

SEED Haematology



Reference intervals – and what Sysmex can offer

The actual meaning of ‘reference intervals’ and their purpose

An isolated result, considered on its own, is not very meaningful. Conclusions can only be drawn when it is compared to other values. A comparison with the same patient’s previous results shows whether the value is stagnant, rising or falling. A comparison with values found in healthy subjects allows an assessment to be made about whether the value is in the typical range for healthy persons or not. A comparison with decision limits allows an assessment to be made about whether other medical measures are indicated.

The range of values typical for healthy persons is called the ‘reference interval’. It is often also loosely referred to as the ‘normal range’. But what is ‘normal’? Laboratory results can be influenced by a number of parameters, from age and gender to dietary habits (more about this below). When talking about the ‘reference interval’, it is important to know what is being referred to. Is the reference cohort actually comparable? Only when this is the case is a reference interval applicable to a specific patient.

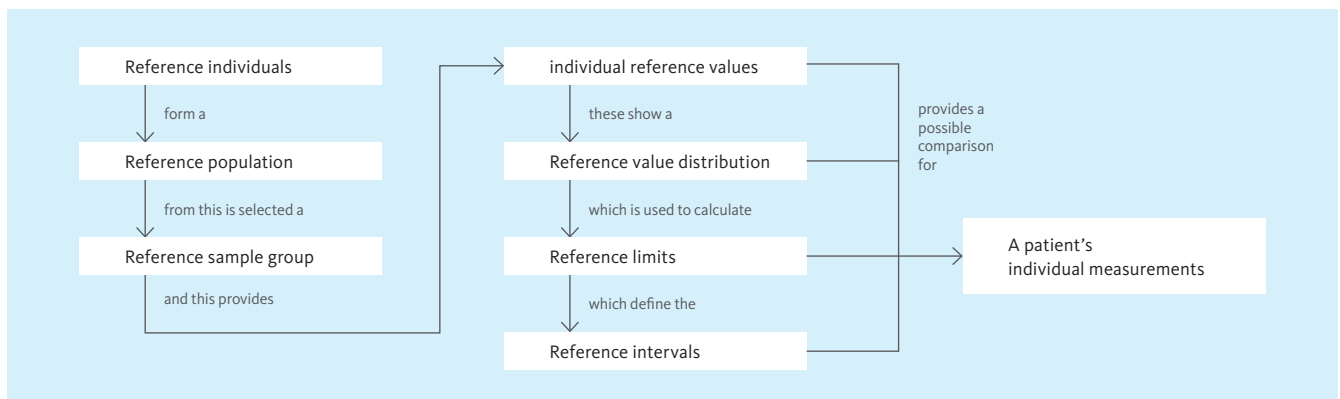


Fig. 1 Definitions

* Revision of the original article published in April 2015

By definition, the reference population consists of all reference individuals [1]. As a special case, this can be solely a particular patient with his or her previous values. However, such a population often consists of a large number of individuals where selecting the representative sample contingent can easily become an obstacle. Many criteria (age, gender, ethnicity, etc.) can be used to further define a reference population. When it is known that measurement results differ, for example in terms of age or gender, it is necessary to define subgroups of reference individuals accordingly. Groups of patients suffering from a certain disease can also serve as a useful reference population for individual patients with this disease. Such special reference intervals are helpful, for instance after bone marrow transplantation, in determining the remission of acute leukaemia, or during pregnancy. As is to be expected, the most frequently used reference intervals are derived from a healthy population. However, this raises the problem of how to define 'healthy'. There are no clear criteria for eliminating 'ill' persons from a reference population.

Prerequisites for determining reference intervals

Reference intervals frequently fall back on supposedly 'healthy' reference groups such as blood donors, young doctors, nurses, paramedics and medical students. However, it has been shown that the results in such readily accessible reference groups differ significantly from those of the general population and are therefore not representative. In short, each of these selections will create different reference intervals, although the differences are sometimes quite small [2–4]. A clear description of the procedure for determining the reference population is necessary for the reference intervals to be useful:

1. A patient's measurements must be compared with the reference intervals that apply to him or her. The same reference intervals cannot be used for different purposes (e.g. physiological studies in athletes or monitoring the treatment of a defined disease condition), so the description must include the purpose of the reference intervals.
2. Moreover, the criteria by which individuals are included in or excluded from the reference population must be explained. If the population has to be divided into subgroups (e.g. by age or gender), these characteristics must be known for each reference individual.
3. The reference individuals should always be as comparable as possible with the patients for whom the reference intervals are to be used.

The main factors that are known to influence the reference intervals and which may have to be considered are:

- Gender, age, ethnicity, social status and occupation, environmental conditions
- Nutritional status
- The circumstances of sample collection

Factors that can lead to different reference intervals in haematology include:

- Preanalytical logistics, sample age
 - Transport
 - Exposure to heat
- Different dietary habits
 - Iron status
 - Dehydration
- Different physical activity
- Marked differences in altitude above sea level
- Exposure to certain chemicals due to work, environmental pollution, smoking, etc.
- Others

Fig. 2 Factors that can influence haematologic reference intervals.

Procedure for determining applicable reference intervals

In light of this multitude of factors mentioned, the question may arise as to how reference intervals applicable for particular patients can be ensured. The simplest method to do so, and the one recommended by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) [3, 8], is an independent survey of reference intervals by the laboratory. To do this, suitable reference groups (e.g. a group of men and a group of women) are selected from a suitable reference population. Each investigated group should include at least 120 subjects after excluding subjects who do not meet the inclusion criteria. In order to exclude samples from persons who are not suitable for the reference group – for example, because they are under medication that influences the measurement results being investigated or because they are on an unusual diet – a questionnaire on this is useful, such as the one found in the Clinical and Laboratory Standards Institute CLSI/IFCC recommendation C28–A3, pages 10–11. The reference interval is then defined as the range in which 95% of the results derived from the subjects are located. If a parameter can show both pathologically elevated and decreased values, as is the case with most haematological

parameters [6–8], both the top 2.5% and the bottom 2.5% of the values are cut off and the remaining range is used as the reference interval. If the measurement result can only be pathologically elevated but cannot assume values that are too low, the top 5% of the values are discarded to obtain the reference interval. There is also a method for calculation based on only 80 subjects, but this is more complex mathematically (see Fig. 3). If different subgroups are considered, for example men and women, the values obtained can be examined to see whether there is a statistically significant difference. If this is not the case, the values can be combined into joint reference intervals. However, both 80 and 120 subjects are often regarded as an unacceptable workload, especially when several subgroups are considered so that 80 or 120 subjects are needed for each.

The above-described method for determining reference intervals is known as a 'non-parametric method'. Parametric methods are also used. These determine a reference interval through a calculation of the mean \pm double standard deviation. However, these methods assume that the investigated parameter follows a normal or Gaussian distribution, which is not the case for many parameters. In a few cases, log transformation, i.e. transformation of the values to their logarithm, can convert a non-normally distributed parameter into a normal distribution. However, this, together with back-transformation, increases the mathematical effort. The theoretical advantage of these methods is that they work with fewer subjects. However, the fact that they are not applicable to many parameters and the extra need to examine for a normal distribution substantially increases the mathematical effort.

Fig. 3 Parametric versus non-parametric methods to calculate reference intervals.

Examination of suitability for reference intervals determined elsewhere

Since, for the reason given above, simply adopting a reference interval published elsewhere is not permissible and can even be dangerous for patients, its suitability as a reference must be examined in every case. To do this, the IFCC suggests the following procedure [9]:

20 local reference samples are taken and compared with the reference interval published elsewhere. If no more than two of the 20 samples are outside this range, it can be used. If 3–4 samples are outside the reference interval, a further 20 new samples must be taken. If no more than two of these 20 samples are outside the range, it can be used. If, in the initial verification, five or more

samples, or more than two of a repeated set of samples, are outside the published reference interval, it is not suitable for use as a reference for local patients. The alternative is then a new survey (see above) or validation of another, different reference interval.

The different meanings of reference intervals and decision limits

If a patient's result is outside the reference interval, he or she does not fit into the reference population, but this does not necessarily mean that medical steps are required.

On the one hand, the values of 5% of the reference population are outside the reference interval anyway (as a result of the calculation) and the patient could simply be part of this percentage (bear in mind that 5% means '1 patient out of 20'). On the other hand, in mild forms of many diseases no intervention is necessary. Instead, the result is compared with decision limits, known as 'cut-off values'. These can be established, for instance, by analysis of a receiver operating characteristic (ROC) curve. Also, in many other areas, it is not crucial if a value can no longer be regarded as 'normal'.

This instead begins when a diagnosis can be established. In chronic diseases, decision limits can also be established, for example, according to therapeutic aspects. The question is then in which range the patient's situation is regarded as stable and when a change in therapy is indicated. In principle, methods for determining a reference interval can be applied analogously, as described above, to a reference group of stable patients. However, such decision limits are often based simply on medical experience and consensus within the corresponding specialty.

An indirect approach to determine reference intervals

A novel approach to determine reference intervals was recently published in an opinion paper by the IFCC Committee on Reference Intervals and Decision Limits (C-RIDL) [9]. This 'indirect' approach to determine reference intervals refers to a statistical analysis of already existing results from specimens which were collected for routine purposes (for example, samples collected for screening, diagnostic or monitoring purposes). This approach is not only a useful adjunct to the use of traditional direct methods, but also has a number of advantages such as basing the outcomes on the analytical and pre-analytical procedures in use, the ability to address a wide range of populations, especially the population served by the laboratory, and, importantly, the relative ease of use and far lower costs. In this opinion paper, the IFCC C-RIDL encourages laboratories 'to use indirect methods to evaluate their reference intervals in use, to estimate new reference intervals and to publish and share their results in appropriate detail and also to continue the search for new and improved techniques for the process'.

XN-Series reference intervals from the Dutch Lifelines cohort

In a multi-disciplinary prospective population-based study by L van Pelt *et al.* 2022 of the Dutch Lifelines cohort, all 105 parameters for XN analysers were evaluated [10]. The reference intervals were calculated in accordance with the statistical methods recommended by the IFCC.

The methods and reference intervals of this study are described in the SEED article ‘The art of defining reference intervals’.

Sysmex reference intervals

Even if Sysmex as a manufacturer has to provide reference intervals as a guide for its haematology analysers and does so in different publications, these cannot be applied blindly to patients for the reasons explained above. Sysmex provides a list of scientific publications that provide information about the reference intervals for Sysmex analysers (see Tab. 1). The publications show that the reference intervals change according to the populations studied. Published reference intervals from older generations of analysers may be used on analysers of newer generations but only after validation. Since there are differences between haematological reference intervals from different populations, the information in the table may facilitate the selection of an appropriate reference interval as a starting point for validation.

Table 1 Published reference intervals from Sysmex analysers

First author	Citation	Analyser	Parameters	Population
L van Pelt <i>et al.</i> [10]	Clin Chem Lab Med	XN-Series	CBC+DIFF+RET+PLT-F	Dutch
Mrosewski I <i>et al.</i> [11]	Clin Chem Lab Med	XN-9000	CBC	German, paediatric
Florin L <i>et al.</i> [12]	Int J Lab Hematol	XN-Series	CBC+DIFF+RET	Belgium, adult
Wilson S <i>et al.</i> [13]	Int J Lab Hematol	XN-3000	CBC+DIFF	Canadian, paediatric
Bohn MK <i>et al.</i> [14]	Int J Lab Hematol	XN-3000	CBC+DIFF	Canadian, paediatric
Ianni B <i>et al.</i> [15]	Arch Pathol Lab Med	XN-1000	CBC+DIFF+RET+PLT-F	US American, newborns
Arbiol-Roca A <i>et al.</i> [16]	EJIFCC	XN-Series	CBC+DIFF+RET	Spanish
Sysmex Corporation [17]		XN-Series	CBC + DIFF + RET	Japanese
Dockree S <i>et al.</i> [18]	EBioMedicine	XN-Series	WBC+DIFF	British, pregnant women (antenatal and postnatal)

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