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4. Analytical Quality Specifications

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4.1 Overall Goal for Analytical Quality Specifications

It has become dogma that reference intervals for biochemical quantities (components) are method dependent and, therefore, are individual to laboratories. This may have arisen largely because a plethora of factors, including laboratory methodology, do affect reference values. The dogma has been further stimulated by external quality assessment schemes and proficiency testing schemes in which considerable differences between analytical methods and commercial kits have not only been tolerated but also stimulated by grouping into so called method dependent or peer groups.

There are a few exceptions from this general approach which include the National Cholesterol Education program in USA and German control schemes (Ringversuche and Indstand). However, more correct approaches seem to be developing in US, and through working groups under the auspices of European External Quality Assessment Organizers. In consequence the future may show a general and more informed attitude to the question of transferability of data and, thereby, also to the development and use of common reference intervals.

It may be that ethnic differences exist for some quantities, as illustrated by Harris *et al.* (2) as well as age- and sex-dependent differences but this does not provide a rationale for method dependent reference intervals. These biological differences, however, should be described in detail so that the information could be shared by all laboratories.

The purpose therefore is to evaluate the analytical quality specifications required for the performance needed for using common reference intervals in laboratories where the populations are homogeneous for a quantity - irrespective of whether there is one single interval or several are required according to known differences in the populations.

Assumptions for the Models

A number of assumptions have to be fulfilled for establishing common reference intervals:

1. The population - or well described subsets - must be homogeneous for the quantity.
2. The inclusion and exclusion criteria for the reference sample group must be clear.
3. The preparation of reference individuals before sampling must be well defined.
4. The sampling technique must be standardized.
5. Handling, preparation, and storage of samples must not influence the quantity, neither the structure nor the concentration or activity.
6. The model for statistical calculations must be in accordance with the actual distribution of data.
7. The number of reference individuals must be sufficient.
8. The assays must be performed with an analytical quality which is better than the quality specifications for sharing common reference intervals - as given below. This infers standardization with traceability of concentration values and specific measurement procedures.

The Models

For homogeneous healthy groups many quantities are distributed symmetrically or with a positive skewness, allowing for application of one of two statistical models, namely Gaussian and log-Gaussian.

The evaluation of the Gaussian model is simpler and it is, therefore, used for the principal evaluation.

IFCC has given recommendations for estimation of reference intervals where a major point is that at least 120 individuals should be used for the calculation of reference intervals in order to keep the uncertainty about these limits low *(5)*. For a Gaussian distribution, this corresponds to a 0.90 confidence interval of each limit of 0.24 times the biological standard deviation, or, that the fraction of individuals outside each limit - due to the uncertainty of the sample variation - with 0.90 certainty is between 0.013 and 0.044 - instead of the 0.025 which is expected from an interval calculated as mean $\pm$ $1.96 * s_{biological}$.

The basis of the model for evaluation of analytical quality specifications is to estimate the reference intervals based on a greater number of individuals (e.g. 800), so as to make the sample uncertainty negligible - and then allow for analytical error instead of sampling error - giving the same maximum uncertainty as allowed by the IFCC.

The Quality Specifications

Based on this concept, the maximum allowable analytical error - combined bias and imprecision - must not decrease or increase the fraction outside each reference limit more than 0.013 to 0.044.

A graphical evaluation *(1)* reveals a functional relationship between maximum allowable analytical bias and imprecision as shown in fig. 4.1.

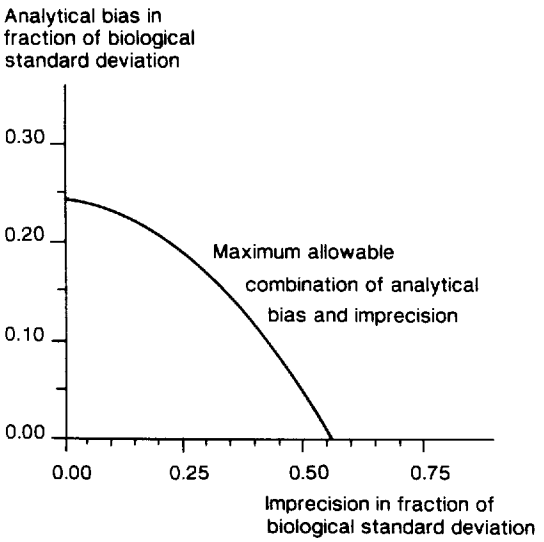

Fig. 4.1. *Relationship between maximum allowable combination of analytical bias and imprecision according to the described concept. The units on the axes are fractions of the biological standard deviation, $s_{biological}$. From Hyltoft Petersen and Hørder (4) with permission.*

The figure shows that the maximum allowable bias is $|B_A| < 0.24*s_{biological}$ when analytical imprecision, s_A, is negligible, and that the maximum allowable imprecision is $s_A < 0.55*s_{biological}$ when B_A is negligible. For all other combinations, the values have to be interpolated from the curve. These are the analytical quality specifications for sharing common reference intervals when the distribution is Gaussian.

For log-Gaussian distributions, the procedure is the same for log-values and the analytical quality specifications are the same on the log-level. The computations, however, are somewhat more difficult. Therefore, a graph may help in making the estimates by reading off from a curve. This functional relationship is given in fig. 4.2. The most important fact from log-Gaussian distributions is that the analytical quality specifications are dependent on the ratio between the upper and lower reference limits, whereby, the specifications are given as a net of curves. Moreover, the specifications are given in terms of percentage bias and percentage coefficient of variation.

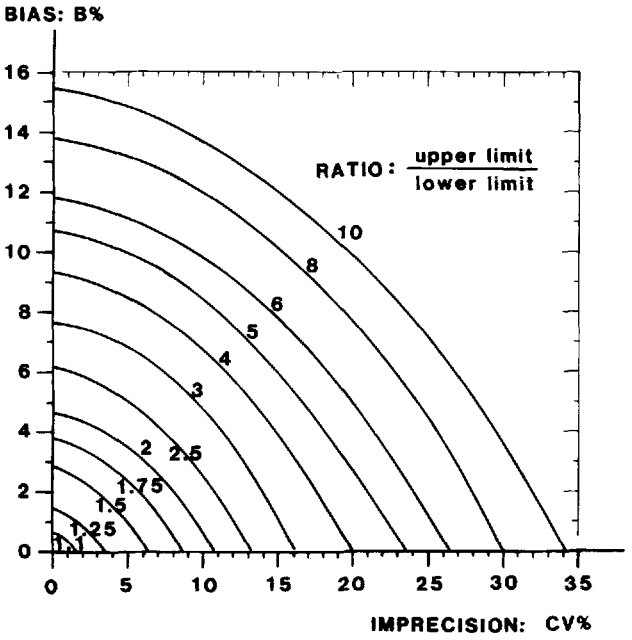

Fig. 4.2. *Approximated maximum allowable combination of analytical bias (%) and coefficient of variation (%) for log-Gaussian distributions. The specifications depend on the ratio between the upper and lower reference limits. From Hyltoft Petersen et al. (3) with permission.*

When analytical quality specifications are interpolated from the curves (fig. 4.2.), then the analytical imprecision should have been subtracted from the distribution in order to get the *isolated* distribution (see below).

Based on the concepts of sharing common reference intervals and of allowing for analytical bias and imprecision instead of uncertainty from samples size it is possible to estimate analytical quality specifications for all endogenous quantities.

Possible Modifications

Even with the best analytical methods, there will be some uncertainty during measurements of the reference values and, in consequence, in the estimate of reference intervals. Furthermore, laboratories using common reference intervals will disclose - at least - some inherent imprecision, which must be included in the stated reference interval. It might, therefore, be considered whether a reasonable low imprecision should be included in the estimation of reference intervals. If so, then the analytical quality specifications are slightly changed, and for laboratories with stable perfomance close to the imprecision obtained during measurements of reference samples, the specification for analytical bias will be of major importance. For quantities with high ratios between the upper and lower reference limits the effect will be negligible as long as CV_A is below 5% but, for quantities with low ratios, it may be necessary to use the combined analytical and biological CV for the estimation of reference intervals. In chapter 7 this pragmatic approach is applied to S-Albumin.

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