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CAP Flow Cytometry Checklist; Difficult and New Items - By Jolene Cardinali and Michael Linden

The College of American Pathologist (CAP) accreditation program depends on peer review of laboratories to ensure quality laboratory testing. As a guideline, there are lab specific Checklists written by experienced technologists and pathologists in the field. Every year CAP adds, changes, or deletes Checklist items to create a more standardized and accurate assessment of acceptable laboratory practices. Continually, and especially as the biennial inspection dates approach, each laboratory diligently prepares for their CAP inspection to fulfill accreditation requirements. There is much organization, preparation and consideration so that the laboratory complies with these Checklist items.

In addition to satiating requirements for the general laboratory Checklists, specialty labs such as flow cytometry have even more specific guidelines. As flow cytometry technology is rapidly changing and advancing, the Checklist evolves. The laboratory standards for the flow cytometry lab are increasingly stringent, and each lab is under constant self-surveillance to meet flow cytometry “standard of care” guidelines as well as CAP Checklist items. In this article, we will address two “hot” Checklist items that flow cytometry laboratories seem to struggle with (validation of new antibodies and new lot validation), and also we will present two new Checklist items (rare event enumeration and reporting).

Validation of new antibodies - Labs must document the validation of new antibodies prior to their utilization in patient flow cytometry panels. Each new antibody received in the lab must be tested on cells of interest, including normal and abnormal cells, within a variety of specimen types.The new antibody should be rigorously tested to ensure that it will optimally perform in all circumstances. It must be evaluated on the lab’s flow cytometers with current voltages and compensation. After testing with the manufacturer’s recommended dilution, titration may be necessary to ensure background fluorescence is optimized without losing signal or creating excess auto-fluorescence. In addition, the signal to noise ratio is important when evaluating the new antibody, and different voltages may help or hinder this separation. Also, if the new antibody is conjugated to a tandem dye fluorochrome, it must be tested under current laboratory practices to ensure that time delay before acquisition, the addition of formalin, or light exposure will not degrade the fluorochrome. Finally, if the new antibody is used in a cocktail, this cocktail must be tested for long term stability before being put into use for patient panels.

New lot validation - When a new lot of antibody is received into the laboratory, it must be verified against the old lot for similar performance before being used for clinical testing. Many labs have developed systems to ensure that there is little lot to lot variability and that the antibodies have similar fluorescence. One method is to use the old and new lots to stain in parallel samples containing identical populations of cells. Typically these samples can be blood; however, some antigens (such as CD71) are only present in marrow. After the tubes are processed, they are analyzed on the flow cytometer(s) to measure the mean fluorescence intensity of each fluorochrome. Typically a Laboratory Director would review the data only if there was a significant decrease in MFI in the new lot. Limits are usually 10-15%. If the new lot is considerably dimmer than the prior lot, a request can be made to the vendor for replacement reagent. Keep in mind

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that the vendor may ask to see histograms of the lot to lot comparison. In contrast, sometimes the new lot is brighter. A brighter antibody/fluorochrome combination is typically a good thing, but keep in mind that the signal can be difficult to compensate or may be saturated if the combination is too bright. Common Laboratory Director limits for brighter fluorochromes hover around 20%. If the new lot of antibody is considerably brighter than the established acceptable limits, the antibody may need to be titrated/diluted.

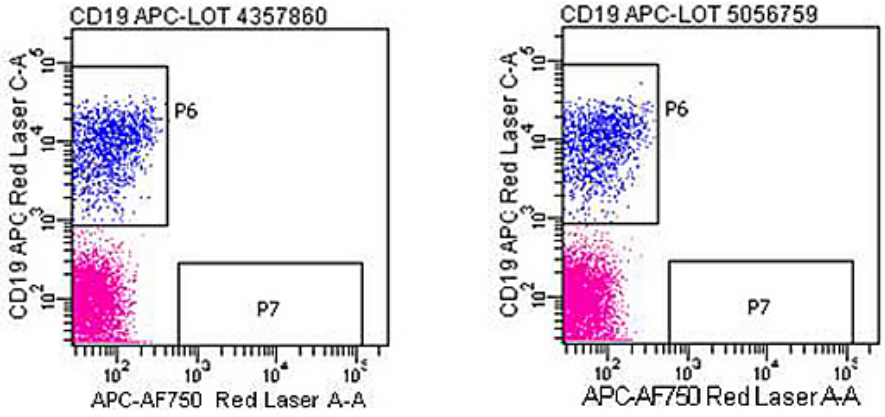

Figure 1. Comparing two lots of CD19 APC.
The MFI of new lot 5056759 (9455) is within 10% of old lot 4357860 (9421)

Rare event analysis/minimal residual disease -

Flow cytometry is a powerful tool to identify small populations within a normal background. The ICCS and other organizations have recommended standards for flow cytometric based testing for rare event assays (such as Paroxysmal Nocturnal Hemoglobinuria (PNH) or minimal residual disease (MRD) testing). While there are many labs that have high sensitivity PNH and MRD assays, there is lab to lab variability in the lower limit of enumeration due to method heterogeneity. Though the CAP Common Checklist has an item on analytical sensitivity, in practice, it is rarely applied to flow cytometry labs. New changes are coming to the Flow Cytometry Checklist to specifically address PNH and MRD assay analytical sensitivity. First, all labs that do such testing must validate/experimentally measure their lower limit of enumeration. For this validation, dilution studies need to be done in a suitable matrix (typically blood or marrow). The preferred method would be to dilute a patient sample containing lymphoma cells, leukemia cells, neoplastic plasma cells, or cells containing a PNH-type clone 10 fold serially. This diluent optimally should be another bone marrow or blood that is “normal” – lacking the neoplastic cells of interest. For example, say a peripheral blood sample has a B cell clone comprising 10% of leukocytes. 10 fold dilutions would be made in normal blood so that the expected recovery would be 1%, 0.1%, 0.01%, 0.001% and 0.0001%. Using the same B cell cocktail for all dilutions, samples would be stained and run in parallel, collecting the same number of events for each tube. If the lab were able to detect the 1%, 0.1%, 0.01%, and 0.001% dilutions, but not the 0.0001%, the limit of detection for clonal B cells with that lab’s assay would be 0.001%. This is only one example. We would expect that if not previously done when setting up the tubes, dilutional studies would need to be performed for all MRD assays that the lab performs (B-lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia, acute myeloid leukemia, plasma cell myeloma, etc.). For samples such as B-ALL, an ideal matrix/diluent would be a normal marrow sample containing hematogones.

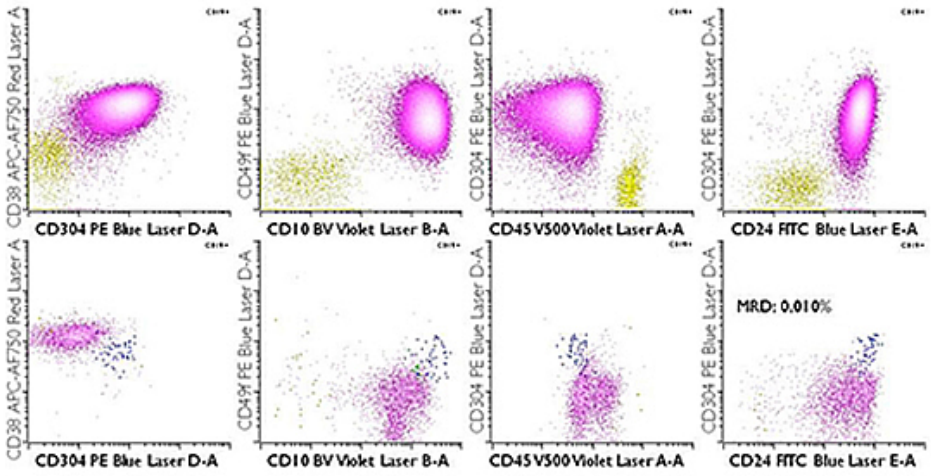

Figure 2. MRD Assay Dilutional Study.
The top row of histograms is displaying new pre-B ALL.
The bottom row of histograms is displaying dilution studies at which 0.01% MRD was found.

Once the lower limit of enumeration has been established, the CAP Checklist has new items that will require that the lab's lower limit of rare events assays is included in the patient's diagnostic report. The intention of the Checklist item is that a lab would report an assay's lower limit of enumeration. Keep in mind, however, that if the lab's assay was developed to collect 500,000 events, a paucicellular specimen (where only 50,000 events could be collected) would have a considerably lower limit of enumeration for that individual patient's test. At this time there is no CAP requirement to state that fewer events were collected for an individual's test; however, we recommend that labs consider creating a policy for such cases. As an example for a case where sufficient events are collected, a case of MRD for B-ALL may be reported as:

Final diagnosis:

Marrow: No abnormal B lymphoblast population detected (see comment)
COMMENT: There is no immunophenotypic evidence of recurrent/persistent B lymphoblastic leukemia. This lower limit of enumeration for this lab's B-ALL MRD assay is 0.001%

In summary, while some of these CAP Flow Cytometry Checklist items may seem cumbersome and increase the time needed for method development and laboratory compliance, the overall goal is to improve patient care and laboratory quality. Moreover, increased standardization will help clinicians compare assays among labs to guide care.

Jolene Cardinali, MT
Special Hematology Laboratory
Hartford Hospital

Michael A. Linden, MD, PhD
Division of Hematopathology,
Department of Laboratory Medicine,
University of Minnesota

Hartford, CT Minneapolis, MN