# THE IMPORTANCE OF ESTABLISHING LABORATORY SPECIFIC REFERENCE RANGES

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***Abstract:*** *The reference ranges provide valuable information for a correct interpretation of the laboratory results. In order to accurately interpret the laboratory test results in correlation with the data from clinical anamnesis and examination, the specialists rely on the availability of the reference ranges. Many laboratories have not established their own reference ranges, but they use those mentioned in the literature. Purpose: This paper aims at analyzing the opportunity of establishing specific reference ranges to the served population by each laboratory. The study is based on a systematic analysis and meta-analysis of the most cited papers of worldwide literature regarding the laboratory reference ranges for hemoglobin, serum iron and serum ferritin. For each parameter, two representative surveys were selected and analyzed in comparison. We have analyzed the benefits and limitations of each research method. The results of the analysis emphasize that there are significant differences between some age groups and the studies published in the scientific literature, thus establishing and validating specific reference intervals for the served population is a very important step. Conclusions: In conclusion, in order to prevent and reduce the risk of errors in diagnosis, it is necessary to establish the reference intervals for the served population. It is also important to establish the reference ranges by age, gender and ethnicity.*

## INTRODUCTION

In 1969 Gräsbeck and Saris introduced the term "reference interval", in order to replace the old definition of "normal values".(1) The reference ranges are useful for providing medical information that ensures correct medical decisions for the patient. This is achieved by generating reliable analytical results on patient samples.(2) Reference ranges provide valuable information for a correct interpretation of the laboratory results.(3) The clinicians use the term "reference range" to differentiate the sick subjects from the healthy subjects in the clinical medical practice.(4,5)

Each laboratory is responsible for ensuring the validity of the issued reference interval.(3) The laboratories are required to establish their own reference intervals according to the analyzed subjects, the analyzer that it is used and the quantitative methods they use.(4)

The concept of reference interval seems simple, but in reality obtaining samples from healthy individuals and establishing the reference intervals is a complex and difficult procedure.(3) In order not to incorrectly classify the results, the reference ranges inspired/provided by the external sources must be validated.(4) In the laboratory, the reference ranges shall not be used without being verified in advance because:

- The population tested by each laboratory is different in structure, age, gender;
- Tools, methods and reagents used in laboratories may differ.

The laboratories using the reference intervals from medical literature must check these intervals through a validation process; this involves the collection of 20 samples from qualified reference patients.(3,6) For the pediatric population, the challenges of establishing benchmarks are related mostly to the child growth and development, values that can severely influence the concentration of several analytes that are typically performed in a laboratory.(7)

## PURPOSE

This paper aims at analyzing the opportunity of establishing specific reference ranges to the served population by each laboratory.

## MATERIALS AND METHODS

The study is based on a systematic analysis and meta-analysis of the most cited papers of worldwide literature regarding the laboratory reference ranges for hemoglobin, serum iron and serum ferritin. For each parameter, two representative surveys were selected and analyzed in comparison. We have analyzed the benefits and limitations of each research method.

## RESULTS

**Table no. 1. Comparative reference ranges for haemoglobin**

| Age | Lothar Thomas – Clinical Laboratory Diagnostics, 1998 Inferior - Superior (g/dl) | Age | Roche Diagnostics, 2004 Inferior – Superior (g/dl) |
|---|---|---|---|
| 2 weeks | 11 – 36 | 1 day | 6.4 – 33.0 |
| 6 mth | 5 – 24 | 1 – 30 days | F: 5.2 – 22.7 |
| 12 mth | 6 – 28 | | B: 5.7 – 20.0 |
| 2 – 12 years | 4 – 24 | 1 – 12 mth | F: 4.5 – 22.6 |
| **Non pregnant women** | | | B: 4.5 – 20.6 |
| 25 years | 6.6 – 29.5 | 1 – 3 years | F: 4.5 – 18.1 |
| 40 years | 4.1 – 24.0 | | B: 5.2 – 16.3 |

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| | | | |
|---|---|---|---|
| 60 years | 7.0 – 26.7 | 4 – 6 years | F: 5.0 – 16.7 |
| **Pregnant women** | | | B:4.7 – 19.7 |
| 25 years | 7.2 – 27.7 | 7 – 9 years | F: 5.4 – 18.6 |
| 40 years | 6.3 – 30.1 | | B: 4.8 – 17.2 |
| 60 years | 7.2 – 21.5 | 10 – 12 years | F: 5.7 – 18.6 |
| | | | B: 5.0 – 20.0 |
| | | 13 – 15 years | F:5.4 – 19.5 B:4.7 – 19.7 |
| | | 16 – 18 years | F:6.9 – 18.3 |
| | | | B:4.8 – 24.7 |
| | | Adults | F:6.6 – 26.0 |
| | | | B:11.0 – 28.0 |

*Source: Guide book, Lothar Thomas – Clinical Laboratory Diagnostics, 1998 (14) and Guide - Roche Diagnostics, 2004 (15)*

**Table no. 2. Comparative reference intervals for serum iron**

| Age | Lothar Thomas – Clinical Laboratory Diagnostics, 1998 Inferior - Superior (g/dl) | Age | Roche Diagnostics, 2004 Inferior – Superior (g/dl) |
|---|---|---|---|
| 1 day | 15.2 – 23.5 | 1 day | 15.2 – 23.6 |
| 2 – 6 days | 15.0 – 24.0 | 2 - 6 days | 15.0 – 24.6 |
| 14 – 23 days | 12.7 – 18.7 | 14 – 23 days | 12.7 – 13.7 |
| 24 - 37 days | 10.3 – 17.9 | 24 – 37 days | 10.3 – 17.9 |
| 40 – 50 days | 9.0 – 16.6 | 40 – 50 days | 9.0 – 15.6 |
| 2 – 2,5 mth | 9.2 – 15.0 | 2 – 2,5 ths | 9,2 – 13,6 |
| 3 – 3,5 mth | 9.6 – 12.8 | 3 – 3,5 mth | 9.6 – 12.8 |
| 5 – 7,0 mth | 10.1 – 12.9 | 5 – 7 mth | 10.1 – 12.9 |
| 8 – 10 mth | 10.5 – 12.9 | 8 – 10 mth | 10.5 – 12.9 |
| 1,5 – 3 years | 10.8 -12.8 | 1,5 – 3 years | 10.8 – 12.8 |
| 5 years | 11.1 – 14.3 | 5 years | 10.7 – 14.7 |
| 10 years | 11.9 – 14.7 | 10 years | 10.8 – 15.6 |
| 12 years | 11.8 – 15.0 | Adults | F: 12.3 – 15.3 B: 14.0 – 17.5 |
| 15 years | 12.8 – 16.8 | > 70 years | F: 11.7 – 16.2 B: 12.1 – 17.6 |

*Source: Guide book, Lothar Thomas – Clinical Laboratory Diagnostics, 1998(14) and Guide - Roche Diagnostics, 2004 (15)*

**Table no. 3. Comparative reference intervals for serum ferritin**

| Age | Lothar Thomas – Clinical Laboratory Diagnostics, 1998 Inferior - Superior (g/dl) | Age | Roche Diagnostics, 2004 Inferior – Superior (g/dl) |
|---|---|---|---|
| 0.5 mth | 90 – 628 | - | - |
| 1 mth | 144 – 399 | 1 mth | 150 – 450 |
| 2 mth | 87 – 430 | 2 -3 mth | 80 – 500 |
| 4 mth | 37 – 223 | - | - |
| 6 mth | 19 – 142 | - | - |
| 9 mth | 14 – 103 | - | - |
| 12 mth | 1 – 99 | - | - |
| 6 mth -15 years | 7.0 – 142 | 4 mth-16 years | 20 – 200 |
| 20-65 years women men | 22 - 112 34 – 310 | 20- 65 years women men | 15 -150 30 – 400 |
| 65- 90 years women men | 13 - 651 4 – 665 | - | - |

*Source: Guide book, Lothar Thomas – Clinical Laboratory Diagnostics, 1998 (14) and Guide - Roche Diagnostics, 2004 (15)*

## DISCUSSIONS

Two standard methods are used to establish laboratory reference intervals:

- The method to test clinically healthy patients, the reference range representing 95% of the values of the tested analyte;
- The method that analyzes the subjects with minimal pathology, taking into account the 5th to the 95th percentile of the values obtained (Hoffmann method).(5)

The first method is considered to be the best, but it is more difficult to apply involving a group of valid reference subjects, divided by age, sex, race, and motivated to participate in testing. This method involves high costs and an adequate organization.(8) In medicine, in order to obtain the reference intervals, the statistical method used is the indirect Hoffman method because it allows the calculation of the reference ranges by using the results from the laboratory database. According to this method, 120 samples from healthy patients are used and by statistical calculation the abnormal vales are eliminated.(5,9)

The Hoffmann method requires the elimination of these values by using Chauvenet criterion. This refers to the removal of the values whose probability of occurrence is lower than ½N, where N is the number of values taken into account and it must be greater than 4. The values considered inappropriate are removed, the data is reviewed and the cumulative frequency is determined. The data are represented on the chart (the cumulative frequency in relation to the values of the studied parameters), then the linear portion is determined by visual assessment together with the deviation maximum. The equation of the regression line is: $Yi = \alpha + \beta + \varepsilon i * Xi$ (where: $\alpha$ is the slope, $\beta$ is the intercept of the line and $\varepsilon i$ is the error). The minimum and maximum values are obtained by solving the equation of the regression line.

Although in respect of the adult population it is possible to collect samples from qualified subjects, with regard to children and teenagers, especially young children and infants, there might be difficulties in obtaining samples, due to difficult sampling and the fact that infants and young children are fed at short intervals.(10) In order to establish the reference ranges the average and standard deviation of the data set is used, the reference limits being between 2.5% and 97.5%.(4,11)

Various manuals as Clinical and Laboratory Standards Institute (CLSI) give specific advice on the statistical methods which can calculate the reference intervals (C28-A3)(12), thus the reference range is considered valid if less than 10% of the reference sample measurements falls outside the calculated reference range.(13)

During the recent past years the study on establishing the reference intervals captivated the interest of the medical research centers, clinics and hospitals. All studies from the literature were conducted in other countries, in environments where patients had different living habits, by using different determination equipment, reagents and methods, and especially with different health policies, all these factors possibly influencing the results severely. Above, is comparison between the reference intervals of hemoglobin (table no. 1), serum iron (table no. 2) and serum ferritin (table no. 3), is exemplified as presented in the literature in two different studies: manual, Lothar Thomas - Clinical Laboratory Diagnostics Guide 1998(14), and Roche Diagnostics, 2004.(15)

Comparing the results (table no. 1) of the two studies, the manual Lothar Thomas - Clinical Laboratory Diagnostics and Roche Diagnostics Guide 1998 and 2004, there are significant differences noticed in the following age groups: 14 to 23 days and 10 years. Roche Diagnostics Guide 2004 presents the age reference intervals up to the age of 10, then it directly specifies the reference ranges for adults, divided by gender. A

comparison of the reference intervals (table no. 2) is difficult to achieve because the above mentioned literature structures the age groups differently. Roche Diagnostics Guide, 2004 presents a different age groups structure and also divided by gender. The comparison of the reference intervals (table no. 3) is difficult to achieve because the above mentioned literature presents the age groups in a different structure, for some age groups there are wider intervals, with a higher upper limit.

Establishing the reference ranges for biochemical, hematological and immunological measurements can be a challenge, also requiring high costs. These challenges are amplified for the pediatric population due to changes accompanying the child's growth and development.

The reference ranges that are used by most laboratories are intervals established many years ago, generally based on superficial studies and done on obsolete laboratory equipment. Many of the benchmarks from the literature show very wide intervals, and which are difficult to interpret in the clinical context. Internationally, there have been numerous studies published on the establishment of the reference ranges but also on the consequences of the wrong categorization of the patients (lack of diagnosis or incorrect categorization due to incorrect reference intervals).

## CONCLUSIONS

- A proper interpretation of results is achieved by setting the reference interval for the served population.
- The Reference Intervals from the literature cannot be applied to other medical facilities without them being validated.
- Many benchmarks from literature show very large intervals, which makes it difficult to interpret in the clinical context.

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