Reference Intervals: Practical Approaches

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Almost as important as the analyses done on clinical samples are the reference intervals that accompany them. Indeed, it is difficult, if not impossible, to interpret any laboratory measurement unless one knows what the reference interval is and how the reference interval was determined. Far too often, rather than establishing our own reference intervals, laboratories use reference intervals provided by manufacturers or found in textbooks, often without even verifying them. Despite our fears about statistics, establishing reference intervals by the most widely recommended method is not overwhelmingly difficult. And verifying for our own use reference intervals established elsewhere is well within the reach of every clinical laboratory.

It is important, at the outset, to distinguish between “medical decision limits” and “reference intervals”. For the former (e.g., cholesterol, hemoglobin A1c), physicians use national (or international) guidelines, so laboratories do not have the luxury of establishing their own reference intervals but must ensure that the values they report are accurate. For the latter, what is required at the start is a group of reference individuals on whom the laboratory tests will be performed. These individuals must be carefully defined and screened. Pre-analytical issues must be carefully controlled (e.g., diet, exercise, time samples are obtained, specimen preparation (centrifugation, storage conditions, etc.). Analytical procedures, especially when trying to replicate values from the literature or when participating in a multi-center trial, must also be precisely defined and followed.

The preferred method for establishing reference intervals is to collect data from 120 reference individuals and then to analyze that data non-parametrically. One needs 120 reference individuals for each partition (e.g., males/females, and/or age intervals, and/or different ethnic groups). The power of non-parametric analysis is that it allows one not to worry about the underlying distribution (e.g., whether or not it is Gaussian).

With fewer than 120 reference individuals, one may be able to use other techniques, including mathematical techniques to render distributions Gaussian as well as more recent techniques such “robust” statistical procedures. Also, if laboratories with existing well-established reference intervals need to update them for new analytical methods, they may be able to use transformation to avoid repeating their reference interval studies from scratch.

In practice, though, most laboratories simply adopt reference intervals established elsewhere. Such laboratories should at least verify the appropriateness of these reference intervals for their methods and their patient populations, which can be accomplished with as few as 20 reference individuals. If no more than 2 of these 20 individuals have values outside the proposed reference interval, then it is reasonable (and statistically valid) to adopt the reference interval. (Of note, if several (e.g., 6 or more) laboratories using the same method were to do such verifications, together they would have a sufficient number of samples to establish a reference interval by the standard non-parametric method, at least for one partition.)

During this session, several concrete examples will be reviewed to help illustrate these techniques and how simple and powerful they can be.