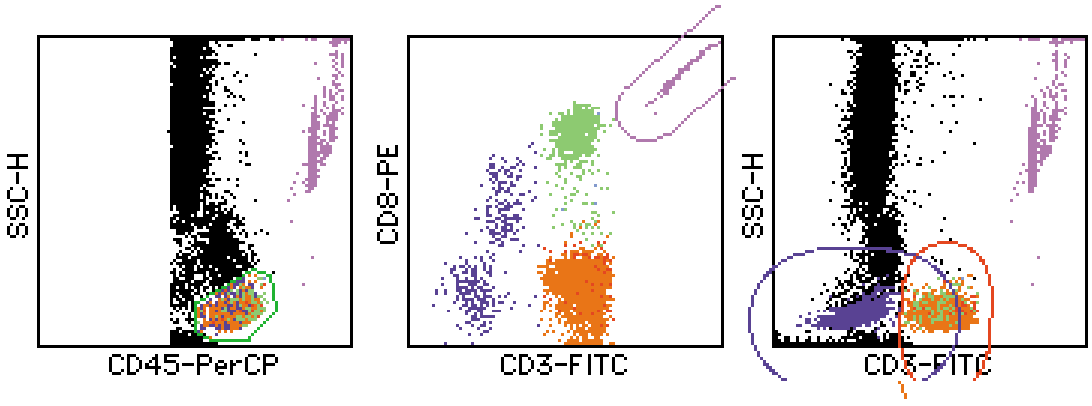

Figure 1c. Preserved process control *after* the $CD3^+$ attractor correction

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Finally, look at the subset attractors in the CD8 PE vs CD4 APC plot. Verify that the $CD4^+$ and $CD8^+$ attractors are in the correct position: the $CD3^+$,$CD4^+$ attractor followed by the $CD3^+$,$CD8^+$ attractor and finally the $CD3^+$,$CD4^+$,$CD8^+$ attractor. It is OK for them to overlap, as long as the $CD3^+$,$CD4^+$ attractor covers all the $CD4^+$ cells, and the $CD3^+$,$CD8^+$ attractor covers all the $CD8^+$ cells. The $CD3^+$,$CD4^+$,$CD8^+$ attractor will likely overlap both of the other attractors in this plot, but the true CD4 and CD8 cells have already been captured by their appropriate attractors before this attractor was placed. The $CD3^+$,$CD4^+$,$CD8^+$ attractor is supposed to capture all events that are dual-positive for CD4 and CD8. If any of the attractors needs to be adjusted to enclose their appropriate populations of interest, then do so.

For the 3/16 + 56/45/19 tube, again the bead attractor is reviewed and adjusted if necessary. Check the expert gate and adjust if necessary. Check and adjust the $CD3^+$ attractor *and* the $CD3^-$ attractor. In this tube, the $CD3^-$ cells are the ones that will be considered to be B or NK cells. Next, review and adjust the $CD19^+$ attractor (B cells) and then the $CD16^+$,$CD56^+$ attractor (NK cells).

Observe any QC messages. The lymphosum should fall within a range of 95 to 105. If not, this is a clue to troubleshoot the results. Again, adjustment of the Expert Gate and attractor positions will most likely be required on fixed samples such as process controls and CAP survey samples.