Q&A Column, 11/13

Editor: Frederick L. Kiechle, MD, PhD

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Submit a Question

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[pulledquote]Q. Can you explain the logic behind doing a full workup for identification and sensitivity on multiple positive blood culture bottles on the same patient, drawn on the same day?[/pulledquote]

A. Blood cultures are considered the gold standard for detection of sepsis. While most episodes of bacteremia and/or sepsis are thought to be monomicrobial, in some instances polymicrobial sepsis, typically in immunocompromised patients, has been described.[1,3] In general, two to three blood cultures should be obtained for patients suspected to have sepsis, the first two sets drawn in sequence from two different peripheral venipuncture sites. The third set should be obtained four to six hours after the initial two sets. For the majority of sepsis patients, no additional blood cultures should be obtained, and likewise obtaining a blood culture for test of cure is not recommended.[1,3] Even when two to three blood culture sets are collected according to published recommendations,[2,3] the length of time to detection for the individual culture bottles in each set and between sets may vary. In patients with sepsis, most blood cultures will turn positive within eight to 20 hours.

Once an index positive blood culture has been identified in a patient, a complete organism identification and antimicrobial susceptibility testing will be performed. For subsequent positive blood cultures, it is not necessary to repeat the completed and detailed organism workup that was done for the index positive culture, when the organism has the same Gram stain and colony morphology as the initial organism. In these instances, performing a few bench tests, such as catalase, coagulase, indole, PYR, and others, should be used to verify that it is the same organism/strain.[2,4] The organism should be reported as "probable genus/species," and antimicrobial susceptibility test results can be referred to the original strain. While this approach is applicable to blood cultures drawn within a 24-hour period, no guidelines have been developed for blood cultures collected past the initial 24-hour time window. Anecdotal, unpublished data suggest that polymicrobial bacteremia may be detected in such subsequent cultures, therefore making repeat full identification and antimicrobial susceptibility testing necessary.

In other instances, it might be necessary to repeat antimicrobial susceptibility testing, when the organism has developed antimicrobial resistance under treatment. Assuming that the initial two to three sets of blood cultures were obtained according to guidelines, there should be in general no need to repeat blood culture collection daily. At the earliest, five days after the first blood culture was obtained and with suspicion of treatment failure, additional blood cultures may be obtained at that point. Should those additional cultures become positive, a "repeat" full workup may be indicated. Good communication between the laboratory and the treating physician(s) is essential in these cases to optimize patient outcomes.

1. Baron EJ, Weinstein MP, Dunne WM, Yagupsky P, Welch DF, Wilson DM. Cumitech 1C. Blood Cultures IV. Baron EJ, coordinating ed. Washington, DC: ASM Press; 2005.

2. Garcia L. Blood cultures. In: Garcia LS, Isenberg HD, eds. *Clinical Microbiology Procedures Handbook*. 3rd ed. Washington, DC: ASM Press; 2010.
3. Riedel S, Carroll KC. Blood cultures: key elements for best practices and future directions. *J Infect Chemother*. 2010; 16:301–316.
4. Clinical and Laboratory Standards Institute. Abbreviated Identification of Bacteria and Yeast; Approved Guideline—Second Edition, M35-A2. Wayne, Pa.: Clinical and Laboratory Standards Institute: 2008.

Stefan Riedel, MD, PhD, D(ABMM)
Assistant Professor, Department of Pathology, The Johns Hopkins University School of Medicine
Director, Clinical Pathology Laboratories, Johns Hopkins Bayview Medical Center, Baltimore, Md.
Member, CAP Microbiology, Resource Committee

[pulledquote]Q. With regard to fasting, what is the gold standard for lipid status assessment? Some people seem to be advocating unrestricted nonfasting.[/pulledquote]

A. The basis for the initial recommendation of using a fasting sample for lipid profile testing is related to the method used at the time for measuring low density lipoprotein-cholesterol (LDL-C), the main atherogenic lipoprotein upon which most cardiovascular risk guidelines are now based.1 Until the advent of direct or homogeneous LDL-C assays about 10 to 15 years ago, almost all labs calculated LDL-C, using the Friedewald equation (LDL-C = Total Cholesterol–HDL-C–Triglyceride/5).2 This equation works because in a fasting sample, the only lipoproteins present are LDL, HDL, and VLDL. If the units are all in mg/dL, the term Triglyceride/5 provides a close approximation of VLDL-C. By using this equation, one can get a reasonably good estimation of LDL-C from a fasting sample without having to resort to a tedious ultracentrifugation step, which would otherwise be needed to separate VLDL from the other lipoproteins. HDL-C can be readily measured from a fasting sample after a precipitation step to remove LDL or VLDL.

The use of direct tests for measuring HDL-C and LDL-C, which do not require the physical separation of lipoproteins, has now made the requirement of using a fasting sample for lipid profile testing somewhat obsolete. Triglycerides can markedly increase after a meal, but for most individuals HDL-C and LDL-C do not significantly differ between the fasting and nonfasting state. Some studies suggest that for some lipid profile tests, the postprandial state may be better for predicting cardiovascular disease risk.3 Simply measuring nonHDL-C (cholesterol on all lipoproteins besides HDL) may also be better as a cardiovascular biomarker than LDL-C, and this parameter is also relatively unaffected by eating.4 Because of the difficulty in getting children to fast, the recent pediatric guidelines for cardiovascular risk testing suggest that a nonfasting nonHDL-C test is suitable as an initial screening test.5
There is concern, however, about the accuracy of some direct LDL-C and HDL-C tests, particularly in patients with dyslipidemias.6 In addition, one study has shown that a particular direct LDL-C test does not seem to be as predictive for cardiovascular disease risk when used on nonfasting versus fasting individuals.7 Because of these concerns and the extra cost of performing a direct LDL-C test, many labs still only offer the calculated LDL-C by using the Friedewald equation and thus require a fasting sample. It is, therefore, important to know what particular lipid tests a clinical laboratory is performing. The new National Cholesterol Education Program guidelines for diagnosing and treating cardiovascular disease are now also under active review, and one should await these new recommendations on the best laboratory practices for cardiovascular disease risk assessment.

1. Executive summary of the third report of the National Cholesterol

Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *JAMA*. 2001;285:2486 –2497.
2. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18:499–502.
3. Mora S, Rifai N, Buring JE, Ridker PM. Fasting compared with nonfasting lipids and apolipoproteins for predicting incident cardiovascular events. *Circulation*. 2008;118:993–1001.
4. van Deventer HE, Miller WG, Myers GL, et al. Non-HDL cholesterol shows improved accuracy for cardiovascular risk score classification compared to direct or calculated LDL cholesterol in a dyslipidemic population. *Clin Chem*. 2011;57(3):490–501.
5. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. *Pediatrics*. 2011;128 (suppl 5):S213–256.
6. Miller WG, Myers GL, Sakurabayashi I, et al. Seven direct methods for measuring HDL and LDL cholesterol compared with ultracentrifugation reference measurement procedures. *Clin Chem*. 2010;56(6):977–986.
7. Mora S, Rifai N, Buring JE, Ridker PM. Comparison of LDL cholesterol concentrations by Friedewald calculation and direct measurement in relation to cardiovascular events in 27,331 women. *Clin Chem*. 2009;55(5):888–894.

Alan T. Remaley, MD, PhD
Section Chief, Lipoprotein Metabolism Laboratory Cardiopulmonary Branch
National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Md.
Member, CAP Chemistry Resource Committee

[pulledquote]Q. We have traditionally verified new lots of reagents by running our two levels of quality control. If we don't see any change in the mean values for either level, we put the reagents into use. On some tests (for example, tumor markers like CEA), we also run a small number of patient samples, covering a medically relevant range, in parallel on the new lot and current lot, but we don't do this for the vast majority of the assays we run. Some of the other CAP-accredited labs in the local area have told us that our practice is not acceptable.[/pulledquote]

A. It is possible that your practice of using QC for lot-to-lot validation is acceptable, but it really depends on the nature of the QC samples you are using. This issue is addressed in two checklists (all common and chemistry and toxicology). They are respectively:
COM.30450 New Reagent Lot Confirmation of Acceptability. "New reagent lots and/or shipments are checked against old reagent lots or with suitable reference material before or concurrently with being placed in service."

CHM.13400 Calibration/Calibration Verification Criteria. “Criteria are established for frequency of recalibration or calibration verification, and the acceptability of results.”

Both require that new reagent lots and/or shipments be tested against old/current lots prior to being placed into service “…to ensure that calibration with the new lot of reagent maintains consistent results for patient specimens.”

Both emphasize that patient specimens are the best material to use. However, several alternatives are specified in COM.30450:

1. Reference materials or QC products provided by the method manufacturer with method-specific and reagent-lot–specific target values.

2. Proficiency testing materials with peer-group–established means.

3. QC materials with peer-group–established means based on interlaboratory comparison that is method specific and includes data from at least 10 laboratories.

4. Third-party general purpose reference materials if the material is documented in the package insert or by the method manufacturer to be commutable with patient specimens for the method. Commutability between reference materials and patient samples can be demonstrated using the protocol in CLSI EP14-A2.

5. QC material used to test the current lot is adequate alone to check a new shipment of the same reagent lot, as there should be no change in potential matrix interactions between the QC material and different shipments of the same lot number of reagents.

If your QC material is provided by the method manufacturer with method- and lot-specific target values, or if it has peer-group–established means based on at least 10 laboratories, then your practice would be acceptable. Otherwise, you cannot use your QC material to verify a new lot of reagent (though it can be used to verify a new shipment of the same lot, as indicated in No. 5 at left).

Gary L. Horowitz, MD
Medical Director, Clinical Chemistry, Beth Israel Deaconess Medical Center
Associate Professor of Pathology, Harvard Medical School, Boston
Chair, CAP Chemistry Resource Committee

David N. Alter, MD
Spectrum Health System, Grand Rapids, Mich.
Vice Chair, CAP Chemistry, Resource Committee

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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.