The Brief Communication by de Carvalho Bittencourt et al. [10.1002/cyto.b.21112] was originally reviewed by three anonymous reviewers. One of the two outside reviewers thought that the suggestion of a robust in-house control did not make sense as a validation approach and recommended rejection. The second reviewer had significant reservations but suggested several changes. A request was made to revise the paper. A second figure demonstrating the variations in absolute CD4 counts was added (Fig. 2). On re-examination, this second reviewer felt that their previous suggestions were not fully implemented. Overall, there was the notion that patient samples are being recommended as robust in-house controls in place of traditional or conventional controls for validation. The authors responded noting that “this type of in-house and patient-base validation indeed provides a good and regular quality control in a field where external QC is scarce and often different from actual patient samples, as stated in the guidelines, although they obviously cannot replace such controls.”

In further discussion, it was noted that although many of the patients did indeed have reasonably consistent or stable data, there would be exceptions. It was noted that patient compliance with therapy would have to be part of such a QC process. It was finally decided that a short report might be warranted but it should be accompanied with appropriate commentary before advocating such an approach to other laboratories.

Toward that end, the following two opposing commentaries have been provided. The first commentary is from Dr. Frank Mandy who was for many years in charge of the QC/QA program for Health Canada. Dr. Mandy also had experience with CD4 T-cell counting in the developing world. With financing provided by the U.C.L.A. Fogarty Institute, Dr. Mandy ran Quality Assessment and Standardization for Immunological measures relevant to HIV/AIDS (QASI) from Canada, an external quality management program without cost to participants. QASI included approximately 500–600 laboratories from 50 countries, mostly from Africa, the Americas, and Asia.

Dr. Mandy’s commentary begins. “The authors go back to the classic dilemma surrounding management of quality control and quality assessment strategy differences confronting scientists in clinical chemistry versus immunophenotyping of leukocyte subsets in infectious immunology. The authors do not dissect the two types of daily internal quality assessment that the operator must conduct. First, demonstrate that the instrument is performing within acceptable limits and next, validate specimen processing and reagent stability. The authors suggest that the daily immunophenotyping processing assessment can be eliminated without compromising repeatability and reproducibility of test results. This position is supported by accumulative data from 32 HIV-infected individuals over a period exceeding a decade based on CD3/CD4/CD8 values and previously published information by Bender (1992) and O’Gorman (2008) regarding antiretroviral therapy (ART) efficacy monitoring. This unorthodox approach to use the robustness of the immunophenotyping data of HIV-infected individuals under treatment is open to criticism. The authors base their conclusion on accumulative results of individuals on ART to serve as longitudinal in-house process control over 12 years with variable follow-up frequency from 7 to 63. To convince quality laboratory managers, a much larger study is required where the noncompliant individuals and drug resistance cases are confirmed independently from CD4 T-cell counts and are removed from the data. The remarkable consistency of CD4 T-cell counts over long time is open to suspicion. Patients were probably preselected, as getting the common cold will cause dramatic drop in CD4 T-cell count, smoking or exercises can also have significant impact. In addition, over a decade, many individuals must have had change in ART cocktail composition, which usually has impact on CD4 T-cell levels.

There is a fundamental distinction between specimens analyzed for immune status assessment and monitoring assay performance. Addressing such disregard to conventional quality management approach will require more rigorous support to substantiate credibility. The role of external quality assessment was ignored. However, the author’s data suggest some promise and it should be considered as a valuable technical observation.
This study has significant merit in situations where immunophenotyping is performed in a rural and remote location with extremely limited infrastructure. If the options are to run the assays without stabilized whole blood and/or fresh volunteer’s blood as daily control, or shut down immunophenotyping, such a makeshift daily control, is a justifiable alternative solution.

The second commentary comes from Thomas Denny from the Duke Human Vaccine Institute and Center for HIV/AIDS Vaccine Immunology; Dr. Denny’s IQA team has more than 15 years combined experience administering a NIAID IQA Program.

Dr. Denny indicated that “while this cohort selected for this study shows consistent absolute CD4 counts over a period of time, I cannot recommend application of this method of QC for resource poor settings for a variety of reasons. As noted by the previous reviewer, there are multiple levels of QC involved in Flow Cytometry: QC of instrumentation, QC of specimen processing, and QC of reagent stability. The authors of this manuscript suggest that internal QC of all three parameters can be assessed using routine patient immunophenotyping results. The premise of such analysis is supported by their own dataset of 32 patients monitored between 1999 and 2012. However, the patient dataset provided here is retrospective, and therefore patients that have long periods of stability can be selected after the fact. However, in real-time, it will not be possible to determine what patient would exhibit such stability. In particular, in resource poor settings it may be difficult to identify a suitable cohort of patients—compliant with ART therapy, visits routinely and consistently.

It is unclear what is the acceptable level of variability (RSD, robust standard deviation) for this method of proposed QC. For example, UPN #3 which is shown in Figure 1 has an AC CD4 range from 585–1040 with an RSD of 15%, which is lower than the average RSD reported for the cohort (18%). If a reading falls out of the acceptability range, it would not be clear whether the reason for change is due to a change in the patient, or a problem with instrumentation, reagent, and/or processing. This underscores the importance of having internal QC practices for the different parameters associated with flow (instrument, reagent, processing) and of external quality assurance programs. QC is only useful when the root problem can be identified, and therefore can be addressed.”

Editorial Comment: Now you the reader can decide for yourself. This section of the journal is open to present controversies and provide a forum for multiple viewpoints. One of the goals of this section is to promote discussion and thought in areas of controversy or where consensus development is needed. Also welcome are discussions of questions, or concerns, relating to the daily practice of clinical cytometry. This includes standards for quality control/quality assurance, safety issues, laboratory management, professional considerations, and results reporting.

Sincerely

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