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.

Q. Is it a requirement that routine bacteriology cultures (for example, urine, sputum) be plated in a biological safety cabinet in your typical hospital biosafety level 2 laboratory? Is it safe to read these cultures on an open bench?

A.March 2024—Generally, all specimens sent to a clinical microbiology laboratory are considered potentially infectious. Specimens may contain a variety of pathogens that can cause a plethora of infectious diseases. Therefore, clinical laboratories must be primed to process these specimens.

Because laboratories may not be prepared for every pathogenic agent and laboratory personnel may not fully recognize the risks associated with handling clinical specimens, it is important that laboratories follow standard precautions and biosafety level 2 practices to minimize exposure to pathogens.

Biosafety in Microbiological and Biomedical Laboratories, published by the Centers for Disease Control and Prevention and the National Institutes of Health, recommends that all procedures that have the potential for creating infectious aerosols or splashes be conducted within a biological safety cabinet or other physical containment devices.

In addition, the American Society for Microbiology, in its "Interim Clinical Laboratory Guideline for Biological Safety," states that: "Microorganisms with a low infectious dose, such as *Brucella* spp., *F. tularensis*, and *Y. pestis*, pose the highest risk of infection from primary specimens; however, specimens containing these organisms can still be handled safely using BSL-2 precautions. Exception is reserved for manipulations with a high risk of droplet or aerosol generation, in which case escalated BSL-3 precautions should be considered."

Clinical laboratories should implement a risk-management approach to biosafety and execute mitigation actions based on the risks identified within their laboratories.

Regarding the reader's second question, routine bacteriologic cultures derived from clinical specimens in BSL-2 laboratories can be safely manipulated on an open bench unless there is suspicion of a highly infectious pathogenic agent, in which case appropriate physical containment equipment should be used.

Buchan BW, Relich RF, Mahlen SD. Interim clinical laboratory guideline for biological safety. American Society for Microbiology. Jan. 11, 2019. www.asm.org/Guideline/Interim-Clinical-Laboratory-Guideline-for-Biologic

Centers for Disease Control and Prevention, National Institutes of Health. *Biosafety in Microbiological and Biomedical Laboratories*. 6th ed. U.S. Dept. of Health and Human Services; Rev. June 2020. www.cdc.gov/labs/BMBL.html

World Health Organization. *Laboratory Biosafety Manual*. 4th ed. World Health Organization; 2020. www.who.int/publications/i/item/9789240011311

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Q. What source should a laboratory use for reference intervals for analytes?

A. A reference interval—also known as a reference range, normal range, or expected value—is the span of results for a specific analyte found in a healthy population. The bounds of the reference interval are often determined by the central 95 percent of a healthy population; the 2.5th percentile and 97.5th percentile define the lower and upper limits, respectively. Reference intervals allow a physician to determine whether a test result indicates potential underlying pathology. It is a CAP checklist requirement (COM.29950) to provide them, if they exist.[1] Good laboratory practice requires labs to periodically review reference intervals and update them as applicable.[2]

Reference interval data may come from a population-based study conducted by the laboratory or from an assay manufacturer or another laboratory, or the data may be pulled from textbooks and literature. Arguably, establishing one's own reference interval is considered the gold standard. Detailed guidelines for determining, establishing, and verifying reference intervals are available from the Clinical and Laboratory Standards Institute.[3] Identifying healthy people using strictly defined inclusion criteria is key, as is taking into consideration preanalytic variables (for example, fasting versus nonfasting) and analytic variables (for example, the analytic sensitivity and specificity of an assay), detecting and excluding outliers, and deciding whether to partition based on sex, age, and/or ethnicity.

However, the cost and logistics of establishing a reference interval likely exceed the capabilities of the vast majority of laboratories. This is due in part to the CLSI recommending a minimum of 120 people for each partition or subgroup. Therefore, it is more common to adopt a reference interval from an external source. When doing so, laboratories should evaluate the original study to identify factors in the study design, such as demographics, sample size, preanalytic and analytic variables, and statistical methodology, that may produce bias. For example, a published interval may have been performed decades ago solely on a Caucasian population by a method no longer widely in use, leading to a range that is far too wide and, therefore, increasing false-negative results.

Laboratories should perform a small verification study to determine the final acceptability of the chosen external reference interval by collecting samples from 20 people. If no more than two of the 20 results from this group fall outside the reference interval, then the interval is considered valid. If more than five fall outside the interval, the laboratory should consider finding an alternative reference interval. If three or four results fall outside the interval, the laboratory should collect samples from an additional 20 individuals. If no more than two results from this new set fall outside the interval, the interval is considered valid. However, if three or more results fall outside the interval, the laboratory should reconsider its chosen reference interval.[3]

An a posteriori, or indirect, sampling approach for establishing a reference interval is another option, although it is not generally recommended by the CLSI due to the likelihood of including people who have a disease in the study population.[3] This procedure relies on harnessing vast amounts of testing data that the laboratory collected during routine clinical care and applying filters to it to arrive at a set of supposedly healthy individuals from which to extrapolate a reference interval.[4]

As mentioned, logistical and financial challenges often preclude laboratories from routinely establishing their own reference intervals, making the adoption of a previously published reference interval more common. In the end, the decision to adopt one interval versus another comes down to evaluating a multitude of variables, including analytical methods, patient demographics, and the statistical analysis employed, to identify which interval is most comparable and applicable to the laboratory's instrumentation and patient population.

Once a candidate reference interval is identified, conducting the verification study is the final check before using it

in patient care. However, nothing is ever static. Assays evolve, instruments get replaced, and the patient population itself can change. Therefore, it is perhaps most important for a laboratory to continually assess the appropriateness of its reference intervals and make adjustments as needed. Sometimes a suitable published interval can no longer be found, and the laboratory has little alternative but to establish its own.

1. College of American Pathologists. COM.29950 Reference intervals. In: All common checklist. Aug. 24, 2023.
2. International Organization for Standardization. ISO 15189 Medical laboratories—requirements for quality and competence. Dec. 2022.
3. Clinical and Laboratory Standards Institute. EP28-A3: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory, Approved Guideline, 3rd ed. 2008.
4. Farrell CJ, Nguyen L. Indirect reference intervals: harnessing the power of stored laboratory data. *Clin Biochem Rev*. 2019;40(2):99–111.

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