January 21, 2010

NIAID DAIDS FLOW CYTOMETRY LABORATORY CERTIFICATION

Laboratories performing flow cytometric enumeration of lymphocyte subsets for NIAID-sponsored studies must participate in the NIAID DAIDS Flow Cytometry Quality Assessment Program.

Every other month, laboratories receive from the Quality Assessment Program a shipment comprised of 5 coded whole blood specimens. For these coded specimens, laboratories are required to measure and report the percent of T cell lymphocytes and the count [per micro liter] for the following phenotypes:

CD3+CD4+ using FSS/SSC gate
CD3+CD8+ using FSS/SSC gate
or

CD3+CD4+ using CD45 as an anchor gate
CD3+CD8+ using CD45 as an anchor gate

measured using the same tubes (same markers and fluorochromes) as are used on specimens from patients enrolled in NIAID-sponsored investigations. Laboratories are evaluated every four months (a trimester) for their ability to perform lymphocyte subset Phenotyping on a total of 10 coded specimens.

Some of the 10 specimens are replicates, allowing for evaluation of both intra-lab (within-lab) performance and inter-lab (between-lab) performance.

Inter-lab performance for a specimen is unacceptable if residual values for CD4 (CD3+CD4+) or CD8 (CD3+CD8+) are $\geq$5%, or $\leq$-5%, with deviates $\geq$2 or $\leq$-2. The residual value is the lab's value minus the median of all participating laboratories analyzing blood from that donor on that shipment. The deviate is the residual divided by the inter-quartile range (IQR). The IQR is three-quarters of the difference between the 25th and the 75th percentiles of results from laboratories analyzing blood from that donor on that shipment. The median and IQR of a replicate set are determined by including all labs and all specimens in that set of replicates.

Intra-lab performance is unacceptable if the range of the replicate values (highest minus lowest) within a given lab is $\geq$4%.

EXAMPLE OF AN INTER-LAB PERFORMANCE EVALUATION ON A SINGLE SPECIMEN

(Assuming that the median reported result on the singleton (not replicate) specimen is 40% and that only 7 laboratories analyze blood from that donor on this shipment and if that the IQR of the results from the 7 labs is 3%)

Lab	Reported CD4% value	Median Reported	Residual = value -med	Deviate= residual/3	performance based on this sample
A	**29**	**40**	**-11**	**-3.67**	**bad**
B	**38**	**40**	**- 2**	**-0.67**	**good**
C	**38**	**40**	**- 2**	**-0.67**	**good**
D	**40**	**40**	**0**	**0**	**good**
E	**41**	**40**	**1**	**0.33**	**good**
F	**42**	**40**	**2**	**0.67**	**good**
G	**45**	**40**	**5**	**1.67**	**good**

The table above shows the values obtained by the individual laboratories (A-G), their residuals, their deviates and whether or not their performance for that sample is acceptable. [Note that (7 + 1)/2 = 4, so that the median is the fourth smallest value, or 40%. (7+1)/4 =2 so the 25th percentile is the second smallest value, or 38%. The 75th percentile is the second largest, or 42%. Therefore, the inter-quartile range (IQR) is 0.75*(42-38) = 3.]

In each trimester there are a total of 6 possible inter-lab determinations and 2 intra-lab determinations. Each determination receives a mark of "good" or "bad" (please see example below). A set of replicate specimens is counted as only one mark, no matter how many replicates are in the set. Thus, there are a total 8 possible marks for the trimester. A lab that receives one-third or more bad CD4 T-cell marks or one-third or more bad CD8 T-cell marks has failed the trimester. If a lab fails for either phenotype (CD4 or CD8), the lab is considered to have failed the trimester.

If a lab fails to analyze any specimens in the trimester due to exclusions (e.g. mailing problems), the "one-third" rule will be applied to the number of marks possible based on the specimens that were analyzed by the lab.

EXAMPLE OF INTER-LAB AND INTRA-LAB PERFORMANCE EVALUATION
(hypothetical results, raw data not provided)

	CD4 T-CELLS	CD8 T-CELLS
INTER-LAB EVALUATIONS: Residual ≥5% and deviate ≥2 = bad		
Spec. #A	Bad	Good
Spec. #B-1 Spec. #B-2 Spec. #B-3	Bad	Good
Spec. #C	Bad	Good
Spec. #D-1 Spec. #D-2 Spec. #D-3	Good	Good
Spec. # E	Good	Good
Spec. #F	Good	Good
INTRA-LAB EVALUATION: Highest value minus lowest value ≥4% = bad		
Spec. #B-1 Spec. #B-2 Spec. #B-3	Good	Bad
Spec. #D-1 Spec. #D-2 Spec. #D-3	Good	Good
BAD MARKS/TOTAL MARKS	3/8	1/8
PERCENT BAD MARKS	37.5%	12.5%
TRIMESTER PERFORMANCE	Unsatisfactory	satisfactory

In the above example, for %CD4, the lab received 3 bad marks for *inter-lab* evaluation and none for *intra-lab* evaluation, totaling 3 bad marks out of a possible 8, which is considered unsatisfactory. For %CD8, the lab had no bad marks for *inter-lab* evaluation, but received one bad mark for *intra-lab* evaluation, totaling 1 bad mark out of a possible 8, which is considered satisfactory. The hypothetical lab in this example failed the trimester based on CD4 T-cell performance.

Late Reporting: Laboratories that are late in reporting their proficiency testing data will be notified via email in within 10 working days following the due date. For each late-reporting notification, laboratories will be penalized equivalent to **one bad** mark for CD4 and **one bad** mark for CD8.

Late Answers to Queries: In the course of the analysis of a shipment, the IQA may notice some results from a laboratory that are far enough from the median that there appears to be a possibility that either the specimen numbers were confused or that the results were mistyped. The IQA will call or email the laboratory to ask that they double-check their data. The laboratory has a total of 5 working days to answer these queries. If the answer is not received within this time period, the laboratory will be penalized equivalent to **one bad** mark for CD4 and **one bad** mark for CD8.

PERFORMANCE RATING

Laboratories are rated Certified, Provisionally Certified, Probationary or Suspended.

I. **Certified Status:** These laboratories may accept and analyze patient specimens from their own site and other NIAID DAIDS study sites.

Demotion from Certified Status: Failure to perform at the satisfactory level for 1 trimester will result in a change of status from Certified to Provisionally Certified at which time the Site Immunologist/Flow Cytometry Laboratory Director will receive the following notification:

> *As the result of your unsatisfactory performance in the NIAID DAIDS Flow Cytometry Quality Assessment Program in the last trimester [DATES], your laboratory moved from Certified to Provisionally Certified. Until your laboratory again attains the status of Certified, you may assay patient specimens from your own study site but may accept specimens from outside sites **only** from NIAID-sponsored study sites for whom you are **currently** analyzing patient specimens. Certified status may be regained after 2 consecutive trimesters of satisfactory performance in the NIAID DAIDS Flow Cytometry Quality Assessment Program.*

II. **Provisionally Certified Status:** Laboratories are Provisionally Certified when they first enroll in the Program or when demoted from Certified Status. Provisionally certified laboratories may assay patient specimens from their own clinical site and may accept specimens for analysis from other NIAID-sponsored study sites ***only*** if the laboratory currently performs analyses for these sites.

Promotion to Certified Status: Certified status may be regained after 2 consecutive trimesters of satisfactory performance.

Demotion to Probationary Status: Unsatisfactory performance for 2 consecutive trimesters will result in a change of status from Provisional to Probationary and the Clinical Site(s) /Flow Cytometry Laboratory Director and study site Principal Investigator will receive the following notification:

> *As the result of your unsatisfactory performance in the NIAID DAIDS Flow Cytometry Quality Assessment Program in the last 2 trimesters [DATES], your laboratory moved from Provisionally Certified to Probationary status. You are prohibited from analyzing patient specimens for any **new** single-center or small (less than 6 study sites) NIAID-sponsored trials even if the sites are your current clients, until your laboratory attains the status of Provisionally Certified. You may continue **current studies** (small or large) and*

accrue new patients to these studies, and you may participate in any ***new large*** *multicenter NIAID-sponsored trials. Provisionally Certified Status may be regained after 2 consecutive trimesters of satisfactory performance in the NIAID DAIDS Flow Cytometry Quality Assessment Program.*

III. Probationary Status: Laboratories are in a Probationary Status following demotion from Provisionally Certified Status. Probationary labs are prohibited from analyzing patient specimens for any new single-center or small (fewer than 6 study sites) NIAID-sponsored trials. Such a laboratory must send all patient specimens on such studies to another laboratory that currently holds Certified Status from the NIAID Flow Cytometry Quality Assessment Program. Such a laboratory may, however, continue to analyze patient specimens from on-going studies on which their site has already entered some patients, and they may participate in any new large (6 or more study sites) multicenter NIAID-sponsored trials. The Site Immunologist/Flow Cytometry Laboratory Director and Principal Investigator will be notified of Probationary status letter via Federal Express. Progress of these laboratories will be closely monitored.

Promotion to Provisionally Certified Status: Provisionally Certified status may be regained after 2 consecutive trimesters of satisfactory performance.

Demotion to Suspended Status: Laboratories that remain Probationary for 3 consecutive failed trimesters the laboratory will be recommended for Suspended status.

IV. Suspended Status: Laboratories are in a Suspended Status following demotion from Probationary Status. Suspended laboratories must send all NIAID DAIDS study specimens for analysis to a laboratory which currently has Certified Status from the NIAID Flow Cytometry Quality Assessment Program. Laboratories will be suspended by action of the Executive Committees) of the relevant NIAID DAIDS study group(s) [e.g. AIDS Clinical Trials Group (ACTG) Executive Committee; International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) Multicenter AIDS Cohort Study. The NIAID DAIDS Site Flow Cytometry Laboratory Director and Principal Investigator and network will be notified of Suspension by email and followed up with a Federal Express letter sent to the laboratory.

Promotion to Probationary Status: The criteria for regaining Probationary status will be determined by a joint recommendation of the NIAID DAIDS Flow Cytometry Advisory Committee and the appropriate Executive Committee.