Q & A Column, 2/14

Editor: Frederick L. Kiechle, MD, PhD

Submit your pathology-related question for reply by appropriate medical consultants. CAP TODAY will make every effort to answer all relevant questions. However, those questions that are not of general interest may not receive a reply. For your question to be considered, you must include your name and address; this information will be omitted if your question is published in CAP TODAY.

Submit a Question

[hr]

Q. We are thinking about using a reference laboratory for HER2 FISH testing of breast carcinomas with an arrangement in which that lab performs the technical component and we perform the interpretation. A "frequently asked question" from 2011 on the CAP Web site seems to say that we must perform bright-field ISH proficiency testing to be in compliance, since we are not performing the hybridization and cannot refer PT to another laboratory. Can you clarify the PT requirement, if any, for this situation? The vendor we are dealing with has offered to establish its own FISH PT program.

A. Laboratories performing HER2 by FISH that refer the hybridization to another facility and interpret the results in-house cannot participate in commercial proficiency testing because this would violate the prohibition against PT referral. Laboratories would need to perform an alternative assessment twice per year. This is outlined in CAP checklist requirement ANP.22973 and in the frequently asked questions for HER2 and ER/PgR on the CAP Web site, as follows:

Is participation in proficiency testing (PT) required for all sites that do HER2 testing?
Yes. In order to be compliant with the CAP/ASCO HER2 guidelines, any laboratory that reports results of such testing must participate in an accepted PT program (see exception below). The CAP Accreditation Program requires participation in a CAP-accepted PT program.

Exception: Laboratories that interpret and report the results of HER2 testing by FISH in which the hybridization is performed at an outside laboratory should not enroll in proficiency testing for that assay due to prohibitions on proficiency testing referral by CMS; such laboratories must perform alternative assessment. This exception does not apply to laboratories that interpret and report the results of HER2 testing by immunohistochemistry when staining is done at an outside facility.

The ASCO/CAP guidelines for HER2 testing apply only to breast carcinoma. HER2 testing on other tumor types (e.g. gastric carcinoma) is not covered by these guidelines at the current time.

Linda Palicki, MT(ASCP)
Director, Continuous Compliance, Laboratory Accreditation Program

College of American Pathologists, Northfield, Ill.

[hr]

Q. In the HIV testing article in the December 2013 issue of cap today, the HIV testing algorithm proposes confirming all the HIV-positive or indeterminate samples using nucleic acid amplification testing. Why not screen all the negative samples as well? According to the article, RNA assays turn positive at 10 days after infection. Third- and fourth-generation assays turn positive 22 and 17 days after infection. Why leave this seven- to 12-day gap? Is it just the matter of cost, because the NAT assays are more expensive?

A. The algorithm proposes NAT to test specimens that are reactive by fourth-generation (antigen-antibody combination) assays but negative or indeterminate by the antibody differentiation test. Using NAT to test all specimens that are negative by antibody or antigen-antibody assay would identify a small number of additional cases but increase turnaround time and substantially increase cost. Several programs have used NAT on specimens that are negative by the initial immunoassay. To reduce the cost of NAT, pools of 10 to 20 specimens are tested with a single NAT; specimens from NAT-reactive pools are then tested individually to determine which one is reactive.[1] In the Emerson study, they stopped testing specimens from low-risk patients because of cost and lack of yield. NAT is not suitable as the initial assay. In addition to the increased cost, two percent to five percent of specimens that are positive for HIV antibody have undetectable RNA.[2] Therefore, both an antibody test and NAT must be performed to detect all persons with HIV infection. The proposed algorithm minimizes the number of tests that need to be performed and, when initial testing is performed with an antigen-antibody combination immunoassay, detects 75 percent to 83 percent of specimens that are NAT-reactive but antibody-negative.

1. Emerson B, Plough K. Detection of acute HIV-1 infections utilizing NAAT technology in Dallas, Texas. *J Clin Virol.* 2013;58 Suppl 1:e48–53.
2. Owen SM, Yang C, Spira T, et al. Alternative algorithms for human immunodeficiency virus infection diagnosis using tests that are licensed in the United States. *J Clin Microbiol.* 2008;46(5):1588–1595.

Bernard M. Branson, MD
Associate Director, Laboratory Diagnostics, Division of HIV/AIDS Prevention
Centers for Disease Control and Prevention, Atlanta

[hr]

Q. With automated CSF/body fluid cell counting becoming more the norm, is it acceptable to report the automated RBC count with a qualifying comment from the cytospin of "RBC ghosts present," rather than resort to a manual RBC count, in which some technologists do not include the RBC ghost cells in the RBC count, so there is a potential discrepancy in automated versus manual RBC counts?

A. We do not count degenerating, or ghost, cells—including red blood cells and white blood cells—under any circumstances. This question raises the issue of the proper verification of an automated analyzer for the reporting of body fluid cell counts. Verification is confirmation of the validation the manufacturer performed that provides evidence that the analyzer meets specific requirements. If a laboratory is using an automated analyzer to report body fluid cell counts, then that analyzer must be properly verified using actual body fluid samples (for example, CSF, synovial, serous). Performance specifications that must be confirmed include accuracy, precision, patient correlation, carryover, lower limits of detection, linearity/analytical reportable range, and evaluation of interfering substances. In most laboratories this will require correlation of RBC and WBC/TNC counts from a hemocytometer to

the automated analyzer. A laboratory that has previously established body fluid cell counting on an automated analyzer may be comparing one analyzer to another. If this method verification has been properly done, and lower and upper RBC and WBC/TNC counts have been established for the reporting of body fluids on the automated analyzer, then there should be no discrepancy between automated versus manual RBC counts—if the counts are within the analytical reportable range for the instrument. If the RBC count is higher than the upper threshold, then dilutions will need to be performed. If the RBC count is lower than the lower threshold, then a hemocytometer cell count will be necessary. In this latter scenario, the hemocytometer may disclose ghost/degenerating RBCs, and therefore an accurate RBC count cannot be reported. In this case, a technologist may issue a comment stating that many ghost/degenerating RBCs were present and they were unable to perform a count.

1. Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Standard—Second Edition. H26-A2. Clinical and Laboratory Standards Institute. 2010.
2. Body Fluid Analysis for Cellular Composition; Approved Guideline. H56-A. Clinical and Laboratory Standards Institute. 2006.

Tracy I. George, MD
Associate Professor of Pathology, Chief, Hematopathology Division
Director, Hematopathology Fellowship, University of New Mexico Health Sciences Center, Albuquerque
Former chair and immediate past advisor, CAP Hematology/Clinical Microscopy Resource Committee

[hr]

Dr. Kiechle is medical director of clinical pathology, Memorial Healthcare, Hollywood, Fla. Use the reader service card to submit your inquiries, or address them to Sherrie Rice, CAP TODAY, 325 Waukegan Road, Northfield, IL 60093; srice@cap.org.