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Evidence-based approach to harmonised reference intervals

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ABSTRACT

Although we are in the era of evidence-based medicine, there is still a substantial gap between theory and current practice with the application of reference intervals as decision making tools. Different laboratories may have different reference intervals for the same tests using the same analytical methods and platforms. These differences have the potential to confuse physicians making the assessment and monitoring of patients more difficult by providing discordant information.

This paper attempts to demonstrate how to use evidence-based approach for harmonising reference intervals. In order to consider harmonisation we must first have an appreciation of the various factors that influence the determination of that reference interval such as the choice of individuals within the population studied, biological variability of the analyte studied, partitioning, sample collection, analytical aspects such as bias and statistical models.

An *a priori* approach for determining reference intervals, whilst recommended, may be beyond the scope of most laboratories and consideration should be given to the use of a validated indirect *a posteriori* approach. Regardless of method used, the continuing application of an evidence-based approach in harmonised reference intervals to meet the quality expectations of physicians should be pursued.

1. Introduction

It has been 50 years since Schneider made the statement that comparison is the foundation of practical medicine [1]. The most widely used decision making tool in that comparison for laboratory medicine is the reference interval [2]. But to provide an adequate degree of confidence for the clinical decision making process, determine whether or not an individual is healthy and to ensure that we are able to provide appropriate comparison we must understand the basis of how we derive such intervals [3]. Reference intervals should be determined in a systematic and scientific manner [4] using evidence-based approach, and where appropriate and the evidence allows, harmonise to allow commutability between laboratories, regions and countries.

The use of the range of results from a reference population for a reference interval, *i.e.* the spread from lowest to highest, is considered inappropriate as this will give too wide range to be useful and has a high likelihood of including individuals with underlying disease. The width of the 100% reference interval also depends on the size of the population tested with extreme values more likely in larger groups. The convention is to take the central 95%, although variations have been introduced for specific circumstances. For example where there is no medical value in a lower reference value we may report to the 95th or 97.5th percentile. For troponin the 99th percentile is recommended [5] to obtain higher clinical specificity. Clinical decision limits for analytes such as glucose and HbA1c have been recommended by expert bodies.

Although we are in the era of evidence-based medicine, there is still a substantial gap between theory and practice with respect to the application of reference intervals as decision making tools [3].

Closing this gap between theory and current practice is not easy. The many complex issues surrounding reference intervals must be considered. The determination of an appropriate reference interval is time consuming, and requires significant data and considerable professional judgement [6]. Addressing this gap can be considered from a number of key stakeholder perspectives. Ceriotti [3] identifies these as:

- Manufacturers of diagnostic tests who operate in an increasingly global market;
- Clinical laboratory professionals who wish to ensure they provide high quality analyses and reports;
- Clinicians who want to base clinical decisions on valid data;
- Patients who want to minimise confusion caused when the same test undertaken in different laboratories produces conflicting results.

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The appropriateness of using the reference intervals supplied by diagnostic reagent and equipment manufacturers in their Instructions for Use (IFU) for global clinical decision making is questionable. The manufacturers are often North American, quote very small sample populations from which the 'expected values' are derived, do not include health status data for those populations and in some cases draw the reference populations from hospital-based groups. Significant differences may exist in disease, ethnic makeup of the population selected, as well as specimen collection and handling procedures, test performance and statistical processes. As the information about these factors is not generally provided by manufacturers it is difficult to assess the suitability of the supplied interval for local use. The chemistry of the analytical system may also have changed over time without revision of the interval.

The basic principle underlying any diagnostic test and reference interval should be that it provides a valid and reliable basis to distinguish between a result that is most likely to lie within a "healthy" category and a result that is most likely to lie within a "disease" category. Reference intervals can be supplied by any "reference population" which may be defined using criteria other than verified good health. However for this paper they will be considered as health-associated reference intervals unless otherwise specified. Some of the factors affecting the determination of reference intervals. Some of these are shown in Table 1.

We now have implicit acceptance that an evidence-based culture underpins the practice of laboratory medicine. This is now perceived as the scientific foundation of medicine [8].

2. Evidence-based medicine

What is generally accepted as the definition of evidence-based medicine (EBM) is "the conscientious, explicit and judicious use of current best evidence in making decisions about the care of patients" [9]. If we accept that there is a clinical question for which the relevance of a test result will lead to a decision being made and an action taken which leads to an improved health outcome, then Sackett's definition can be applied to the clinical laboratory [8]. Evidence-based medicine and laboratory medicine are essential in the assessment of the clinical effectiveness of those decisions on the clinical outcome [10].

Table 1
Factors affecting the determination of reference intervals adapted from Jones 2004 and C28-A3 [6,7].

Difficulties with laboratory-based reference intervals	A lack of direct comparability of results from different laboratories. ▪ Differences in laboratory reference intervals are commonly greater than can be explained by differences in the assays which are not related to standardisation ▪ Combining results from different laboratories in electronic databases is difficult to support ▪ The expense of performing effective reference interval studies in all laboratories for all analytes is prohibitive ▪ Development of some reference intervals, *e.g.* sex hormones, is beyond all but the most well-resourced laboratories.
Technical problems with reference intervals.	▪ True standardisation differences ▪ True local population differences ▪ Agreement on format of results & reference intervals
Practical issues with reference intervals.	▪ Requires organisation & support of a qualified body to oversee the project ▪ Ensure that population sampling is adequate to derive quality local data ▪ Use agreed statistical approaches for deriving reference intervals ▪ Disseminate both the criteria and derived reference intervals for use by all laboratories to encourage wide-spread adoption

In clinical medicine the highest level of evidence is related to patient outcome. For pathology tests the contribution to clinical outcome is the decision made based on the test results. Whilst the purpose of reference intervals is to provide a comparator for interpreting individual patient results, the effect of the reference interval on clinical outcomes has not been widely studied. The best available evidence may then be whether the interval provides the expected information *i.e.* that a result outside the interval indicates a level of specificity for the patient which is different from the reference population.

The specific topic of this paper is the use of an evidence-based approach for harmonising reference intervals. But first we must have an appreciation of the various factors that influence the determination of that reference interval.

The values associated with a reference interval for any particular analyte should be representative of a specific population and include consideration of a number of factors [11]:

- The choice of individuals within that population. These must be clearly defined to represent the population for which the reference values are to be determined.
- The biological variability of the analyte within that reference population and any need to partition into age and gender subgroups.
- Pre-analytical aspects such as sample collection and type of sample required.
- Analytical aspects such as method bias.
- Statistical models, including the choice of outlier exclusion methodology.

For common reference intervals there are additional aspects to consider:

- Differences in the populations served by different laboratories
- Differences in pre-analytical factors
- Differences in analytical processes.

3. Selecting the reference population

It is essential to decide in advance which individuals to select and how to partition them — as in the *a priori* approach recommended by the IFCC [7] Most commonly the reference population is one free from disease, *i.e.* "healthy". However the reference interval concept can be applied to any population. If deviating from the use of a healthy population it is important to convey this to clinicians as this is a deviation from the usual concept.

What is a healthy population? This is a concept that has been difficult to define over many decades. Health has been described as a "relative condition lacking a universal definition" [7] and becomes the initial conundrum in any Reference Interval study. There will be some level of uncertainty associated with the "health" of individuals within the study cohort because of the probability that some of these individuals may have subclinical disease. To reduce this possibility the scope of the reference interval should be clearly defined [12]. Once this is determined and understood the method of selecting the reference population can be defined. The requirements of the recruitment of individuals should include:

- A questionnaire that is specific for the studied analyte(s)
- A physical examination to identify those subjects who may have disease processes that would bias the outcome
- Further investigations such as laboratory tests or imaging to aid in the identification of underlying disease processes that may bias the outcome
- Ideally a regime to define who is included or excluded in the population studied (*a priori*).

Ethnic differences, including racial and cultural differences, cannot be neglected as has been demonstrated by Johnson et al. [13], where Caucasian and Asian Indians living in the same community in