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.
When reporting reference ranges for absolute differential counts, should the ranges be age specific or is a single reference range acceptable?

A.January 2022—Laboratories should evaluate healthy people within such categories as gender, age group, and genetic background when establishing institutional reference intervals for a given analyte. This is necessary because various intrinsic and extrinsic factors can influence RIs in a laboratory or patient population, or both. This evaluation also helps determine where certain populations may overlap and, therefore, where groups may be combined.[1] Reference intervals are usually based on Gaussian distribution and defined as the range of values into which 95 percent of healthy (nondiseased) individuals fall. Accordingly, five percent of healthy people will have laboratory results above or below the established RI.

Recruiting healthy individuals, particularly from the pediatric population, to establish institution-specific RIs can be costly and time intensive and may preclude laboratories from running robust studies. Laboratories that cannot perform institution-specific studies may use previously established RIs from a laboratory database of test results.[2] It takes a much smaller pool of participants to verify previously reported RIs, making these studies more palatable to most laboratories. Regardless of the method employed, the reporting laboratory should independently verify or validate the RIs and tailor them to its patient population. Independent verification is important as normal ranges vary slightly depending on the automated analyzer used.

Immune cellular constitution matures throughout childhood and adolescence and is relatively stable in adulthood (over 18 years old). The total white blood cell count and absolute differential count for cell types tend to be higher in newborns and decrease until about five to 10 years of age. Because of these variances, most published RIs are partitioned into different groups for neonates through 18 years old. These partitions vary by institution and depend on the initial studies performed or prior studies verified. Total WBC counts and differential counts show small variances between males and females, with secondary partitions provided for gender. Large studies have found no significant differences between males and females with regard to WBC counts and differential counts and no age dependency between 18 and 100 years old. Therefore, RIs for the age interval of 18 to 100 years for men and women are usually combined. Age-dependent RIs more accurately represent the patient population being examined and, therefore, provide clinicians with a better platform for diagnosing and managing various disease states.[3]

A common question is whether to provide WBC RIs as proportional amounts (percentages) or absolute counts, or both. According to the Clinical and Laboratory Standards Institute, providing absolute concentrations of WBCs is preferred given that these are the medically significant values.[4] In accordance with the CLSI recommendation, the College of American Pathologists hematology and coagulation checklist item HEM.36820 states, “For WBC differential counts, the CAP recommends that laboratories report absolute cell counts, along with their corresponding reference intervals. The CAP discourages the reporting of percent cell counts without absolute counts on WBC differentials.”[5]

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2. Soldin SJ, Brugnara C, Wong EC. *Pediatric Reference Intervals*. 6th ed. AACC Press; 2007.
3. Valiathan R, Ashman M, Asthana D. Effects of ageing on the immune system: infants to elderly. *Scand J Immunol*. 2016;83(4):255–266.
4. Clinical and Laboratory Standards Institute. H20-A2: Reference Leukocyte (WBC) Differential Count (Proportional) and Evaluation of Instrumental Methods; Approved Standard, 2nd ed.; 2007.
5. College of American Pathologists. HEM.36820 Reference intervals. In: Hematology and coagulation checklist. Sept. 22, 2021.

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Q. Is it acceptable to use polystyrene tubes for aliquotting plasma for coagulation tests, such as platelet aggregation, and factor-related studies requiring serial dilutions of plasma? I recall seeing recommendations for using nonpolystyrene tubes for frozen plasma aliquots but did not see a reason for the recommendation.

A. The Clinical and Laboratory Standards Institute document “H21-A5: Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays” indicates in section 5.3.1.2 that secondary tubes used for plasma-based coagulation assays should be composed of nonactivating material, such as polypropylene, and not polystyrene.[1] However, during the review and comment period for this document, a commenter pointed out that the warning against polystyrene containers is not

supported by the literature. I reviewed the reference provided for this section to confirm that it does not mention polystyrene tubes.

While many laboratory collection guidelines recommend using polypropylene tubes, I could not find definitive studies in the literature indicating that polystyrene should not be used for plasma-based coagulation testing. An archived CLSI document titled “H58-A: Platelet Function Testing by Aggregometry” does not mention polystyrene tubes either.[2] However, a 2019 publication by Hechler, et al., indicates that platelet preparation for laboratory platelet-function testing can involve polypropylene or polystyrene tubes.[3]

If a laboratory is considering changing tube types for a given coagulation or platelet assay, I recommend performing validation studies to compare the existing tube type to the new tube type.

1. Clinical and Laboratory Standards Institute. H21-A5: Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline, 5th ed.; 2008.
2. Clinical and Laboratory Standards Institute. H58-A: Platelet Function Testing by Aggregometry; Approved Guideline; 2008.
3. Hechler B, Dupuis A, Mangin PH, Gachet C. Platelet preparation for function testing in the laboratory and clinic: historical and practical aspects. *Res Pract Thromb Haemost. 2019;3(4):615–625*.

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