January 12, 2015
U.S. Department of Health and Human Services
Food and Drug Administration
Center for Devices and Radiological Health
Office of In Vitro Diagnostics and Radiological Health
Division of Immunology and Hematology
Hematology Branch


To whom it may concern:

We are writing on behalf of the International Clinical Cytometry Society (ICCS) to comment on the above-referenced draft guidance. ICCS is a non-profit professional organization dedicated to promoting education and advancements world-wide in the application of flow cytometry to clinical needs (http://www.cytometry.org/web/index.php). It is the largest organization of its kind and its membership is comprised of practicing physicians, scientists, medical technologists, and laboratory personnel focused on promoting the highest standards of clinical practice in this field. Although the majority of ICCS members are not the specified target audience, we do consider ourselves to be crucial stakeholders affected by this guidance. Furthermore, should the recently published “Draft Guidance for Industry, Food and Drug Administration Staff, and Clinical Laboratories: Framework for Regulatory Oversight of Laboratory Developed Tests (LDTs)” eventually be adopted, ICCS members would become de facto manufacturers of cytometric devices. We thank you for the opportunity to contribute to this important document and look forward to ongoing engagement with the FDA.

Our overarching concerns around this guidance are essentially all related to its clarity and timeliness, particularly as regards the distinction between qualitative (i.e. leukemia/lymphoma testing) and quasi-quantitative (i.e. CD34 enumeration) assays. An excellent summary of this distinction and the many fundamental ways in which these types of assays differ may be found in Wood et al.’s 2013 “Validation of cell-based fluorescence assays: practice guidelines from the ICSH and ICCS - part V - assay performance criteria.” [2] Given that the guidance does not include this reference, any of the other related and co-published ICSH/ICCS fluorescence assay validation references (3-8), or indeed any reference more recent than 2007, it is not surprising that the guidance content is somewhat dated and does not always reflect current thinking or practice regarding flow cytometry. The guidance also ignores many technological advances over the last decade. We have included a number of more recent references at the end of this letter, and urge you to consider them when drafting the next version of this guidance.
Insofar as guidance specifics are concerned, we summarize here a number of issues. We start with what we consider to be the most important omissions from the current draft and subsequently address statements that we find to be inaccurate, incomplete, or unnecessarily prescriptive. Note that we use line numbers to reference specific points of the draft guidance, as the section labeling format is not applied uniformly across the document, and does not correspond to the Table of Contents.

As indicated earlier in this letter, we consider the distinction between qualitative and quasi-quantitative assays to be of paramount importance (9), and strongly encourage emphasis of this concept in the guidance. Separate sections of the guidance (or possibly even separate guidance documents) addressing the two types of assays would be particularly helpful, as certain concepts such as linearity, reference ranges, and reference intervals are not relevant to qualitative assays. Similarly, the guidance regarding qualitative assays needs to acknowledge that limit of detection (LOD) and limit of blank (LOB) for qualitative assays such a leukemia/lymphoma immunophenotyping is closely linked to the phenotype of a specific patient’s aberrant/malignant population (as well as to the number of simultaneously-assessed parameters and the reagents used), and the patient-to-patient variability is such that LOD/LOB is very nearly patient/sample-specific. Consequently, the cut-off attainable for one patient may not be appropriate for another patient.

This issue becomes especially relevant in the case of minimal residual disease (MRD). The section entitled “Enumeration of Rare Events” (lines 626 – 649), cites suggested target detection levels from the “2006 Bethesda International Consensus Recommendations on the Immunophenotypic Analysis of Hematolymphoid Neoplasia by Flow Cytometry” (10) as follows: B cell, 0.1%; T cell, 1%; Myelomonocytic, 0.5%; and Plasma cell, 0.1%. The guidance appears to suggest that these levels might be informative for minimal residual disease. These targets, however, were not intended to be used in the MRD context but instead reflected the experience of the authors when surveyed regarding typical lineage-specific limits of detection when using their usual reagent combinations and their own, laboratory-developed methods for routine diagnosis and monitoring of patients with hematolymphoid neoplasms. We do not recommend use of these ballpark figures as benchmarks for minimal residual disease assays, and instead encourage the use of more rigorous and clinically-relevant, disease-specific decision points as documented in the extensive medical literature on this topic. (11-18)

Other statements that we find to be inaccurate, incomplete, or unnecessarily prescriptive include but are not limited to the following:

1. “Specimen and Data Analysis”, line 480
   The use of “paired isotype matched control antibodies” is suggested here and elsewhere (lines 536 and 895) in the guidance. A large body of literature describes the limitations and failings of isotype controls (19,20) and we do not recommend their use. This section also ignores Fluorescence-Minus-One controls for gating (21) as well as newer computational data analysis tools such as probability state modeling (22,23).
2. “Analytical Specificity: Reagents”, lines 430 - 454
This section suggests the use of Western blots to evaluate monoclonal antibodies without noting that Western blots present antigens in their denatured format. Only linear epitopes can therefore be assessed using this technique; epitopes that are presented conformationally on the cell surface will not be recognized by Western blot (24). This section also states that “specificity should be demonstrated by showing that the binding of each antibody in the multi-color reagent cocktail (single parameter analysis) is equal to that of the individual antibody-conjugates when used to stain the antigen expressing cells at the same concentration as in the final cocktail.” From a practical perspective this requirement may not be possible to meet, as some antigens cannot be reliably detected by themselves. There is no discussion regarding reagents for defining signal to noise ratio, a basic but important aspect of many flow cytometric assays, nor is there any discussion of reagent optimization (25). Non-antibody reagents for flow cytometry assays are not discussed.

3. “Detection Sensitivity “, lines 511 – 623, Figure 1
The procedure described in this lengthy and prescriptive section is not referenced, and is not widely used by the flow cytometry community.

4. "Linear Range", lines 651 - 662
We suggest inclusion of guidance for demonstration of linearity for assays measuring antigen density (8,26).

5. “Repeatability” & “Reproducibility”, lines 664 – 736
These sections state that repeatability and reproducibility “should be demonstrated using clinical specimens that are prepared 20 times and analyzed in batch fashion” and further indicate that this testing be carried out on “blood, bone marrow, lymph nodes, or other specimen types”. Not only is this suggestion not even remotely feasible given the constraints of patient sample size (27,28), it is unnecessary: recent publications demonstrate that 3 to 6 replicates over three analytical runs are adequate to demonstrate repeatability and reproducibility (2,8,29). Similarly, patient samples that “span the reportable range from low, medium and high” are essentially irrelevant in the context of qualitative assays for leukemia/lymphoma. We suggest that the statement “Where specimen availability and stability permit, you should include at least 20 days and at least 2 replicates per day” be deleted, as there are no instances in which specimen availability and stability would permit this testing, and currently no clinical setting to which it would apply.

In addition to the above specific issues, we would like to make several additional points regarding revision of this draft guidance. First, recent advances in flow cytometry technology should be discussed, including cytometers with greater than 4-color capacity, cytometers with digital (rather than analog) signal processing, cytometers and assays that do not require compensation, new fluorphors, and new blood collection tubes. Note also that Clinical Laboratory Standards Institutes (CLSI) has adopted the term “measurand” to replace “analyte” (30); we encourage the use of current
terminology. Reference ranges in general are similarly no longer considered to be relevant by the clinical laboratory community (31).

In conclusion, we would like to thank the FDA for the opportunity to provide constructive criticism on this draft guidance. We encourage you to take advantage of the wealth of experience and expertise within the clinical flow cytometry community in general, and particularly within the International Clinical Cytometry Society. Please do not hesitate to contact any of us for clarification or assistance regarding this letter. We look forward to working together with the FDA on future versions of this guidance.

Best regards,

The ICCS Advocacy Committee

Bruce Davis, brucedavis@trilliumdx.com
Jeannine Holden, jeannineholden@gmail.com
Jerry Hussong, jerry.hussong@aruplab.com
Virginia Litwin, virginia.litwin@covance.com
Teri Oldaker, toldaker@cox.net
Angela Salazar, SalazarA@Sysmex.com
Elizabeth Stone, estone@CSILaboratories.com
Paul K Wallace, paul.wallace@roswellpark.org
References


12. Rawstron AC, Child JA, de Tute RM, Davies FE, Gregory WM, Bell SE, Szubert AJ, Navarro-Coy N, Drayson MT, Feyler S and others. Minimal residual disease assessed by multiparameter flow cytometry in multiple myeloma: impact on...


