# Q&A column

## 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.

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**Q. Can bronchoalveolar lavage specimens from multiple lobes be pooled for culture? Can multiple biopsies from the same joint be pooled for culture?**

A.June 2022—These are straightforward questions with somewhat complex answers. In general, the advantages of pooling samples include saving time, labor, raw materials, laboratory space, and money. Yet there are downsides. One potential disadvantage is the dilution of microbes within a specimen to below the limit of detection or threshold of clinical significance. Another potential drawback is the loss of sampling-site information that could be used for clinical-pathologic or radiologic-pathologic correlation.

With these factors in mind, it is not uncommon for laboratories to pool samples for fungal or mycobacterial cultures, including from bronchoalveolar lavage (BAL) and joint tissue biopsies. However, there is a lack of literature that examines this practice. For other types of organisms, a more nuanced discussion is needed, and practices may vary across laboratories. Additional considerations for pooling also differ between BAL specimens and joint tissue biopsies.

For routine microbiological analysis of BAL fluid, literature and guidelines predominantly center around the diagnosis of hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP). Infectious Diseases Society of America (IDSA) guidelines describe the use of quantitative BAL cultures for diagnosing HAP/VAP, employing a cutoff of $10^4$ CFU/mL for clinical significance, though the guidelines favor noninvasive methods, such as endotracheal aspiration with semiquantitative culture.[1]

The guidelines do not address the practice of pooling samples from different bronchial segments, but there are a handful of papers that indirectly address this practice. Zaccard, et al., calculated that pooling samples from the right and left lung would have allowed detection of all bacterial species originally above the $10^4$ CFU/mL cutoff in 92.5 percent (160/173) of samples in their study.[2] In 3.5 percent (6/173) of samples, at least one, but not all, bacterial species would have been detected. Four percent (7/173) of the samples would have been falsely negative. As would be expected, failure to detect bacterial species occurred with lower concentrations of bacteria near the $10^4$ CFU/mL cutoff in the nonpooled samples. A study by Jonker, et al., found 74 percent discordance between radiologic findings and sites of BAL culture positivity, and there is disagreement in the literature about whether the presence or absence of infiltrates on radiography correlates with culture positivity at all.[3-5] These and other studies have been largely conducted in surgical and trauma patients. The methodologies for BAL fluid collection vary. Depending on the methods used, dilution due to pooling may be comparable to dilutions observed in higher volume BAL procedures.[6,7] Ultimately, laboratories that want to pool BAL samples might first discuss this undertaking with relevant stakeholders or conduct an internal study to evaluate the effects of pooling on culture quantitation and sensitivity, or both.

Laboratories may have the same concerns about pooling joint tissue biopsies for routine culture as pooling BAL specimens, but, in practice, the former is more straightforward. Past and present guidelines for diagnosing prosthetic joint infection, including those from the MusculoSkeletal Infection Society, IDSA, European Bone and

Joint Infection Society, and American Academy of Orthopaedic Surgeons with the endorsement of the IDSA, recommend performing as many as five or six tissue biopsy cultures, with a threshold of two or more positive cultures indicating infection versus contamination.[8-11] As a result, pooling samples could reduce sensitivity and the ability to discriminate between contaminants and pathogens.

Recommendations for diagnosing native joint infections, on the other hand, do not mention performing multiple biopsies and instead generally advise using clinical findings, radiologic findings, synovial fluid cultures, and blood cultures.[12] However, orthopedists may apply the prosthetic joint infection criteria to native joints. In such cases, it would be worth discussing with requesting orthopedists how best to proceed.


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**Q. We verify our reference intervals with each new reagent lot for coagulation tests (PT, APTT, fibrinogen, and TT). What difference in values between lots necessitates establishing a new reference interval?**

**A CAP TODAY Q&A from January 2015 mentions limits within 1.5 seconds of each other between new and old reagent lots for human recombinant PT. What about limits for APTT and fibrinogen?**

A.The January 2015 Q&A answered by Russell A. Higgins, MD, and John D. Olson, MD, PhD, discussed initial determination of coagulation test reference intervals. The response addressed how many reference individuals should be tested (based on statistical concepts and practical considerations, such as laboratory resources), how to analyze the distribution of coagulation test data (nonparametric versus parametric) to determine appropriate statistical methods, and how to determine data outliers, as well as the clinical significance of the upper limit of the normal reference interval for coagulation testing.

In my experience, most coagulation laboratories verify their existing reference intervals for new lots by testing 20 to 40 carefully selected healthy reference individuals who have no history of a bleeding or thrombotic disorder or medical condition that may affect the coagulation system. As an example of this type of evaluation, Drs. Higgins and Olson mentioned an approach wherein they accept the reference interval for prothrombin time (PT) new reagent lots if the new and old limits are within 1.5 seconds of each other.

Because definitive guidance specific to each coagulation test (PT, activated partial thromboplastin time [APTT], fibrinogen, etc.) is not available, I recommend following the Clinical and Laboratory Standards Institute general guidance for defining, establishing, and verifying reference intervals. This CLSI document indicates that an existing reference interval can be verified by transference by testing as few as 20 reference individuals. If no more than two of the 20 values (10 percent of results after ensuring the data is free of outliers) fall outside the reference interval, the reference interval is considered acceptable for use with the new lot number. If more than 10 percent of the values fall outside the reference interval, an additional 20 healthy reference individuals can be tested. If less than 10 percent of the new results fall outside the reference interval, the reference interval is considered verified. If five or more of the original 20 values fall outside the reference interval or if more than 10 percent continue to fall outside the reference interval after testing additional reference individuals, the laboratory should consider

establishing a new reference interval after confirming the reference individuals were from a representative population and excluding preanalytical issues.

Our laboratory generally tests 40 reference individuals over five days to incorporate typical run-to-run variation. We have had great success verifying reference intervals based on the CLSI recommendations.

Clinical and Laboratory Standards Institute. EP28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline, 3rd ed.; 2008.

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