

**Western Pathologist Quality Assurance Association**  
SM. Hossein Sadrzadeh, Ph.D., FACB

**A Practical Approach to Establish Reference Ranges**

To establish a diagnosis, laboratory (and other clinical) data from a patient are compared to reference values. The terms reference values, reference limit, reference interval, and observed values, were recommended by the International Federation of Clinical Chemistry (IFCC) to replace the obsolete term normal ranges. The reference range for a laboratory test can be defined as a range in which at least, 95% of test results from apparently healthy individuals would fall. Determination of reference ranges is a costly and cumbersome task, which is clearly defined in the guidelines by the National Committee for Clinical Laboratory Standard. Fortunately, most manufacturers in diagnostics industry follow those guidelines to establish reference ranges for their analytical methods. Therefore, it is not necessary to “re-invent the wheel”. A laboratory can simply adopt or transfer the reference ranges established by the manufacturer or another literature for its own methodologies, as long as the methodologies used by both institutions are identical. Indeed, CLIA and CAP guidelines allow transference of reference ranges from manufacturers, if the adopting laboratory can verify the ranges. According to CAP commentary 01:42080 for many analytes (e.g., therapeutic drugs and CSF total protein), literature reference or a manufacturer’s package insert information may be appropriate.” To verify the previously established reference range, the laboratory should randomly select 20 apparently healthy individuals from their native population (medical technologists, medical students, volunteer blood donors, etc.). Also, it is acceptable to use “normal” population from hospitalized patients. However, it is imperative to apply certain criteria such as selecting only patients hospitalized for cosmetic surgery, cataract operation, etc. The selected population should represent both genders and be adjusted for age. Thus, specimens should be collected considering the effects of pre-analytical variables such as age, gender, fasting status, diet, medications, posture, circadian rhythm and medical history. Obviously, the specimens should be analyzed using the identical systems used to establish the original reference range. Following the analysis, the collected data should be displayed graphically and viewed. If the data represent a bell-shaped or Gaussian distribution (i.e., parametric), then mean  $\pm$  2 SD (standard deviation) should be calculated and the range should be compared to the established range. However, it is more likely that data will be nonparametric or skewed. In that case, the high and low 2.5 percentile (outliers) are thrown out and the central 95% reference range should be selected and compared to the established reference range. The outliers should be examined more carefully and if determined that they are affected by the above pre-analytical variables, they should be excluded and the new subjects should be recruited and data should be analyzed again. If the laboratory’s range is “close” to the manufacturer’s range, the donor’s range can be adopted. Generally, if two or less or the subjects fall outside the range then the reference can be adopted. If three or more subjects fall outside the reference range the subjects should be reviewed carefully for interference by pre-analytical variables, and if such variables detected the new “normal” subjects should be recruited and the study should be repeated. If three of the repeated values fall

outside the reference range, the laboratory should follow the NCCLS guideline to establish its own reference test.

References:

1. Sasse EA. Determination of reference intervals in the clinical laboratory using the proposed guideline National Committee for Clinical Laboratory Standards. C289-P. Arch Pathol Lab Med, 116:710-713, 1992.
2. Solberg, H. Establishment and use of reference values. In: Burtis, C, Ashwood, E. eds. Tietz, Textbook of Clinical Chemistry. Philadelphia, Penn: W.B. Saunders Company: 336-356, 1999.
3. Young Ds. Determination and validation of reference intervals. Arch Pathol Lab Med. 116: 704-709,1992.