

Quality Control in Clinical Laboratories

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1. Introduction

1.1 The automated analyzers in clinical laboratories

Nowadays, the overwhelming majority of laboratory results in clinical laboratories is being generated by automated analyzers. Modern automated analyzers are highly sophisticated instruments which can produce a tremendous number of laboratory results in a very short time. This is achieved thanks to the integration of technologies from three different scientific fields: analytical chemistry, computer science and robotics. The combination of these technologies substitutes a huge number of glassware equipment and tedious, repetitive laboratory work. As a matter of fact, the laboratory routine work has diminished significantly. Today laboratory personnel's duties have been shifted from manual work to the maintenance of the equipment, internal and external quality control, instrument calibration and data management of the generated results.

1.2 Statistical Quality control in industrial production

Quality control is an ancient procedure. For centuries manufacturers checked the quality of their products trying to find early any defect. At that time, manufacturers checked every product, one by one, without exception. Today, in industrial business, monitoring the quality of the each product is unattainable due to the large-scale production of different goods. Modern quality control is used to check the quality of a minimum number of samples from the total production. The procedure is called **statistical quality control (SQC)** or **statistical process control (SPC)**. SQC is faster and more efficient than single checking. The most general definition of SQC is: "SQC is process that minimizes the variability of a procedure" although it would be wiser to define SQC as "The process that focuses on revealing any deviations from well defined standards".

1.3 SQC in clinical laboratories' automated analyzers

SQC can be used in every automated production, like the laboratory determinations which are performed by biomedical analyzers. Unlike the industrial business where all products are similar, the laboratory determinations are totally different because of the huge biological differences among human beings. As a result, SQC can be done only for the equipment and the analytical methods and rarely for each laboratory result. SQC of automated analyzers

uses as samples not the patients' results but the results of some special samples, the control samples.

The aim of this chapter is the introduction to the statistical quality control for automated analyzers in biomedical sciences such as haematology and biochemistry. The most important relevant laboratory SQC methods will be described.

2. Basic terms and definitions

2.1 Types of laboratory errors and mistakes

In laboratory practice many non-conforming results may appear. These results are divided in two major categories:

- **Errors:** Non-conforming results with "statistical meaning". This category includes all the "wrong" laboratory measures due to non-human action.
- **Mistakes:** Non-conforming results with "no statistical meaning". This category contains all the human errors e.g. mixing up samples.

Another classification of errors and mistakes is based on the time and the stage they appeared in laboratory practice.

1. The **pre-analytical stage** encompasses all the procedures which are take place before the analysis of the patients' samples on the automated analyzers (e.g. blood drawing, sample transportation, centrifugation, dilutions etc).
2. The **analytical stage** includes the analytical methods
3. The **post-analytical stage** refers to transmission of data from analyzers to the LIS, validation of results that have been produced and posting of the results to physicians or patients.

According to the previous classification, errors and mistakes are divided in three corresponding categories: I) pre-analytical, II) analytical, III) post-analytical. The majority of pre-analytical and post-analytical outliers are "mistakes" in contrary to analytical outliers which are considered as "errors"¹.

Tables 1–3 contain a list of the most common errors in hematology and biochemical analyzers. Although many kinds of errors can be detected with various methods in laboratory practice, the laboratory staff focuses on detecting and eliminating the analytical errors.

The reasons are:

- The analytical errors are attributed to the laboratory staff.
- The analytical errors can be detected with SQC methods.
- Statisticians have helped in establishing certain limits for the analytical errors for every laboratory determination.

1. Inappropriate specimen (e.g. wrong specimen-anticoagulant ratio)
2. Wrong anticoagulant (e.g. sodium citrate in place of EDTA)
3. Improper conservation method
4. Inappropriate patient's preparation (e.g. wrong diet)
5. Mistakes in patients' identification

Table 1. Common pre-analytical errors

¹ In this chapter, we will use the term "errors" for all kinds of outliers except special circumstances.

1. Expired reagents which may lead to erroneous results
2. Expired controls or calibrators
3. Calibration curve time-out elapsed
4. Failure in sampling system
5. Failure in aspiration system of reagents
6. Changes in analyzer's photometric unit / flow cell / measuring unit
7. Any other analyzer's failure

Table 2. Common analytical errors

1. Wrong matching between sample and laboratory's files
2. Wrong copy of results from the analyzer's report to the laboratory report (in cases of manual transfer)
3. Delay in delivering the results to the physicians, clinics or patients
4. Loss of the results

Table 3. Common post-analytical errors

2.2 Precision and accuracy

Analytical errors influence the repeatability, reproducibility, precision, trueness and accuracy of the analytical methods. Precision and accuracy can be defined with many different ways.

1st definition (EURACHEM/CITAC Guide CG 4)

Precision is the closeness of agreement between independent test results obtained under stipulated conditions.

Accuracy is the closeness of the agreement between the result of a measurement and a true value of the measurand.

According to the same guide **true value**² is the "value consistent with the definition of a given quantity". In fact true value is any measurement with no errors random or systematic (see paragraph 3.4).

Measurand is a "particular quantity subject to measurement" or simply any substance or analyte which can be assayed in a clinical laboratory.

2nd definition

$$\text{Precision} = \text{repeatability or reproducibility}^3 \quad (1)$$

$$\text{Accuracy}^4 = \text{trueness} + \text{precision} \quad (2)$$

² According to the EURACHEM/CITAC Guide CG 4 the indefinite article "a" rather than the definite article "the" is used in conjunction with "true value" because there may be many values consistent with the definition of a given quantity.

³ According to ISO 5725-3:1994 the definition of precision concludes and the "intermediate precision" (s_{zi}) with i denoting the number of factors. Intermediate precision relates to the variation in results observed when one or more factors, such as time, equipment and operator, are varied within a laboratory; different figures are obtained depending on which factors are held constant.

⁴ Recently the term analytical uncertainty has been used to encompass several aspects of accuracy.

Repeatability is the degree of consensus between successive measurements which have been done on the same sample with very similar conditions (same analyzer, same user, same laboratory, same methods, same lot of reagents) in a very short time (e.g. same day). It is approached by within run or within day experiments and often is symbolized as s_r .

Reproducibility is the degree of consensus between successive measurements achieved on the same sample with different conditions (e.g. different analyzer, different user, different lot of reagents) in a long time. It is approached by between day experiments, can be either intra-laboratory or inter-laboratory, and often is symbolized as s_R .

Trueness is often used to describe the classical term bias.

3rd definition

$$\text{Precision} = x_i - \bar{x} \quad (3)$$

$$\text{Trueness} = \bar{x} - \mu \quad (4)$$

x_i : a single measurement, μ : a true value, \bar{x} : the average of successive measurements⁵

Based on equations 3 and 4 the equation of accuracy is transformed as follows:

$$\text{Accuracy} = (\bar{x} - \mu) + (x_i - \bar{x}) = (x_i - \mu) \quad (5)$$

2.3 Types of analytical errors

Analytical errors fall into two subcategories according to EURACHEM/CITAC Guide CG 4 as follows:

Random Errors (RE) (Fig. 1, RE)

Result of a measurement minus the mean that would result from an infinite number of measurements of the same measurand.

The mathematical definition is:

$$\Delta\text{RE} = x_i - \bar{x} \quad (6)$$

In fact random errors affect the **precision** (see paragraph 2.2) of all measurements. Random errors are attributed to either undetermined reasons (inherent error) or well defined causes. Their magnitude (ΔRE) is equal to the precision of a measurement and it is always greater than zero ($\Delta\text{RE} > 0$). ΔRE can be diminished by increasing the number of measurements (it influences the \bar{x}). Large ΔRE increases the dispersion of the results around a true value.

The experimental standard deviation of the arithmetic mean or average of a series of observations is not the random error of the mean, although it is so referred to in some publications on uncertainty. It is instead a measure of the uncertainty of the mean due to some random effects. The exact value of the random error in the mean arising from these effects cannot be known.

⁵ Although, accreditation bodies use the term trueness for the difference $\bar{x} - \mu$ in daily SQC practice is very common to relate the difference $\bar{x} - \mu$ with the term "accuracy" which is expressed. We will use this term for accuracy later in this chapter.

Systematic Errors (SE) (Fig. 1, SE)

Systematic error is defined as a component of error which, in the course of a number of analyses of the same measurand, remains constant or varies in a predictable way. Quite often is attributed as:

Mean that would result from an infinite number of measurements of the same measurand carried out under repeatability conditions minus a true value of the measurand and is expressed mathematically as:

$$\Delta SE = \bar{x} - \mu \quad (7)$$

Systematic errors can be attributed to certain reasons and therefore can be eliminated much easier than random errors. ΔSE cannot be diminished by increasing the number of measurements. As opposed to the random errors their magnitude can be zero ($\Delta SE \geq 0$).

There is also another kind of analytical errors but it cannot be detected easily with SQC methods. These errors are called "**gross errors**" (GE) and they are classified to the category of mistakes (Fig. 1, GE). Gross errors can result from mixing up of samples, clots in the analyzers' sampling system etc.

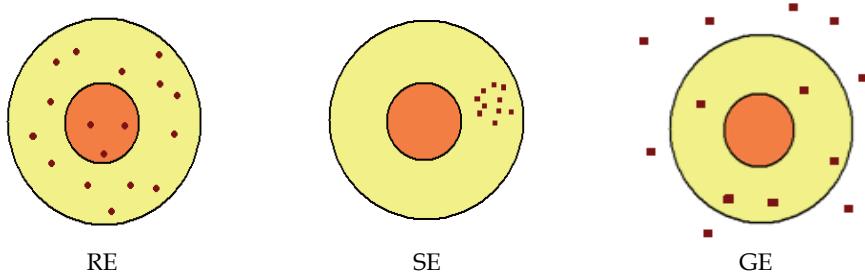


Fig. 1. Representation of the values' dispersion in random errors (RE), systematic errors (SE) and gross errors (GE)

Random and systematic errors can be detected very effectively by means of SQC methods such as Levey-Jennings charts, Westgard rules, Cusum charts e.t.c.

2.4 Total analytical error

The mathematical definition of the total analytical error (TE) is:

$$\text{Total analytical error} = \text{Random Error} + \text{Systematic Error} \quad (8)$$

According to the definitions of random and systematic errors (paragraph 2.3):

$$TE = \Delta RE + \Delta SE \quad (9)$$

Under ideal circumstances, total analytical error equals to zero, but this cannot be achieved in daily practice. Only ΔSE can be zero ($\Delta SE \geq 0$) where as ΔRE is always greater than zero ($\Delta RE > 0$) because of the existence of the inherent error.

Since $TE > 0$ is unavoidable, TE of every single determination must be lower than a specified limit. This limit is called "**allowable total analytical error**" (aTE) and it is different for each analyte being determined in a clinical laboratory.

2.5 Internal and External SQC

Random and Systematic errors must be detected at an early stage and then every effort should be taken in order to minimize them. The strategy for their detection consists of specific SQC methods which are divided in two categories:

Internal Quality Control (IQC). *It concludes all SQC methods which are performed every day by the laboratory personnel with the laboratory's materials and equipment. It checks primarily the precision (repeatability or reproducibility) of the method.*

External Quality Control (EQC). *It concludes all SQC methods which are performed periodically (i.e. every month, every two months, twice a year) by the laboratory personnel with the contribution of an external center (referral laboratory, scientific associations, diagnostic industry etc.). It checks primarily the accuracy of the laboratory's analytical methods. However, there are certain EQC schemes that check both the accuracy and the precision.*

Other terms for external quality control are: interlaboratory comparisons, proficiency testing (**PT**) and **external quality assessments schemes (EQAS)**.

The metrics of internal and external quality control are based on statistical science (e.g. SDI, CV, Z-score) and they are graphically represented by statistical charts (**control charts**). Some of them are common in other industries while others specific for internal or external quality control in clinical laboratories.

2.6 Control materials

Control materials (or simply “**controls**”) are all the materials which can be used for error detection in SQC methods. Although this term is considered equal to “**control samples**”, several SQC methods have been deployed based on patients’ results.

Control samples are **pools** of biological fluids (serum, whole blood, urine or other materials). They contain analytes which are determined by the laboratory, ideally in concentrations/activities close to the decision limits where medical action is required. In internal and external SQC, it is common practice that laboratories use two or three different control samples which contain different quantities of analytes e.g. low, normal, high concentrations/activities. Control samples with the same analytes but different concentrations/activities are called “**levels**”. Different levels check the performance of laboratory methods across all their measuring range. In most cases control samples are manufactured by analyzers’ or reagents’ manufacturers, but they can also be made by the laboratory personnel.

Before control samples are assayed for IQC reasons, each laboratory has to estimate their limits. **Control limits** are an upper and lower limit (see paragraph 3.2) **between** which the **control values** are allowed to fluctuate.

3. Internal quality control

3.1 Normal distribution

Normal or Gaussian distribution (N) is the basis of SQC theory. Distribution chart is a biaxial diagram (x/y). X-axis represents the values of a variable’s observations and y-axis the frequency of each value (the number of each value’s appearance). It has a bell-shaped form with its two edges approaching asymptotically the x-axis. The highest point of normal distribution corresponds to the value with the higher frequency (mode value). In any normal distribution, the mode value is equal to mean and median value of the variable.

Mode value (Mo) is the value with the highest frequency. It is always on the top of every distribution curve.

Median value (M) is the value which divides the variable's observations in two equal parts. It represents the "center" of the distribution.

Mean value or average value (μ or \bar{x}) is equal to the value which all the observations should have if they were equal. In normal distribution, mean, median and mode values coincide. The mean value or average can be calculated by the next formula:

$$\mu = \frac{\sum_{i=1}^N x_i}{N} \quad (10)$$

Where: x_i = Single value, $\sum x_i$ = Sum of values, N = Total number of values

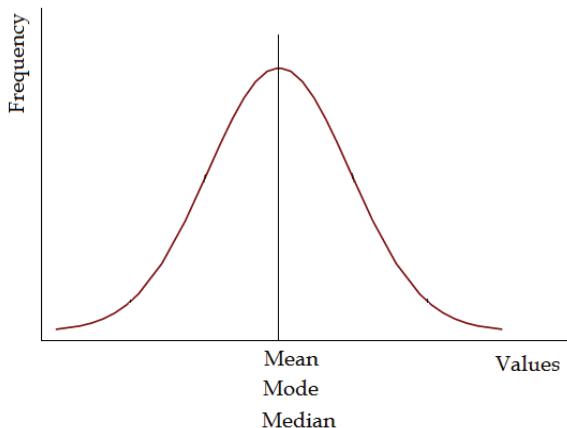


Fig. 2. The normal distribution

The length of a distribution curve defines the variance of the variable. The most common measure of variance is standard deviation (SD or s). **Standard deviation** can be calculated by the next formula:

$$s = \sqrt{\frac{\sum_{i=1}^N (x_i - \mu)^2}{N-1}} \quad (11)$$

The distance between the upper (UL) and lower limit (LL) of a normal distribution is six standard deviations ($6s$). Since mean value is in the center of normal distribution, the total range of a normal distribution is $\mu \pm 3s$ (to be more exact, not all, but nearly all (99.73%) of the values lie within 3 standard deviations of the mean). Every normal distribution can be defined as $N \sim (\mu, s)$. For instance $N \sim (76, 2.3)$ means a normal distribution with mean value = 76 and standard deviation = 2.3.

Mean value and standard deviation allow the statisticians to calculate the distance of each observation from the center (mean), using as measuring unit the s . This distance is called Z-score. It is measured in standard deviation's units by the next formula:

$$Z\text{-score} = \frac{x_i - \mu}{s} \quad (12)$$

Where: x_i = Single value, μ = Mean value, s = Standard deviation

Supposing we are looking the distance of value $x_i = 80$ from the mean of the normal distribution $N \sim (100, 5)$. According to equation 12 Z-score is:

$$Z\text{-score} = \frac{x_i - \mu}{s} = \frac{80 - 100}{5} = -4$$

So the value 80 is 4 standard deviations lower than mean value 100. In Fig. 3 the location of $Z\text{-score} = -4s$ seems to be lower than the lower limit of the distribution.

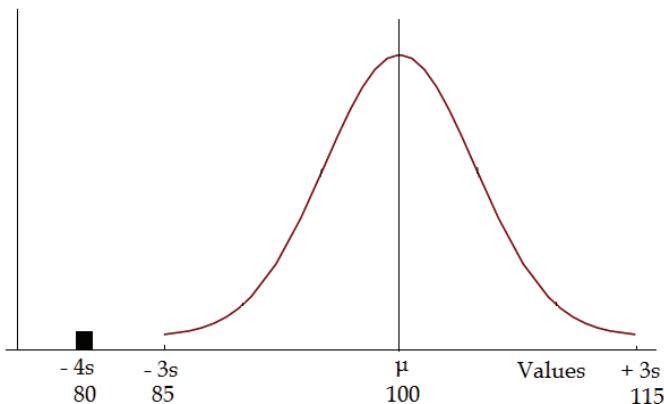


Fig. 3. The location of the value 80 with $Z\text{-score} = -4$

Because of the symmetric bell-shaped form of normal distribution its surface can be divided in six parts containing a specific percentage of its observations ("empirical rule", Table 4, Fig. 4).

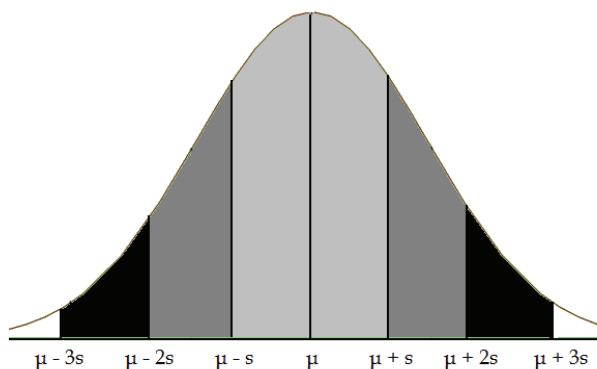


Fig. 4. The division of a normal distribution in six parts

1. The part $\mu \pm s$ contains 68,26% of the observations.
2. The part $\mu \pm 2s$ contains 95,46 % of the observations.
3. The part $\mu \pm 3s$ contains 99,73 % of the observations.

Table 4. The “empirical rule” of the normal distribution

Standard deviation depicts the variation in the same units as the mean (e.g. cm, L, mol/L). So standard deviation cannot be used to compare the variance of different distributions or distributions with different mean. Therefore for comparison reasons the **coefficient of variation** (CV%) is being used. CV% is a normalized measure of dispersion of a probability distribution. It is defined as the ratio of the standard deviation to the mean and expressed as a percentage:

$$CV\% = \frac{s}{\mu} \cdot 100 \quad (13)$$

The normality (the normal distribution) of a variable is crucial for any statistical study. The “**normality test**” is the first task of the statisticians before the application of any statistic test. If the variable’s values are compatible with the normal distribution then “**parametric tests**” are employed. Otherwise “**non-parametric tests**” are used. The most known normality tests are Kolmogorov-Smirnov & Shapiro-Wilk.

3.2 Calculation of control limits

Control limits are necessary for any SQC method in internal and external quality control (see paragraph 3.1). They consist of a center value (CL) and an upper and low control limit (UCL & LCL). Generally, they are created by repetitive measurements of control samples. In internal SQC two or more control samples are assayed every day and at least once per day before the patients’ samples. Then the laboratorians check if all control values lie within the control limits. If at least one of the controls’ measurements is outside of one of the two control limits then further actions may be required until random or systematic errors are under control.

Control limits vary depending on the control samples, the automated analyzer and the method of determination. They can (and should) be calculated by the laboratory itself, although many laboratories use the control limits established by the analyzer’s manufacturer.

The steps for their calculation are the following:

1. The laboratory’s staff collects 20 – 30 successive measurements from any control level.
2. Standard deviation (s) and mean value (μ) are calculated. The range $\mu \pm 3s$ is considered as “trial limits”
3. The laboratory’s staff checks if any of the measurements exceed the range $\mu \pm 3s$. If so, the outlier is rejected the standard deviation and mean value are calculated once more.
4. The laboratory’s staff repeats the previous procedure until no measurement exceeds the range $\mu \pm 3s$. The final μ and s are the mean value and the standard deviation of the control limits.

Control limits correspond to a normal distribution. The mean value of the control limits is symbolized as “ μ ” and it is considered a true value of the daily control values (see paragraph 3.4). Their standard deviation is symbolized as “ s ” and it is equal to the inherent error. On the contrary the mean value of the daily control values is symbolized as \bar{x} and their standard deviation as “SD”. SD encompasses the inherent error and any other existing random error.

3.3 Random and systematic errors in normal distribution of control values

Successive measurements derived from the same sample have a normal distribution. This is also the case with control values. Control values have always an inherent error, even if the determinations have been done under exactly the same conditions. This inherent error is the minimum random error of the process (Fig 5). If other sources of random errors exist the standard deviation of the measurements will be higher. In systematic errors the mean value of the control values has been moved further up or down from the mean value of the control limits (μ) (Fig 6).

In daily practice a measurement has both random and systematic errors. Random error may be approached by a number of concepts and statistical techniques (precision, repeatability, reproducibility, s , $CV\%$). Another concept for approaching the analytical variation caused by the random errors is the **imprecision** (s_{meas}). The difference $\bar{x} - \mu$, expressed as a percentage of μ , is called **bias_{meas}**.

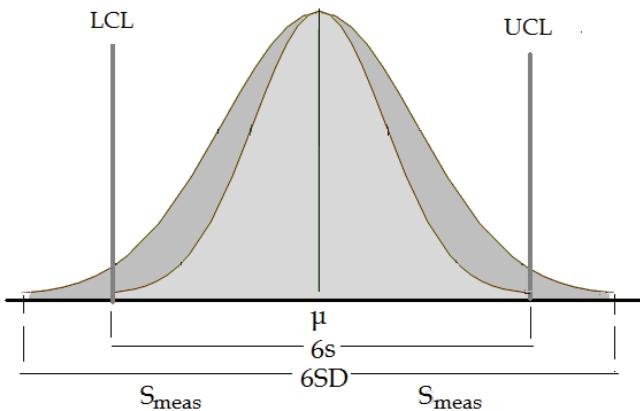


Fig. 5. The random error in normal distribution

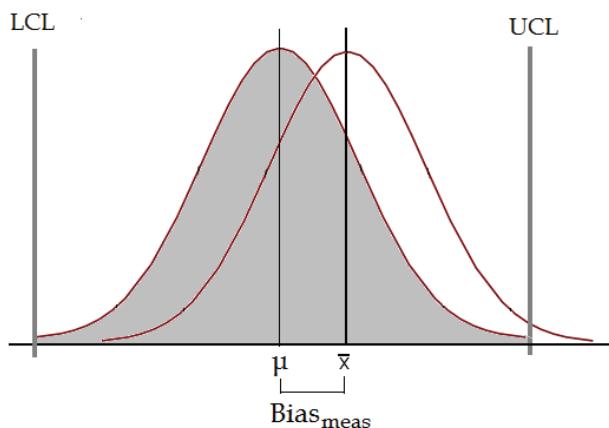


Fig. 6. The systematic error in normal distribution

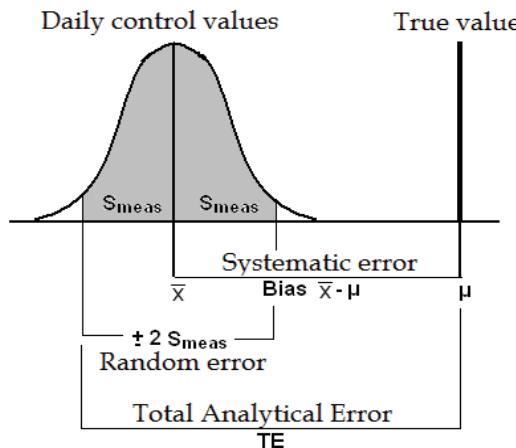


Fig. 7. The graphical display of a total analytical error. It refers to the maximum incidence of RE and SE

3.4 A true value in SQC

Although the previous procedure estimates the standard deviation and mean, with the exception of outliers, the laboratory personnel cannot be sure that the mean is the real one. The mean value of the 20-30 values will approach the true if some other procedure has come before. This procedure is the **calibration** of the automated analyzer with reference materials / calibrators. The validation of the calibration is done with “**recovery tests**” and the external quality control.

3.5 Levey-Jennings chart

Levey-Jennings chart is the most important control chart in laboratory quality control. It can be used in internal and external quality control as well. It detects all kinds of analytical errors (random and systematic) and is used for the estimation of their magnitude.

It was firstly introduced in 1952 (Levey S. & Jennings E, 1950). Stanley Levey and Elmer Jennings were inspired by Walter Shewhart charts, the most effective control charts in industrial business at that time. Shewhart and Levey-Jennings charts are based on **normal distribution** (Fig. 4).

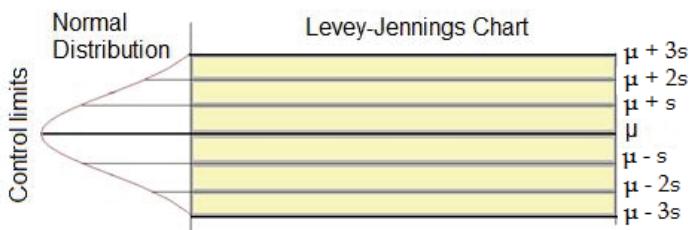


Fig. 8. Plotting of a Levey-Jennings chart from a control limits distribution curve

Plotting of a Levey-Jennings chart starts from the distribution curve of the control limits ($\mu \pm 3s$). The first step is to rotate clockwise the distribution curve of the control limits by 90°. The second step is to draw seven lines which start from the points $\mu + 3s$, $\mu + 2s$, $\mu + s$, μ , $\mu - s$, $\mu - 2s$ and $\mu - 3s$. These seven lines form the Levey-Jennings chart (Fig. 7). For every different control level a different Levey-Jennings chart is being plotted.

3.6 Random and systematic errors in Levey-Jennings chart

The operator of an automated analyzer assays on a daily basis two or three control levels with the same chemical methods and equipment as with the patient's samples. Every control value is plotted on a Levey-Jennings chart. The analyzer's operator checks if any or the daily control values exceeds certain limits. If so, there is either a random error or a systematic error or both.

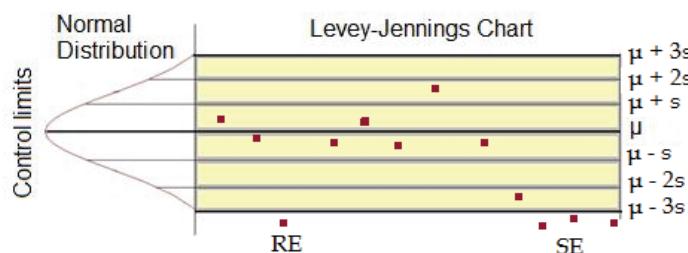


Fig. 9. The daily use of a Levey-Jennings chart with a random error (RE) and a systematic error (SE)

In Levey-Jennings chart a random error is revealed when one control value exceeds the UCL ($\mu + 3s$) or LCL ($\mu - 3s$). The detection of a systematic error is more complicated. In systematic errors two or more successive control values exceed the control limits which can be respectively $\mu + 3s$, $\mu + 2s$, $\mu + s$ or $\mu - s$, $\mu - 2s$, $\mu - 3s$ (Fig 9).

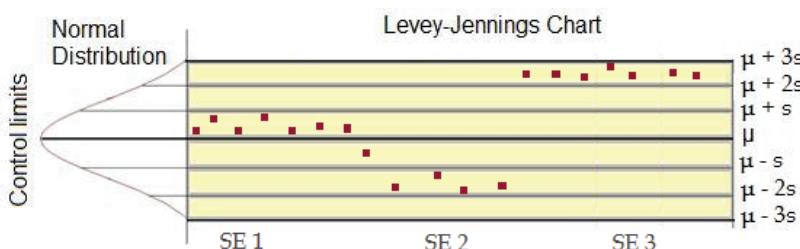


Fig. 10. Three different systematic errors on a Levey-Jennings chart. SE 1 has 7 successive control values between μ and $\mu + s$ (UCL = $\mu + s$), SE 2 has 4 successive control values between $\mu - s$ and $\mu - 2s$ (LCL = $\mu - 2s$), SE 3 has 7 successive control values between $\mu + 2s$ and $\mu + 3s$ (UCL = $\mu + 3s$)

Daily values create their own normal distribution which may be different from the normal distribution of control limits. Some examples with random and systematic errors are displayed in Fig. 10 & 11.

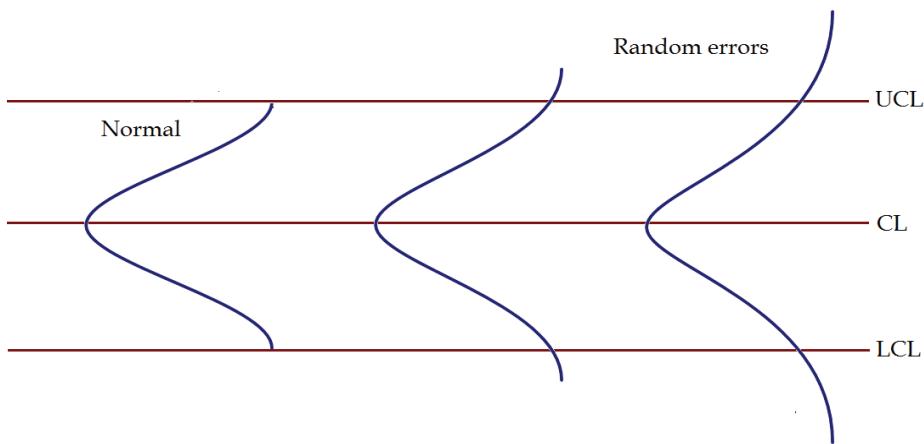


Fig. 11. The random errors on a Levey-Jennings chart

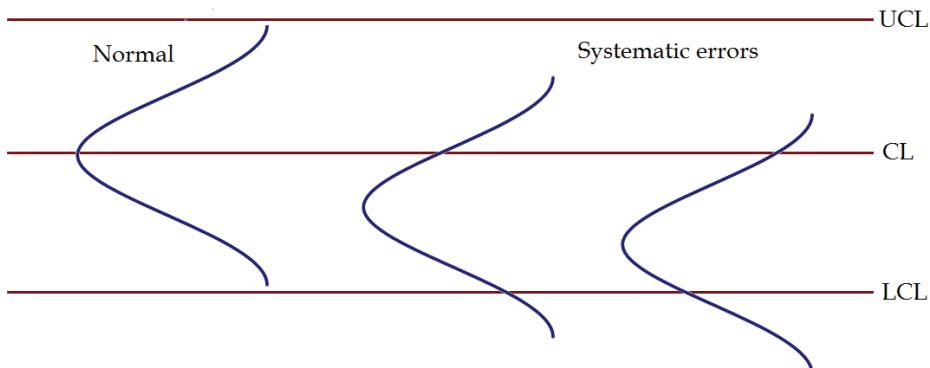
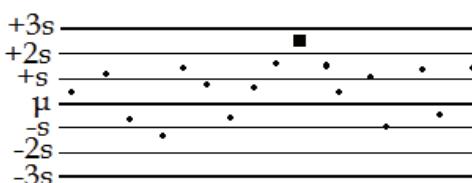


Fig. 12. The systematic errors on a Levey-Jennings chart

3.7 The Westgard rules

Error detection can be done very easily by using quality criteria. Although many of them have been proposed on the past, the most widely used are the so called Westgard rules (Westgard et al., 1981). Westgard rules (or modifications of them) are used today in almost every single biochemical, immunological or hematological automated analyzer. They are symbolized as A_L . A is the number of control values and L the control limits (Table 5).

1_{2s} . One control value lies between $\mu+2s/\mu+3s$ or between $\mu-2s/\mu-3s$. It is only a warning rule.



1_{3s}. One control value lies over $\mu+3s$ or under $\mu-3s$. This criterion is sensitive to the detection of random errors. The results should be blocked and not reported to the patients. The run⁶ is rejected.

2_{2s}. Two successive control values lie between $\mu+2s$ and $\mu+3s$ or between $\mu-2s$ and $\mu-3s$. It defines a systematic error. The results should be blocked and not reported to the patients.

R_{4s}. The distance of two successive control values, values, is over 4s. It is a criterion sensitive to random errors. The results should be blocked and not reported to the patients. (Normally this criterion is used with two different control levels/across runs – when used with one level, as in this example, is applied for two consecutive runs).

4_{1s}. Four successive control values lie between $\mu+1s$ and $\mu+3s$ or between $\mu-1s$ and $\mu-3s$. It defines a systematic error. The results should be blocked and not reported to the patients.

10 \bar{x} . Ten successive control values lie between μ and $\mu+3s$ or between μ and $\mu-3s$. It is a criterion that reveals systematic errors. The results should be blocked and not reported to the patients.

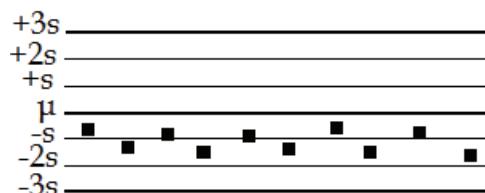
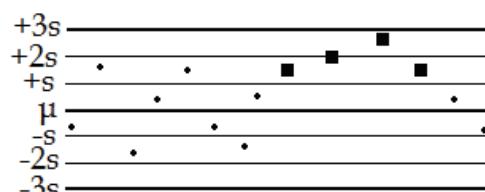
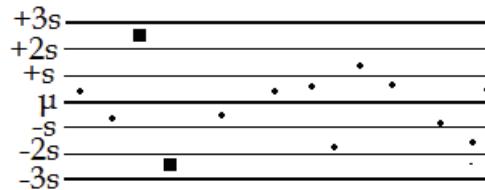
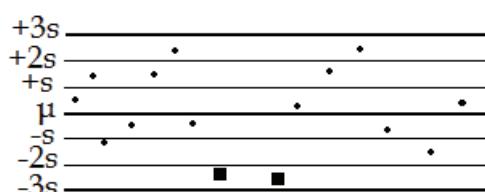
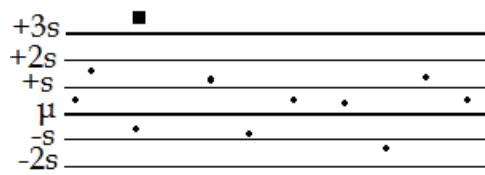


Table 5. Application of Westgard rules in one control level

In internal quality control two or even three different control samples may be used (see paragraph 2.6). In this case Westgard rules are used with a different way (Table 6).

⁶ Analytical run or run is the analysis of patients' samples and controls during the day keeping the same analytical conditions.

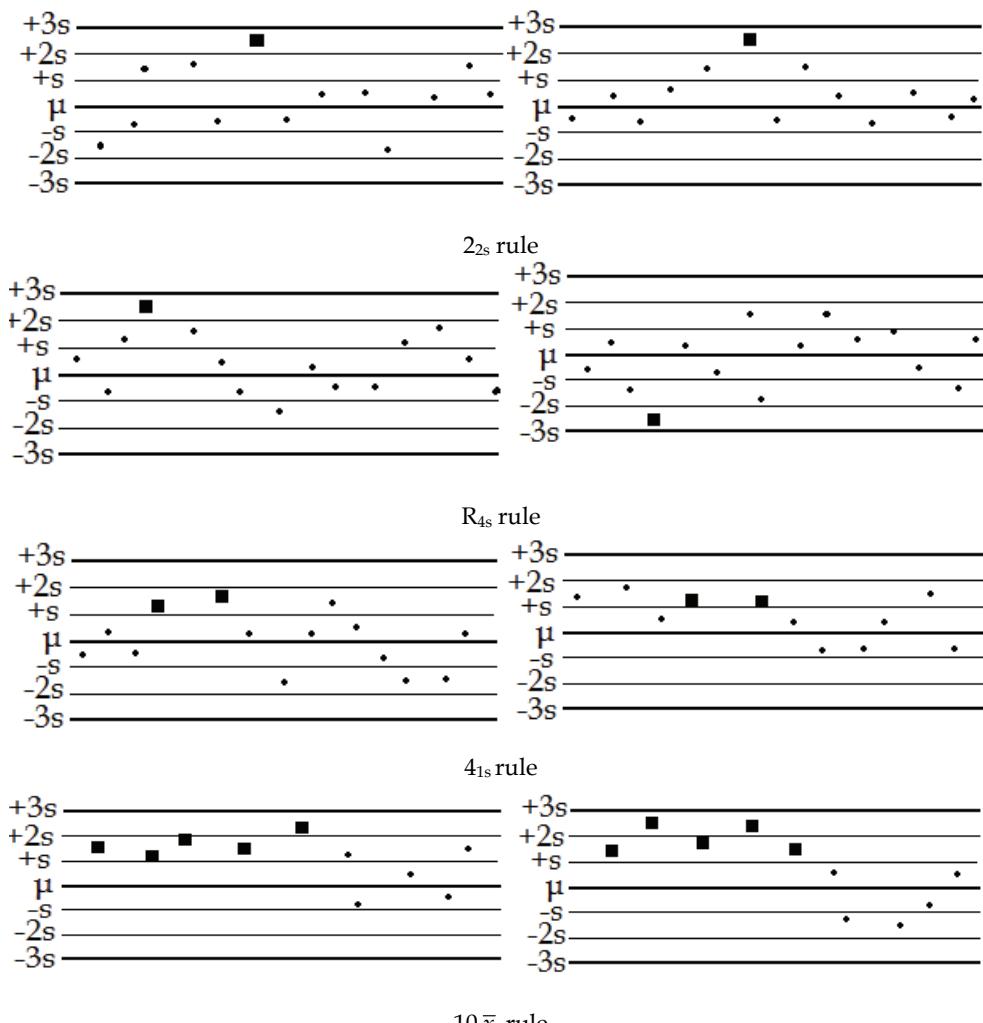


Table 6. Application of Westgard rules in two levels of controls

3.8 The Average of Normals method

Levey-Jennings chart and Westgard rules detect random and systematic errors. This is achieved usually with the daily analysis of two different control levels. But control samples determination has some disadvantages:

- It is costly.
- It is time consuming.
- In many labs is performed once a day, as a rule before the analytical run (although several laboratories perform IQC in well defined intervals depending on the analyte or use bracketing before releasing the results).

These three disadvantages can be minimized with some others methods that use as control materials the patients' results. The most known of these is the **Average of Normals** (AON) with extensive application in biochemistry analyzers. The main disadvantage of AON method is that detects only **systematic errors**. The advantages are that it is free of charge, fast and it is done automatically throughout the day.

AON method based on the principle that the mean value of all normal results fluctuates between well defined limits (Hoffmann & Waid, 1965). The steps for implementation of AON method are:

1. The laboratory calculates the mean value and the standard deviation of the analyte reference values (RV)⁷. The laboratory can use the proposed reference values from bibliography or better estimate its own. For instance, if $RV = 100 - 120 \text{ mmol/L}$ then the mean value (μ) is 110 mmol/L . The standard deviation is $(120 - 100)/6 = 3,3 \text{ mmol/L}$.
2. The laboratory defines which number of normal results (N) will use in AON method every day. This number of normal results will be the "control sample" of the method and it will remain steady.
3. The laboratory calculates the **standard error** (SE) of normal results with the following formula:

$$SE = \frac{s}{\sqrt{N}} \quad (14)$$

4. The laboratory calculates the confidence interval (CI) of the method with the formula:

$$\text{CI or Control limits} = \mu \pm 1.96 \times \frac{s}{\sqrt{N}} \quad (15)$$

The confidence interval will be used for the definition of the control limits of the method.

5. Every day the laboratory calculates the mean value of N normal results. This mean value is symbolized as AON and it is calculated by the next formula (see equation 9).

$$AON = \frac{\sum_{i=1}^N x_i}{N} \quad (16)$$

6. If AON exceed the control limits (equation 15) then the analyte's determination has a systematic error which has to be eliminated before the analytical run.

The effectiveness of AON method depends on the number of normal results (N) which will be used daily. N is relevant to the variance of method (CV_a) and the biological variation of the analyte (CV_b). The variance of the analytical method can be calculated by the coefficient of variation of the control samples. The biological variations for most analytes are available in the scientific literature (see Appendix II & paragraph 5.1).

In Fig. 12 there is a nomogram (Cembrowski et all., 1984) which correlates number N with the ratio CV_b/ CV_a . Such nomograms can be plotted by sophisticated software based on simulations theory.

⁷ Reference values (later normal values) are the range of analyte's values of a health person.

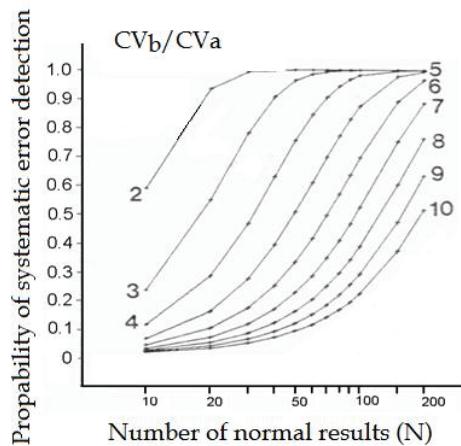


Fig. 13. Cembrowski nomogram which correlates the ratio CV_b/CV_a with the number of normals (N). The nomogram detects systematic errors with $\Delta SE = 2s$ with probability of false alarm 1%

In daily practice AON method has its own control chart which detects only systematic errors. In AON chart each dot represents a daily mean value of the same analyte (Fig. 13).

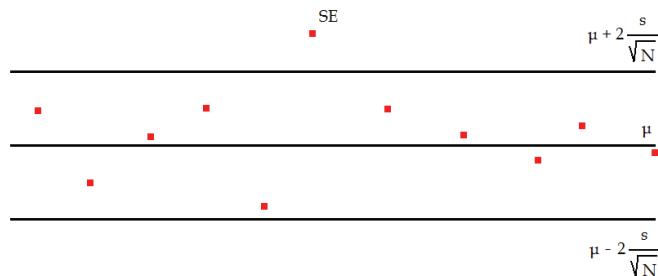


Fig. 14. AON chart. Each dot is a mean value. The point outside the upper limit represents a systematic error and not a random error like in Levey-Jennings chart

3.9 Bull's algorithm

The AON method of Hoffman & Waid is used mainly to biochemical analyzers. Hematology analyzers use another average of normals method, the Bull's algorithm (Bull, 1974). Bull had determined that some hematological parameters have very small biological variation (CV_g) resulting in their mean value remaining steady. Bull applied his idea in erythrocyte indexes (MCV, MCH, MCHC) at the beginning, but today his algorithm is used for the majority of hematological parameters.

In Bull's algorithm a **moving average is calculated** instead of a mean value (equation 6). The moving average is a mean value that contains the mean value of a former moving average. In Bull's algorithm the moving average is usually calculated by a batch of 20 values, 19 of them are previous patients' values and one is the mean value of the previous batch. Bull's moving average is symbolized as \bar{X}_B . It is calculated by the formula:

$$\bar{X}_{Bi} = (2 - r) \bar{X}_{B,i-1} + rd \quad (17)$$

The constant r defines the percentage of the participation of the previous Bull's moving average to the calculation of the current one. For instance if $r = 0.4$ the previous Bull's moving average participate to the calculation of the current moving average with a percentage of 40%.

In contrary to AON method Bull's algorithm uses all the patient values not only the normal ones. It eliminates the outliers with the constant d (difference) which is calculated by the next formula:

$$\begin{aligned} \bar{X}_{Bi} &= (2 - r) \bar{X}_{B,i-1} + rd \\ d &= \operatorname{sgn}\left(\sum_{j=1}^N \operatorname{sgn}(X_j - \bar{X}_{B,i-1}) |X_j - \bar{X}_{B,i-1}|^p\right) \left(\frac{1}{N} \sum_{j=1}^N \operatorname{sgn}(X_j - \bar{X}_{B,i-1}) |X_j - \bar{X}_{B,i-1}|^p\right)^{1/p} \end{aligned} \quad (18)$$

If $P = 1/2$:

$$d = \sqrt{\sum_{j=1}^N (X_j - \bar{X}_{B,i-1})^2} \left(\frac{1}{N} \sum_{j=1}^N \sqrt{(X_j - \bar{X}_{B,i-1})^2} \right)^2 \quad (19)$$

If $r = 1.0$ the differences $|X_j - \bar{X}_{B,i-1}|$ are all positives, then equation 17 is transformed as follows:

$$\bar{X}_{B,i} = \bar{X}_{B,i-1} + \left(\frac{\sum_{j=1}^N (X_j - \bar{X}_{B,i-1})^2}{N} \right)^{1/2} \quad (20)$$

Some manufacturers have developed modified equations for Bull's algorithm. For instance, Sysmex hematology analyzers use a variation of the previous equations. Instead of the symbol \bar{X}_B it uses the symbol \bar{X}_M .

The equation 17 is transformed as follows:

$$\bar{X}_{M(i)} = \bar{X}_{(i-1)} + \operatorname{sgn} \left\{ \sum_{i=0}^N \operatorname{sgn}[x_{(i,i)} - \bar{X}_{M(i-1)}] \sqrt{|x_{(j,i)} - \bar{X}_{M(i-1)}|} F \right\} \quad (21)$$

The constant F is calculated by the formula:

$$F = \left\{ \frac{\sum_{i=0}^N \operatorname{sgn}[x_{(i,i)} - \bar{x}(i-1)] \sqrt{|x_{(j,i)} - \bar{X}_{M(i-1)}|}}{N} \right\}^{1/2} \quad (22)$$

Bull's algorithm like AON method detects only systematic errors and it has its own control chart and its own rules. If Bull's algorithm is used for the quality control of erythrocyte

indexes the control limits of Bull's chart are $\bar{X}_B \pm 3\%$. The range $\pm 3\%$ comes from the biological variation of the erythrocyte indexes which is almost 1%. The center line or target of Bull's chart is the Bull's average (\bar{X}_B) which has been calculated after nine successive calculations (equations 17, 21).

The three erythrocyte indexes are:

$$\text{MCV} = (\text{Hct}(\%)) / (\text{RBC(millions}/\mu\text{L})) 1000 \quad (23)$$

$$\text{MCH} = (\text{HgB(g/dL}) / (\text{RBC(millions}/\mu\text{L})) 1000 \quad (24)$$

$$\text{MCHC} = (\text{HgB(g/dL}) / (\text{Hct}(\%))) 100 \quad (25)$$

Where: MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, Hct: Hematocrit, Hgb: Hemoglobin, RBC: Number of Red Blood Cells.

From the equations 23 – 25 it is obvious that the combined study of the charts of MCV, MCH and MCHC shows which of the three hematological parameters Hct, Hgb and RBC is increasing or decreasing due to a systematic error (Fig 14).

For the interpretation of Bull's charts two rules have been proposed (Luvetzszy & Cembrowski, 1987):

1. $1_{3\%}$. Bull's moving average exceeds the limit $\pm 3\%$.
2. $\overline{3}_{2\%}$. The mean value of three successive Bull's moving averages exceeds the limit $\pm 2\%$.

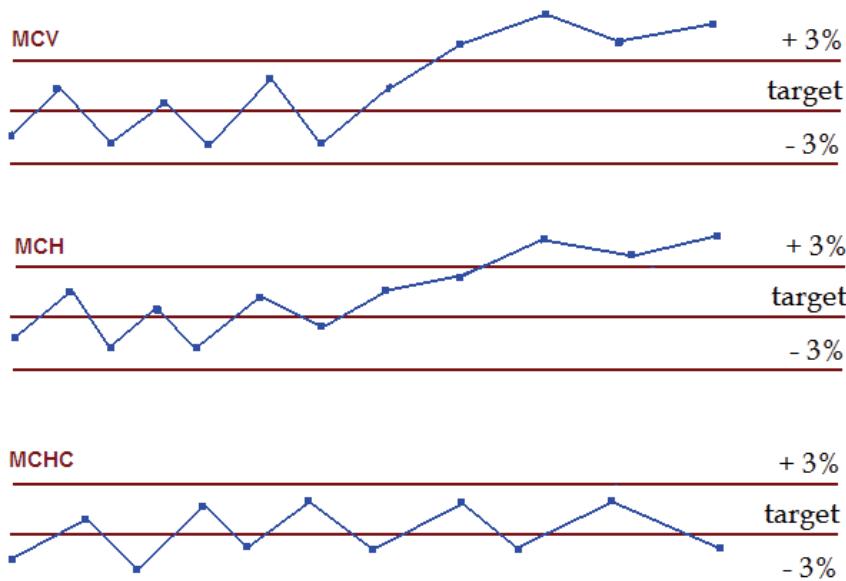


Fig. 15. The MCV and MCH indexes are increasing over the upper control limit ($+ 3\%$) while MCHC is not affected. From the three Bull's charts and the equations 23, 24, 25 it is deduced that RBC is smaller than it has to be

3.10 Delta check method

The AON and Bull's algorithm SQC methods detect systematic errors using a group of patients (their mean value or moving average). There are two other methods that detect random errors using previous values of individual patient. The first of these is the Delta check method (Nosanchuk & Gottmann, 1974). *Delta check is the difference between the current value of one person and the previous one.*

$$\text{Delta check} = \text{Current value} - \text{Previous value} \quad (26)$$

Besides this classic equation, other approaches for Delta check calculation have been developed:

$$\text{Delta check\%} = (\text{Current value} - \text{Previous value}) / \text{Current value} \quad (27)$$

$$\text{Delta check} = \text{Current value} / \text{Previous value} \quad (28)$$

This difference for equation 26 should vary between two limits which are called "Delta check limits". In order to calculate them we must take into consideration the reasons of delta differences:

1. The "intra-individual" biological variation of the analyte (CV_I).
2. The analytical variation (S_{meas}). S_{meas} can be easily estimated by the control values.
3. The pre-analytical variation ($CV_{pre-analytical}$).
4. The improvement or deterioration of the patient's health.
5. Errors and mistakes in the pre-analytical, analytical and post-analytical stage.

The only factors which can be calculated from the list above are the first three. The equation that combines them is:

$$CV_{total} = \sqrt{CV_I^2 + S_{meas}^2 + CV_{pre-analytical}^2} \quad (29)$$

If we don't take into account the pre-analytical CV the equation 29 will become:

$$CV_{total} = \sqrt{CV_I^2 + S_{meas}^2} \quad (30)$$

Although the range $\pm \sqrt{CV_I^2 + S_{meas}^2}$ can be considered as the total variation (CV_{total}) of a result, we have to take into considerations some other points. In paragraph 3.1, the table 4 referred to the probability which have any observation of a normal distribution to be on a certain place around the mean value. For instance, the probability of an observation to be within ± 2 s from the mean value is 95%. This distance is equal to z-score (equation 11). So the range of a single result (x_i) must be:

$$x_i \pm Z - score \sqrt{CV_I^2 + S_{meas}^2} \quad (31)$$

In most cases for z-score (or simply z) the number 2 is chosen.

In delta check method, there are two results: the previous and the current one. Since total variation is the sum (with the statistical mean) of the two variations.

$$\text{Total variation} = \sqrt{(CV_{first\ result})^2 + (CV_{second\ result})^2} \quad (32)$$

or

$$\text{Total variation} = \sqrt{\left(Z\sqrt{CV_l^2 + S_{meas}^2}\right)^2 + \left(Z\sqrt{CV_l^2 + S_{meas}^2}\right)^2} \quad (33)$$

or

$$\text{Total variation} = \sqrt{2} Z\sqrt{CV_l^2 + S_{meas}^2} \quad (34)$$

The last calculation formula of Total variation will be the control limits of Delta check which will be calculated by the next formula:

$$\text{Control limits of Delta check} = \text{Current Value} \pm \sqrt{2} Z\sqrt{CV_l^2 + S_{meas}^2} \quad (35)$$

4. External quality control (EQC)

4.1 Calculation of EQC control limits

External quality control or external assessment scheme (EQAS) or proficiency testing program (PT)⁸ refer to the process of controlling the accuracy of an analytical method by interlaboratory comparisons.

Its basic idea can be synopsized in the following steps:

1. The EQAS coordinator prepares and sends to the participants of the scheme one or two samples from the same pool.
2. The samples are assayed by the laboratories using the same equipment and reagents as they do in routine for the patients' determinations.
3. The EQAS coordinator gathers all the results and it groups them (**peer groups**) according the laboratories analytical methods, analyzers or any other criteria.
4. The EQAS coordinator calculates the target value (**consensus mean**) and its total variation (expressed as standard deviation) of the laboratories results.
5. If any of the laboratories has values outside of the control limits (target value \pm allowable variation) then this laboratory is considered "**out of control**".
6. The "out of control" laboratories have to correct their analytical procedures.

In many countries the participation of the laboratories in EQC is mandatory. In any case, EQC is a very important part of SQC. The calculation of the consensus mean is similar to calculation of the control limits in internal quality control. Although there are many calculation methods, an EQAS coordinator normally takes following steps:

1. The EQAS coordinator calculates the mean value (μ) and the standard deviation (s) of each peer group.
2. It calculates the range $\mu \pm 3s$.
3. If there are laboratory results which are over the previous range, these results are rejected.
4. It calculates the range $\mu \pm 2s$.
5. If there are laboratory results which are over the previous range, these results are rejected.
6. The steps 4 – 5 are repeated until there will be no value outside the new range $\mu \pm 2s$.

⁸ The term PT is used in USA in spite of EQAS which is used for the majority of the European countries.

4.2 EQAS charts and statistics

4.2.1 Standard Deviation Index

EQAS has its own charts and statistics. One of the most important statistics is the “**Standard Deviation Index**” (SDI). SDI shows the distance of the laboratory results from the consensus mean. It quantifies the inaccuracy of the analytical method. It is similar to Z-score (equation 12) and it is calculated by the formula:

$$SDI = \frac{\text{laboratory result} - \text{Mean value of peer group}}{\text{Strandard deviation of peer group}} \quad (36)$$

The control limits of SDI are zero ± 2 SDI. The SDI value of each laboratory can be located on a proper chart (SDI chart) as follows:

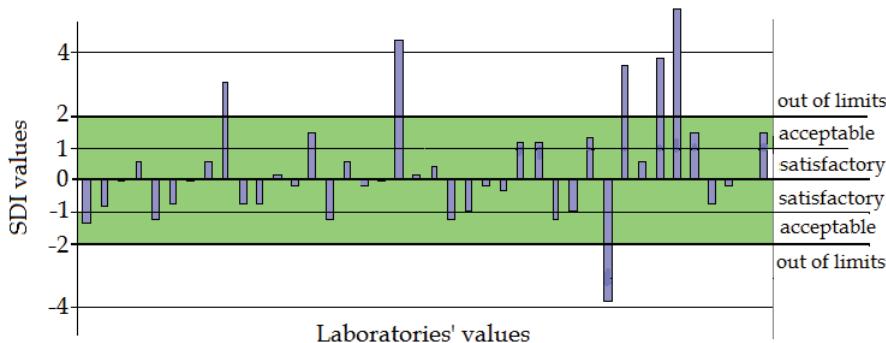


Fig. 16. SDI chart for a given analyte. Each bar corresponds to a different laboratory

Four rules are usually employed for SDI evaluation:

1. **2/5_{SDI}**. Two from five successive control limits exceed 1 SDI. It is a warning rule.
2. **X_{1.5 SDI}**. The mean value of five SDI values exceeds the limits ± 1.5 SDI. It reveals a lasting systematic error.
3. **1_{3 SDI}**. One value exceeds the limits ± 3 SDI.
4. **R_{4 SDI}**. The range (R) between the lower and higher SDI values is over ± 4 SDI.

4.2.2 Precision Index and Coefficient of Variation Ratio (CVR)

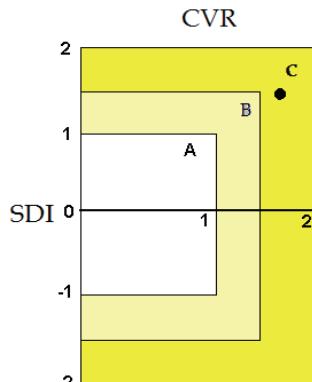
External quality assessment schemes quite often use two different statistics for the measurement of precision, the Precision Index (PI) and the Coefficient of Variation Ratio (CVR).

$$PI = \frac{\text{Standard deviation of laboratory}}{\text{Strandard deviation of peer group}} \quad (37)$$

The control limits of PI are < 2 . Its chart is similar to SDI chart.

$$CVR = \frac{CV \text{ of laboratory / month}}{CV \text{ of peer group / month}} \quad (38)$$

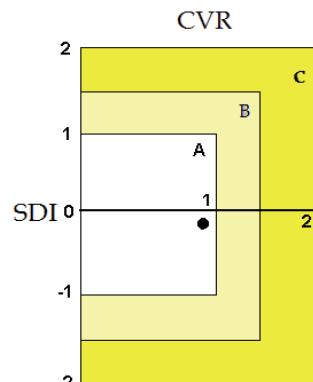
The combined SDI/CVR chart has the ability to evaluate the total analyte's performance (precision and accuracy).



SDI = 1.5

CVR = 1.6

Fig 17.1



SDI = - 0.4

CVR = 0.9

Fig 17.2

Fig. 17. CVR/SDI chart. The surface A corresponds to acceptable performance, the surface B to the grey-zone performance and the surface C to the rejected performance. In Fig. 16.1 the laboratory has unacceptable performance while in Fig. 16.2 the laboratory has acceptable performance

4.2.3 EQC normal distribution charts

More often than not, EQAS coordinators represent graphically the total performance of all the laboratories in a normal distribution chart. This normal distribution chart consists of bars (histogram) which correspond to certain groups of values. The bars may have different colors depending on the distance from the consensus mean. The bar that corresponds to each laboratory's result is marked by various methods (Fig. 17).

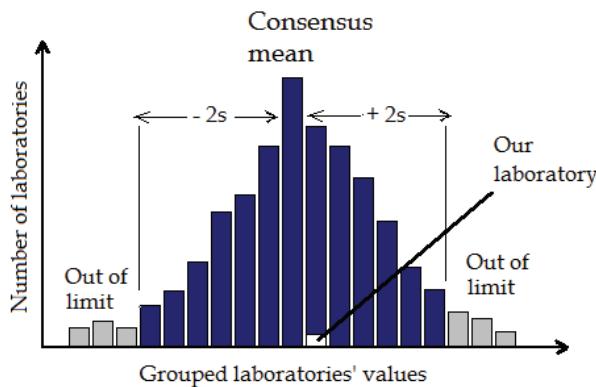


Fig. 18. An usual histogram from an EQAS report

In paragraph 4.1 we mentioned that EQAS coordinators usually group the laboratories according their analytical method and their automated analyzer. This is necessary so that the consensus mean will be more reliable and representative. Histograms containing two or more peer groups have bars with two or more different colors respectively (Fig 18).

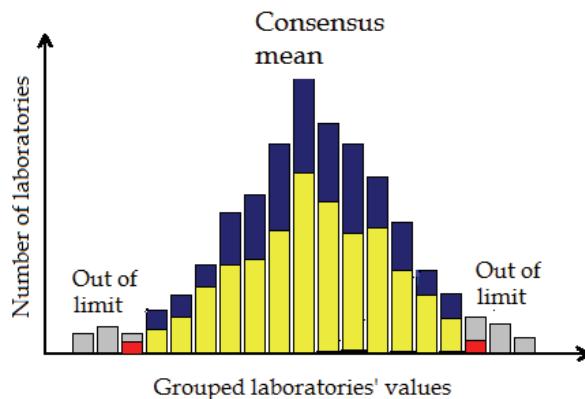


Fig. 19. A histogram from an EQAS report with two different peer groups

4.2.4 Youden plot

Most EQAS schemes use two control samples of different levels in order to check the performance of the analytical method in different concentration/activities, and preferably close to the decision limits. Youden plot is a rectangular chart of which the four angles correspond to the control limits of the two control levels [-4SD - +4SD] (Fig. 19). The acceptable part, the grey-zone and the rejected part have different colors. Each dot represents a different laboratory and therefore Youden plot describes the whole EQAS scheme. Dots (laboratories) that lie across the diagonal of the rectangular, at 45°, but are far for the center correspond to laboratories with proportional analytical error. The greater the distance from the center, the greater the proportional error. Dots restricted in the central rectangular, correspond to laboratories of which the performance is considered acceptable for this specific analyte.

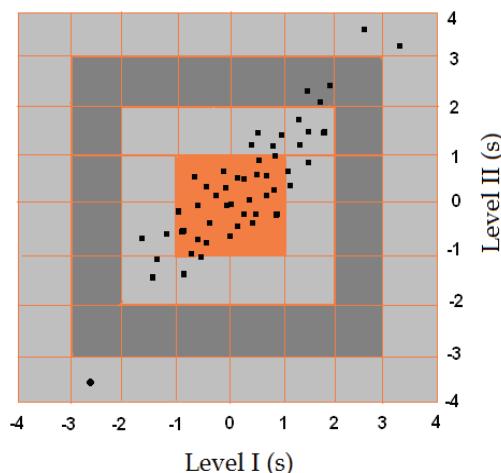


Fig. 20. A typical example of Youden plot. Each dot represents a different laboratory and its performance for the analyte in question

4.2.5 Yundt chart

Yundt chart helps to illustrate the performance of an analytical method across all its measuring range⁹. It needs at least three control levels to be plotted (Fig 20 & 21). If the line across the dots of the three levels is a straight one then the laboratory has a very good linearity. If not, there may be several issues with the linearity of the method.

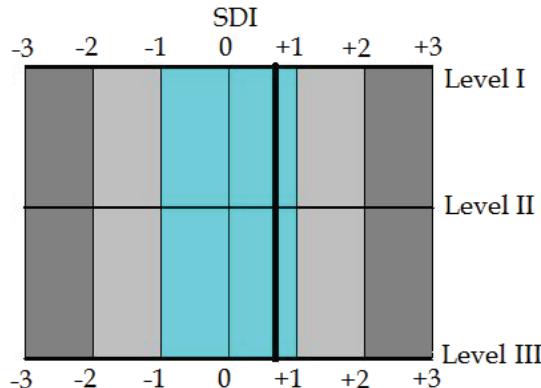


Fig. 21. A Yundt chart with three control levels. In this example the laboratory has a very good linearity

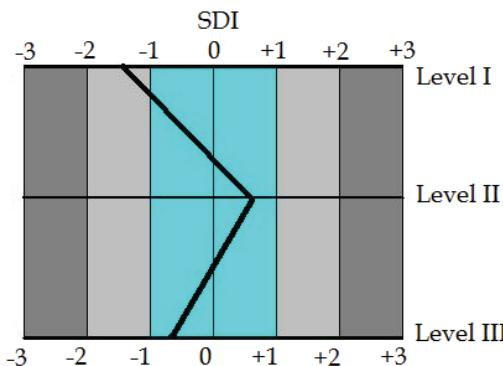


Fig. 22. A Yundt chart with three control levels. In this example the performance varies across the measuring range

5. Quality specifications

SQC's goal is to detect random and analytical errors or better, to ensure that total error is lower than the allowable total analytical error (aTE). aTE depends on some characteristics of the analyte and the analytical method, e.g. imprecision, bias, biological variation. According to these characteristics some analytes need more or less rigorous SQC rules than others. The

⁹ Measuring range is the ability to perform within acceptable analytical error throughout the defined measuring range of an analytical method.

laboratorians choose the SQC methods (statistics, charts and rules) according to these characteristics of the analyte and the analytical method.

There are two common practices:

- aTE depending on the analytical performance of the analytical method (imprecision, bias%). This practice is extensively used in the United States. In US the laboratories are obliged to follow the restrictions of CLIA (Clinical Laboratory Amendments Act). Among others obligations their determinations must have TE less than the aTE which CLIA proposes (Appendix I).
- aTE depending on the biological variation of the analyte (intra-individual variation and within-individual variation). This is usually the European practice where most laboratories, on a voluntarily basis, choose their SQC methods so as their TE to be less than aTE (Appendix II). aTE when comes from biological variations is symbolized as TE%_b.

5.1 Criteria of acceptable performance

A lot of European laboratorians have accepted some criteria for performance of the analytical methods. These criteria are called "criteria of acceptable performance" and they are based on the human biological variation (Ricos et al., 2000). Two kinds of biological variations have been calculated:

- The intra-individual variation (CV_I).
- The inter-individual variation (CV_w)

On the other hand the analytical variation can be divided in two subcategories:

- Within-day analytical variation (CV_{w-day})
- Between-days analytical variation (CV_{b-day})

The total analytical variation is calculated by the formula:

$$CV_{an-total}^2 = CV_{w-day}^2 + CV_{b-an}^2 \quad (39)$$

1st Criterion of acceptable performance: The total analytical variation must be lower or equal from the half of the intra-individual variability.

$$CV_{an-total} \leq 0.5 CV_I \quad (40)$$

2nd Criterion of acceptable performance: The within-day analytical variation must be lower or equal from 1/4 of the allowable total analytical error TE%_b.

$$CV_{w-day} \leq 0.25 TE\%_b \quad (41)$$

3rd Criterion of acceptable performance: The total analytical variation must be lower or equal from 1/3 of the allowable total analytical error TE%_b.

$$CV_{an-total} \leq 0.33 TE\%_b \quad (42)$$

4nd Criterion of acceptable performance: The bias% of the analytical method must be:

$$Bias\% < 0.25 \sqrt{CV_I^2 + CV_w^2} \quad (43)$$

5th Criterion of acceptable performance: The allowable total analytical error TE%_b of the analytical method must be:

$$TE\%_b \leq k(0.5 CV_w + 0.25\sqrt{CV_w^2 + CV_i^2}) \quad (44)$$

For $k = 1.65$ the equation 44 is equal to:

$$TE\%_b \leq 1.65 \Delta RE + \Delta SE \quad (45)$$

6. Appendix

Appendix I.

Allowable total errors from CLIA (USA) for some common biochemical parameters.

AST/SGOT	$\mu \pm 20 \%$
ALT/SGPT	$\mu \pm 20 \%$
ALP	$\mu \pm 30 \%$
LDH	$\mu \pm 20 \%$
AMY	$\mu \pm 30 \%$
ALB	$\mu \pm 10 \%$
CK	$\mu \pm 30 \%$
Gluc	$\mu \pm 10 \%$ or $\mu \pm 6 \text{ mg/dL}$
Urea	$\mu \pm 9 \%$ or $\mu \pm 2 \text{ mg/dL}$
UA	$\mu \pm 17 \%$
Creat	$\mu \pm 15\%$ or $\mu \pm 0.3 \text{ mg/dL}$ (greater)
Chol	$\mu \pm 10 \%$
TRIG	$\mu \pm 25 \%$
HDL	$\mu \pm 30 \%$
TP	$\mu \pm 10 \%$
ALB	$\mu \pm 10 \%$
TBILI	$\mu \pm 20 \%$ or $\mu \pm 0.4 \text{ mg/dL}$ (greater)
Ca	$\mu \pm 1.0 \text{ mg/dL}$
IRON	$\mu \pm 20\%$
Na	$\mu \pm 4 \text{ mmol/L}$
K	$\mu \pm 0.5 \text{ mmol/L}$
Cl	$\mu \pm 5\%$
Mg	$\mu \pm 25\%$
pO ₂	$\mu \pm 3 \text{ SD}$

Appendix II.

Desirable Specifications for Total Error (TE%), Imprecision (s_{meas}), and Bias%, derived from intra- ($CV_I\%$) and inter-individual ($CV_w\%$) biologic variation for Common Biochemical parameters.

Analyte	CV _I %	CV _w %	s _{meas}	Bias%	TE%
TBILI	12.8	10	12.8	10.0	31.1
DBILI	18.4	14.2	18.4	14.2	44.5
ALB	1.6	1.3	1.6	1.3	3.9
TP	1.4	1.2	1.4	1.2	3.4
GPT/ALT	5.8	4.5	5.8	4.5	14
GOT/AST	6.0	5.4	6.0	5.4	15.2
γGT	6.1	10.7	6.1	10.7	20.8
LDH	3.7	5.0	3.7	5.0	11.1
ALP	3.2	6.4	3.2	6.4	11.7
Ca	1.0	0.8	1.0	0.8	2.4
Phos	4.3	3.2	4.3	3.2	10.2
Chol	3.0	4.0	3.0	4.0	9.0
Trig	10.5	10.7	10.5	10.7	27.9
Creat	2.2	3.4	2.2	3.4	6.9
Urea	6.2	5.5	6.2	5.5	15.7
UA	3.7	5.0	3.7	5.0	11.1
Cl	0.6	0.4	0.6	0.4	1.4
CK	11.4	11.5	11.4	11.3	30.3
CK MB	9.9	7.8	9.9	7.8	24.1
Gluc	2.5	2.3	2.5	2.3	6.3
HDL	3.6	5.2	3.6	5.2	11.1
LDL	4.2	6.8	4.2	6.8	13.6
Fe	13.3	8.8	13.3	8.8	30.7
Mg	1.6	1.7	1.6	1.7	4.3
K	2.4	1.8	2.4	1.8	5.8
Na	0.4	0.3	0.4	0.3	0.9
AMYL	4.4	7.4	4.4	7.6	14.6
CHE	3.5	3.1	2.7	2.9	7.4

Disclaimer: The views described here are the authors' personal interpretation and approach, which may differ from those of the institutions they work.

7. References

- Bishop J & Nix A. (1993). Comparison of Quality-Control Rules Used in Clinical Chemistry Laboratories. *Clin Chem*; 39(8): 1638-49.
- Bonini P, Plebani M, Ceriotti F, Rubboli F. (2002). Errors in Laboratory Medicine. *Clin Chem*; 48(5): 691-8.
- Carey N, Cembrowski G, Garger C, Zaki Z. (2005). Performance characteristics of several rules for self-interpretation of proficiency testing data. *Arch Pathol Lab Med*; 129(8): 997-1003.
- Cembrowski G, Chandler E, Westgard J. (1984). Assessment of "Average of normals" quality control. Procedures and Guidelines for implementation. *Am J Clin Pathol*; 81(4): 492-9.
- Fraser G. (1988). The application of theoretical goals based on biological variation data in proficiency testing. *Arch Pathol Lab Med*; 112(4): 404-15.
- Harris E, Kanofsky P, Shakarji G, Cotlove E. (II) (1970). Biological and Analytic Components of Variation in long-term studies of Serum Constituents in Normal Subjects. *Clin Chem*; 16(12): 1022-27.
- Hoffmann R & Waid M. (1965). The "average of normals" method of quality control. *Am J Clin Pathol*; 43:134-41.
- Karkalousos P. (2007). The schemes of external quality control in laboratory medicine in the Balkans. *JMB*; 26:245-7.
- Levey S & Jennings E. (1950). The use of control charts in the clinical laboratory. *Am J Clin Pathol*; 20:1059-66.
- Lewis S. (1998). The trend of external quality assessment in Europe. *Sysmex Journal International*; 8(2): 67- 71.
- Lippi G, Guidi G, Mattiuzzi C, Plebani M. (2006). Preanalytical variability: the dark side of the moon in laboratory testing. *J Lab Med*; 30(3): 129-36.
- Panagiotakis O, Anagnostou-Cacaras E, Jullien G, Evangelopoulos A, Haliassos A, Rizos D. (2006). ESEAP: the national External Quality Assessment Scheme for clinical chemistry in Greece & Cyprus. *Clin Chem Lab Med*; 44(9):1156-7.
- Parvin C. (1991). Estimating the performance characteristics of quality-control procedures when error persists until detection. *Clin Chem*; 37(10): 1720-24.
- Parvin C. (1992). Comparing the power of Quality-Control Rules to Detect Persistent Systematic Error. *Clin Chem*; 38(3): 358- 63.
- Parvin C (1992). Comparing the power of Quality-Control Rules to Detect Persistent Random Error. *Clin Chem*; 38(3): 364- 69.
- Parvin C. (1993). New Insight into the comparative power of quality-control rules that use control observations within a single analytical run. *Clin Chem*; 39(3), 440-47.
- Plebani M & Carraro P. (1997). Mistakes in a stat laboratory: types and frequency. *Clin Chem*; 43(8): 1348-51.
- Radin N. (1996). The multi-rule Shewhart Chart for Quality Control. *Clin Chem*; 30(6): 1103-4.
- Ricós C, Baadenhuijsen H, Libeer J-C, Petersen PH, Stockl D, Thienpont L, Fraser C. (1996). External quality assessment: currently used criteria for evaluating performance in European countries and criteria for future harmonization. *Eur J Clin Chem Biochem*; 34: 159-65.

- Ricós C, Alvarez V, Cava F, García-Lario JV, Hernández A, Jiménez CV, et al. Current databases on biological variation: pros, cons and progress. *Scand J Clin Lab Invest* 2000; 20(8): 733-5.
- Ricós C, Alvarez V, Cava F, García-Lario JV, Hernández A, Jiménez CV, et al. Integration of data derived from biological variation into the quality management system. *Clin Chim Acta* 2004; 346(1): 13-8.
- Ricós C, Garcia-Victoria M, de la Fuente B. (2004). Quality indicators and specifications for the extra-analytical phases in clinical laboratory management. *Clin Chem Lab Med* 2004; 42(6): 578-82.
- Radin N. (1996). The multi-rule Shewhart Chart for Quality Control. *Clin Chem*; 30(6): 1103-04.
- Westgard J. (1992). Simulation and modeling for optimizing quality control and improving analytical quality assurance. *Clin Chem*; 36(2): 175-8.
- Westgard J, Groth T. (1981). A multirule Shewhart Chart for quality control in clinical chemistry. *Clin Chem*; 27(3): 493-501.
- Williams G, Young Z, Stein M, Cotlove E. (I) (1970). Biological and analytic components of variation in long-term studied of serum constituents in normal subjects. *Clin Chem*; 16(12): 1016- 22.
- Wood R. (1990). A simulation study of the Westgard multi-rule quality-control system for clinical laboratories. *Clin Chem*; 36(3): 462- 65.