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SEED HAEMATOLOGY

Reference ranges – and what Sysmex can offer

The actual meaning of 'reference ranges' and their purpose

First of all, an isolated result, considered on its own, is not very meaningful. Conclusions can only be drawn when it is compared with other values. Comparison with the same patient's previous results shows whether the value is stagnant, rising or falling. Comparison with values found in healthy persons permits assessment of whether the value is in the typical range for healthy persons or not. Comparison with decision limits permits an assessment of whether other medical measures are indicated.

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

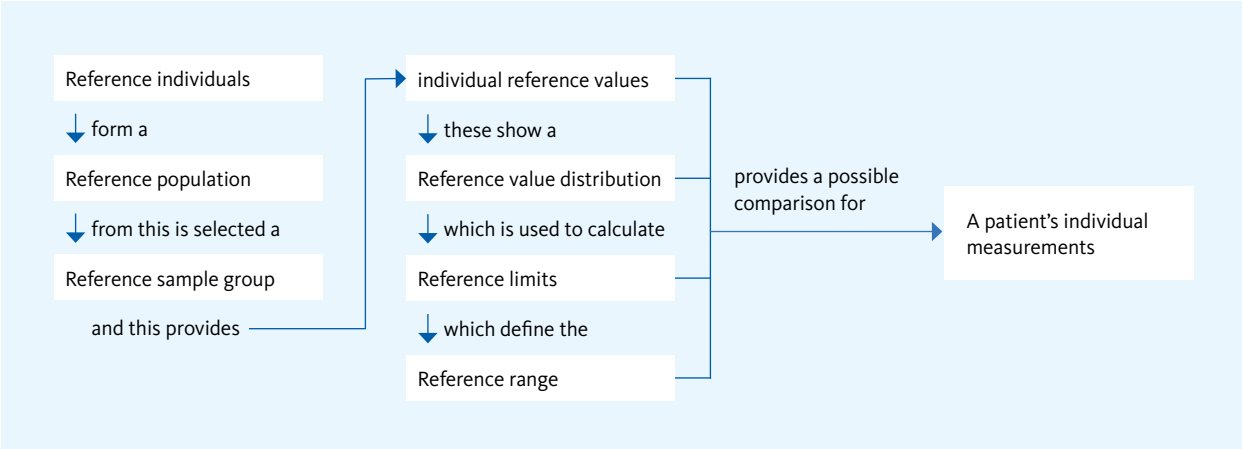

Fig. 1 *Definitions*

The reference population consists by definition of all reference individuals. 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, depending on 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 ranges are helpful, for instance, in determining remission of acute leukaemia, after bone marrow transplantation or during pregnancy. As is to be expected, the most frequently employed reference ranges 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 ranges

Reference ranges 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, all these selections will create different reference ranges, although the differences are sometimes quite small [1–3]. A clear description of the procedure for determining the reference population is necessary for the reference ranges to be useful:

1. A patient's measurements must be compared with the reference values that apply to her or him. The same reference values 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 values.

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 (by e.g. age, 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 ranges are used.

The main factors that are known to influence the reference values 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 ranges 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.

Fig. 2 *Factors that can influence haematological reference ranges*

Factors that can lead to different reference ranges in urinalysis include:

- Preanalytical logistics, sample age
  - Transport
  - Preservatives
  - Exposure to light and heat
- Different dietary habits
- Different physical activity
- Differences in obtaining the sample
  - First vs. second morning urine
  - Midstream urine, catheter urine ...
  - Hygiene of the genital region
- Exposure to certain chemicals due to work, environmental pollution, smoking ...
- Etc.

Fig. 3 *Factors that can influence reference ranges in urinalysis*

Procedure for determining applicable reference ranges

After this multitude of factors mentioned, the question may arise as to how reference ranges that are applicable for particular patients may be ensured.

The simplest method to do so, and the one recommended by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) [2, 7], is an independent survey of reference ranges 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 is useful, such as the one found on pages 10 and 11 of the Clinical and Laboratory Standards Institut CLSI / IFCC recommendation C28-A3. The reference range 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 [4–6], 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 range. If the measurement result can only be pathologically elevated but cannot assume too low values, as is often the case with cell counts in urinalysis, the top 5% of the values are discarded to obtain the reference range.

There is also a method for calculation based on only 80 subjects but this is more complex mathematically (see Fig. 4). 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 a joint reference range. However, both 80 and 120 subjects are often regarded as an unacceptable workload, especially where several subgroups are considered so that 80 or 120 subjects are needed for each of these.

The above described method for determining reference ranges is known as a 'non-parametric method'. Parametric methods are also in use; these determine a reference range through a calculation of the mean ± 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 to 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 increase the mathematical effort.

Fig. 4 *Parametric versus non-parametric methods to calculate reference ranges.*

Examination of suitability for reference values determined elsewhere

Since, for the reason given above, simply adopting a reference range 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 [7]:

20 local reference samples are taken and compared with the reference range published elsewhere. If not more than 2 of the 20 samples are outside this range, it can be used. If 3–4 samples are outside the reference range, a further 20 new samples must be taken. If not more than 2 of these 20 samples are outside the range, it can be used. If in the initial verification, 5 or more samples, or more than 2 of a repeated set of samples, are outside the published range, 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 range.

The different meanings of reference ranges and decision limits

If a patient's result is outside the reference range, he or she does not fit with the majority of the reference population but this does not necessarily mean that medical steps are required:

On the one hand, 5% of the reference population's values are outside the reference range 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 regarded as 'normal'. This rather 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 often are based simply on medical experience and consensus within the corresponding specialty.

Sysmex reference ranges

Even if Sysmex as a manufacturer has to provide reference ranges as a guide for its instruments and does so in different publications, these may not be applied blindly to patients for the reasons explained above.

Currently there are some scientific publications that inform about the reference ranges for Sysmex analysers (see Tab. 1). These studies have been done mainly using X-Class haematology analysers and it could be seen that the reference ranges change according to the populations studied.

Published reference ranges from older analysers may be used on newer analysers 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.

Reference ranges for XN-Series analysers have been established by Sysmex Corporation [14], but customers need to be aware that these are based on a Japanese population.

First author	Analyser	Parameters	Population
Hong *et al.* [8]	XE-2100	PLT	Han Chinese
Qiao *et al.* [9]	XE-2100	CBC	Han Chinese
Ambayya *et al.* [10]	XE-5000	CBC + research	Multi-ethnic Malaysian
Sehgal *et al.* [11]	XE-2100	CBC	Indian
El Graovi *et al.* [12]	XE-2100	CBC	Moroccan
Pekelharing *et al.* [13]	XE-5000	CBC + DIFF + RET	Dutch
Sysmex Corporation [14]	XN-Series	CBC + DIFF + RET + PLT-F	Japanese

Tab. 1 *Published reference ranges from Sysmex analysers*

References

[1] ***Haeckel R, Wosniok W and Arzideh F. (2007):***
A plea for intra-laboratory reference limits. Part 1. General considerations and concepts for determination. Clin Chem Lab Med 45 : 1033 – 1042.

[2] ***Henny J. (2009):***
The IFCC recommendations for determining reference intervals: strengths and limitations. LaboratoriumsMedizin 33 : 45–51.

[3] ***Ceriotti F, Hinzmann R and Panteghini M. (2009):***
Reference intervals: the way forward. Ann Clin Biochem 46 : 8–17.

[4] ***Wakeman L, Al-Ismail S, Benton A et al. (2007):***
Robust, routine haematology reference ranges for healthy adults. Int J Lab Hematol 29 : 279–283.

[5] ***Heil W and Ehrhardt V. (2008):***
Reference ranges for adults and children. Pre-analytical considerations. Mannheim: Roche Diagnostics.

[6] ***Bain BJ and England JM. (1975):***
Normal haematological values: sex difference in neutrophil count. Br Med J. 5953 : 306–309.

[7] ***Solberg HE. (2004):***
The IFCC recommendation on estimation of reference intervals. The RefVal program. Clin Chem Lab Med 42 : 710–714.

[8] ***Hong J, Min Z, Bai-Shen P, Jie Z, Ming-Ting P, Xian-Zhang H, Xiao-Ke H, Lan-Lan W, Xin Z, Wei G, Rui Q, Wen-Xiang C, Xin-Zhong W, Yue-Yun M, Hong S. (2014):***
Investigation on Reference Intervals and Regional Differences of Platelet Indices in Healthy Chinese Han Adults. J Clin Lab Anal; early online.

[9] ***Qiao R, Yang S, Yao B, Wang H, Zhang J, Shang H. (2014):***
Complete blood count reference intervals and age- and sex-related trends of North China Han population. Clin Chem Lab Med; early online.

[10] ***Ambayya A, Su AT, Osman NH, Nik-Samsudin NR, Khalid K, Chang KM, Sathar J, Rajasuriar JS, Yegappan S. (2014):***
Haematological Reference Intervals in a Multiethnic Population. PLoS One 18; 9(3) : e91968.

[11] ***Sehgal KK, Tina D, Choksey U, Dalal RJ, Shanaz KJ. (2013):***
Reference range evaluation of complete blood count parameters with emphasis on newer research parameters on the complete blood count analyzer Sysmex XE-2100. Indian J Pathol Microbiol 56(2) : 120–4.

[12] ***El Graoui O, Ellaabi S, Igala M, Elalloussi FZ, Nourchafi N, Mifdal H, Serhier Z, Othmani MB, Mikou KA, Oukkache B. (2014):***
Hematology reference intervals in Moroccan population. Clin Lab 60(3) : 407–11.

[13] ***Pekelharing JM, Hauss O, de Jonge R, Lokhoff J, Sodikromo J, Spaans M, Brower R, de Lathouder S, Hinzmann R. (2010):***
Haematology reference intervals for established and novel parameters in healthy adults. Sysmex Journal International Vol. 20. No. 1.

[14] ***Sysmex Corporation, Japan (2014):***
Reference ranges analysis document for XN series.

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