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# The quality assurance system in clinical chemistry

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**Summary**. - The quality assurance system in clinical chemistry allows for the identification of errors and control actions to correct them. It is well known that laboratory errors can be classified as: pre-analytical, analytical and post-analitycal. While pre-analytical and post-analytical errors are very difficult to identify, the analytical variability (both imprecision and inaccuracy) can be monitored with internal quality control (IQC) programs and external quality assessment (EQA) schemes. The purpose of IQC is mainly to verify the stability of laboratory estimates with time and therefore it is essentially a control of imprecision. IQC programs are based on the use of control samples which are analyzed in each analytical series. The easiest method of representing IQC data is by the use of Shewhart's chart, although "cusum" chart and Youden plot are often useful. As for the criteria according to which an analytical series should be accepted or rejected, the use of practical control rules is widely spread in laboratories. Participation in EQA schemes allows the laboratory to have a retrospective estimate of its performance in terms of both imprecision and inaccuracy, if definitive or reference methods are available. In lack of definitive or reference methods, consensus mean or median can be derived from the data obtained by all the participants or, in some cases, by the participants using the same analytical method (e.g. for analytes not yet completely characterized and measured with immunoasays).

*Key words*: quality assurance, internal quality control, external quality assessment.

**Riassunto** *(Il sistema di sicurezza di qualità in chimica clinica).* - Il sistema di sicurezza di qualità in chimica clinica permette di individuare le cause di errore e le azioni da intraprendere per la loro eliminazione. E' generalmente accettato che gli errori sono classificabili come: pre-analitico, analitico e post-analitico. Mentre le cause di errore pre- e post-analitico sono di difficile individuazione, la variabilità analitica (sia come imprecisione che inaccuratezza) può essere monitorata attuando programmi di controllo di qualità interno (CQI) e partecipando a schemi di valutazione esterna di qualità (VEQ). Scopo del CQI è principalmente di verificare la stabilità del sistema analitico, cioè monitorare l'imprecisione nel tempo. I programmi di CQI sono basati sull'uso di campioni di controllo inseriti in ogni serie analitica. Il metodo più semplice di rappresentare i dati di CQI è quello della carta di controllo di Shewhart, anche se la carta "cusum" e il grafico di Youden sono spesso di utilità. Relativamente ai criteri decisionali per accettare o scartare una serie analitica, l'applicazione delle regole di controllo è una pratica relativamente diffusa presso i laboratori. La partecipazione a schemi di VEQ permette al laboratorio di avere una stima retrospettiva delle sue prestazioni sia in termini di imprecisione che di inaccuratezza, nel caso che siano disponibili metodi definitivi o di riferimento. In mancanza di questi ultimi, è possibile utilizzare la media o la mediana di tutti i dati o del singolo metodo (ad esempio nel caso di analiti non ancora completamente caratterizzati e misurabili con metodi immunometrici).

*Parole chiave:* sicurezza di qualità, controllo di qualità interno, valutazione esterna di qualità.

## Introduction

The quality assurance system in clinical chemistry lies in identifying causes for error and actions to correct them [1-3]. One should keep in mind, in particular, that some errors occur in the laboratory, and these are the easiest to identify and to correct; other errors may occur outside the laboratory. It is generally accepted that errors, or rather result variability in laboratory assays, depend on three factors: pre-analytical, analytical and post-analytical. Pre-analytical variability concerns whatever may alter the result of analysis and may occur outside the laboratory: exchange of specimens, uncontrolled collection, non-optimal sample preservation, etc. Analytical variability concerns what take place in the laboratory during any of the various analytical steps: calibration of the instruments, sample pipetting, reagent preparation, etc. Post-analytical variability concerns anything that take place after the analysis itself. It is generally very difficult to identify causes for pre- and post- analytical errors, due to the high number of variables involved. It is certainly easier to operate on the analytical variability through suitable evaluation actions. The most widely used among these are internal quality control (IQC) programs and external quality assessment (EQA) schemes. IQC is one of the important elements for

decision of accepting or rejecting an analytical series. On the other hand, EQA is an *a posteriori* control of the laboratory performance.

### Random and systematic errors

Any measurement is subject to errors: random errors (expressing the variation among replicated estimates) which produce imprecision and systematic errors (expressing a bias from the reference, or target value) which cause inaccuracy. When analyzing the same sample several times, the results obtained are arranged according to a gaussian curve whose width (extimated by calculating standard deviation) correlates to imprecision: the narrower the gaussian curve, the lower the imprecision. The difference between the mean obtained and the target value (this difference is commonly indicated as bias%) correlates to inaccuracy: the lower the difference, the lower the inaccuracy. The two kinds of error described (random and systematic) produce the global error. This is represented by the square root of summed squared random error (coefficient of variation, CV%) and squared systematic error (bias%).

### Internal quality control

The purpose of internal quality control (IQC) is mainly to verify the stability of laboratory estimates at the time of testing, and it is essentially a control on imprecision [4]. IQC programs have various procedures, but all are based on the use of selected control samples which are analyzed in each analytical series. Differences in the various programs depend upon the number of control samples required and on the data presentation. In clinical chemistry, control samples have generally two concentration levels (normal and pathological); in immunoassay the use of three concentration levels is advisable. Obviously, the higher is the number of controls, the easier is the decision of accepting or rejecting the analytical series.

Control samples can be prepared in the laboratory [5] or bought from specialized industries. The former ones, being made of untreated human serum, monitor the behaviour of unknown samples better. Nevertheless, they present problems of stability, infection (samples obtained in the laboratory are often positive for HBsAg, anti-HCV and anti-HIV tests) and concentration, which can often be far from the optimal one. Industrially made control samples do not have these drawbacks, although they may behave differently from the unknown samples, as they underwent treatment (deprivation, exogenous analyte addition, preservative addition, filtration, lyophilization).

The easiest system to represent control data, among the many proposed, is the use of Shewhart's chart (Fig. 1). To prepare this chart, it is necessary to analyze control samples in different analytical series (at least 20 determinations are advisable). Successively, the average values achieved and the limits (three standard deviations) are reported on a plot. Any time that an analytical series is run, the control results are recorded on the chart and then appraised as to their position in comparison with the chosen limits defined by plus or minus three standard deviations: therefore about the 0.3 percent of values will be outside the limits.

The use of Shewhart's control chart allows an easy identification of some error situations, i.e., an out-of-control method resulting in an increase of imprecision and of inaccuracy. In the former case measurements are in a position alternatively higher and lower than the set limits, while in the latter case, measurements are outside the set limits always in the same direction (either upwards or downwards). Increase of imprecision may be due to several causes, among which are pipetting problems, lack of stability of measuring instruments. Increase of inaccuracy may be due to wrong or degraded standards.

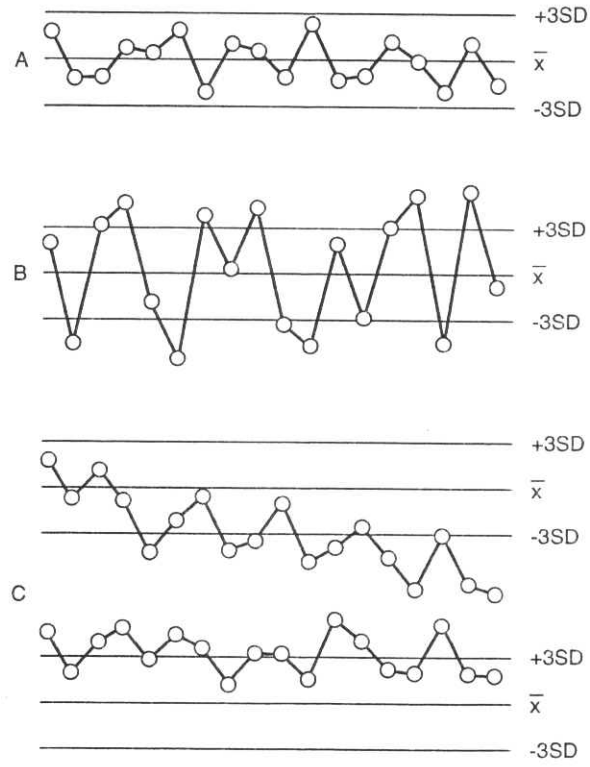


**Fig. 1.** - Examples of Shewhart's control chart. A) in control situation; B) increase of imprecision; C) increase of inaccuracy.

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Other methods have been proposed to point out errors and help operators. In order to demonstrate systematic errors more easily it is possible to use the "cusum" chart (Fig. 2). In this method, systematic deviation and out-of-control situation are identified earlier and more easily for the operator. Amplification is mathematically obtained as it is not the control value which is recorded, but the cumulative sum of deflection between data and target value. In this way, if controls have even slightly higher values than the average ones, the deflection sum will continuosly increase.

Another widely used method, is the Youden plot (Fig. 3 and 4). In this case it is necessary to use two control samples, A and B. The results of control A and B, divided by relevant target values, are reported on the abscissa and ordinate axes, respectively. In the presence of a random error, values are arranged perpendicularly to the equivalence line (Fig. 3), while in the presence of systematic error, values are arranged along the equivalence line (Fig. 4). In both cases Youden plot makes visually more apparent the presence of both random and systematic errors.

Once control charts have been prepared, the problem of decision arises, i.e., the problem of establishing criteria according to which an analytical series should be accepted or rejected depending on control values. In an ideal IQC program, the rejecting signal should always be supplied only in the presence of errors. As both theoretical coded criteria and general consensus are missing, practical control rules, widely spread in laboratories, have been proposed to make the operator's task easier [6-8]. According to some of these practical rules, e.g., using three concentration level samples, an analytical series is rejected if one control out of three deflects by more than three standard deviations from the mean, if two controls out of three deflect by more than two standard deviations, if the three controls deflect from mean by more than one standard deviation.

## External quality assessment

Participation in external quality assessment (EQA) schemes is another aspect that can contribute to improve laboratory performance. Unknown samples are sent to different laboratories at the same time, and the collected test results are analyzed. Generally speaking, these schemes allow measurements of the total variability among laboratories and an estimate of mean imprecision and inaccuracy of laboratories and methods. Each participant receives a retrospective estimate of its own work, in terms of imprecision and inaccuracy. He will also be able to compare his results with the results achieved by other participating laboratories.

These programs are in use throught Europe for general clinical chemistry, while EQA programs for immunoassay, although not yet consolidated, are quickly spreading and in endocrinology, either routine or pilot

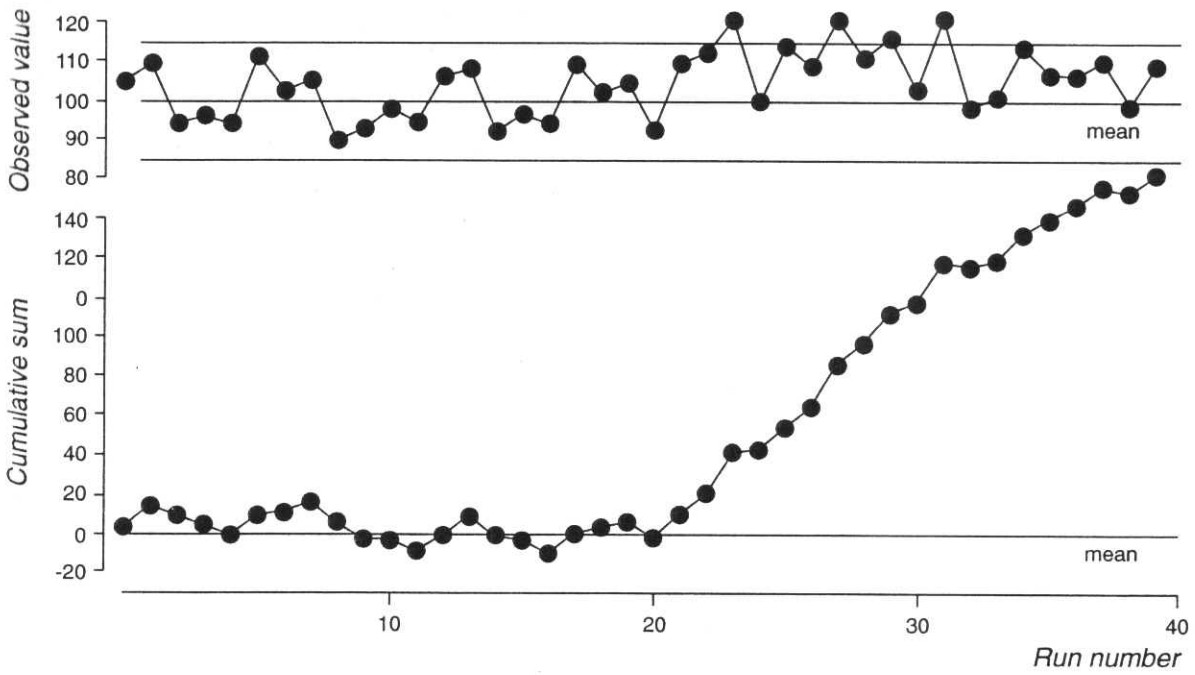


**Fig. 2.** - Examples of Shewhart's control chart (top) and of "cusum" chart (bottom) in the presence of a systematic error. This is more apparent in the "cusum" chart than in Shewhart's.
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programs have begun in most European countries [9-11]. In the United Kingdom and in Germany, EQA schemes have a twenty year history; in France they have a ten year history; in Italy EQA schemes have been operating for some time, although they have not been extended to all Italian laboratories [12-14]. The French situation is more complex: national schemes managed by public authority exist together with those managed by scientific bodies.

Operative details concerning different EQA schemes will be dealt with in other articles in this volume. It might therefore be interesting to explain some general rules rather than to present actual operative aspects. One of the most important points is the choice of the EQA organizer: public authority, professional bodies or industry. In some countries control management is committed to public authority, in other countries to professional societies. The best way is probably to find the right equilibrium between the two, as the importance of both structures is undeniable, in order to avoid indiscriminated proliferation of assessment programs.

On the other hand, anonymous and voluntary participation are undoubtedly very important. It must not be forgotten that the EQA program is intended to be educational and should stimulate laboratories to improve their results. The problem of using EQA programs to identify poor performance by participants in order to revoke their authorizations and to refuse analysis fee refund, is much more delicate. Participation to these programs should be only one of the criteria by which a laboratory performance is evaluated. Other criteria might be based upon instrumental equipment, staff qualification, scientific updating.

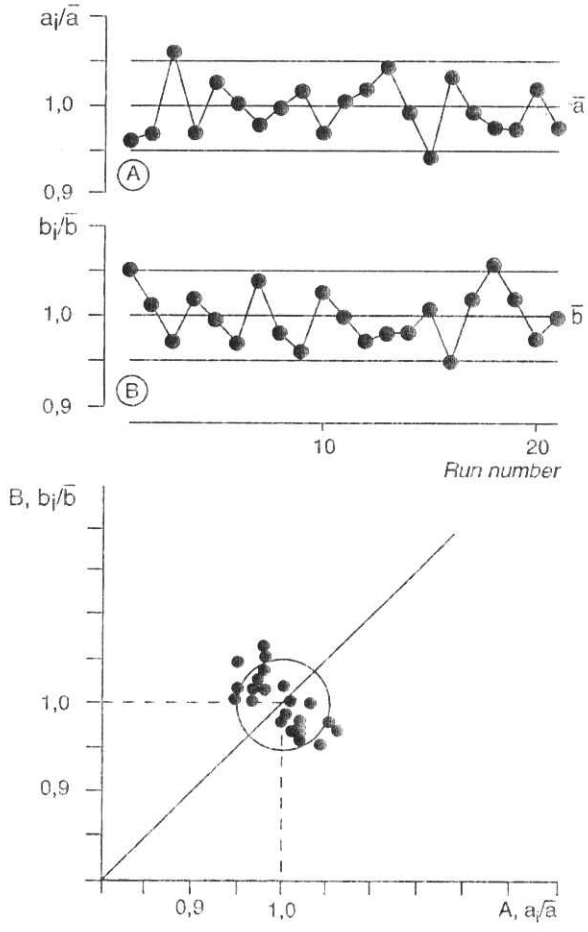


**Fig. 3.** - Examples of Shewhart's chart (top) used for two control samples (A and B) and their Youden plot (bottom). In the presence of random error, in Youden plot, values are arranged perpendicularly to equivalence line.
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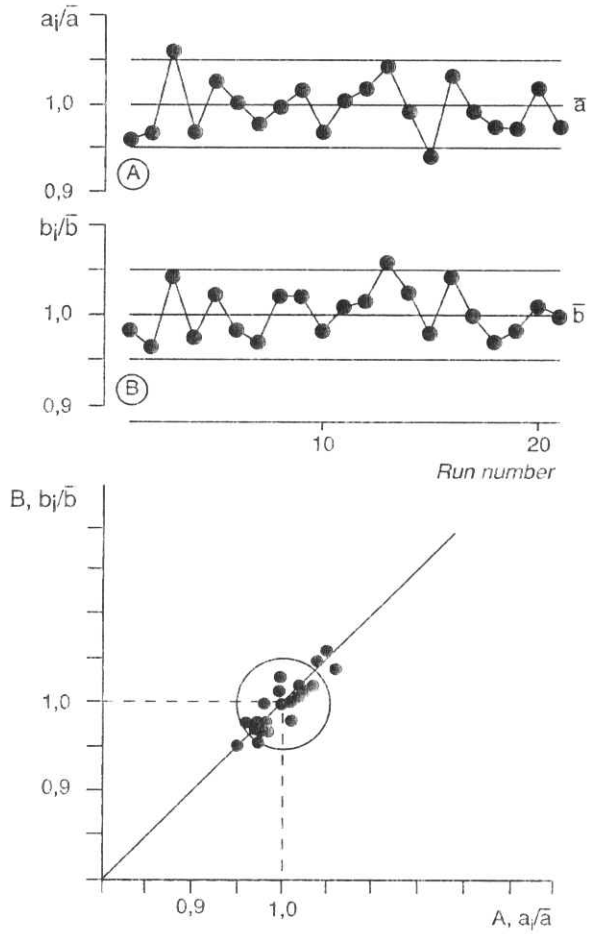


**Fig. 4.** - Example of Shewhart's chart (top) used for two control samples (A and B) and their Youden plot (bottom). In the presence of systematic errors, in Youden plot, values are arranged along equivalence line.
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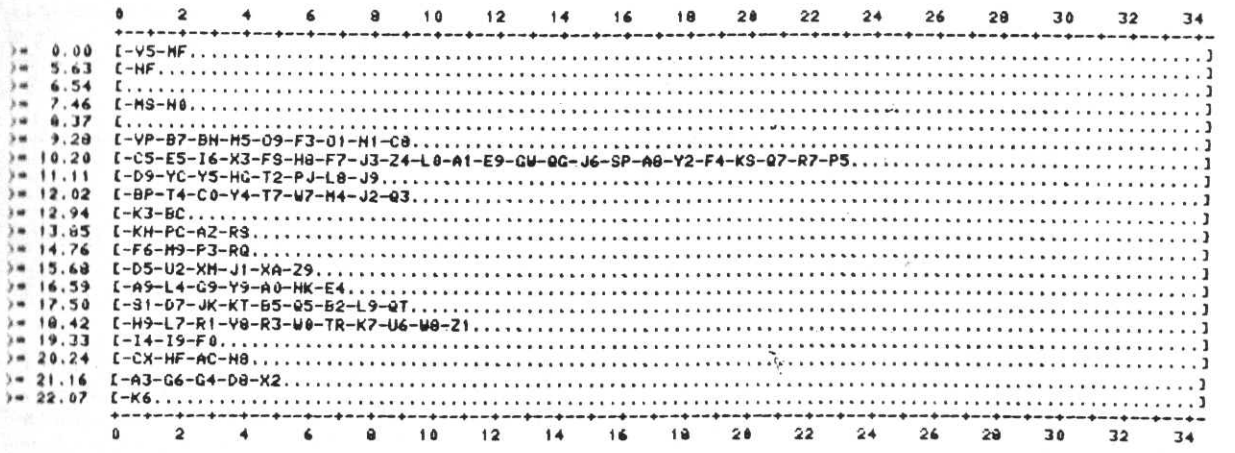


**Fig. 5.** - Example of LH histogram obtained in the Italian (CNR Tecnostandard) EQA scheme for a sample sent in October 1989 (x axis: number of laboratories; y axis: LH concentration in IU/l 1st IRP 68/40). Laboratory data are quite scattered and can be approximately divided into two groups: the first (mean value about 10 IU/l) refers to laboratories using monoclonal antibody based kits, the second (mean value about 18 IU/l) refers to laboratories using less specific, polyclonal based kits. The number of data around the general mean (about 15 UI/l) is low and the mean cannot be considered as a target value.

From an organizational point of view, there are various critical points of EQA programs. The assigning of a reference value is an important question. Definitive and reference methods are only available for a limited number of analytes [15-17]. In endocrinology, only some analytes (steroids and drugs) can be measured by other methods other than immunoassay (e.g. isotopic dilution, gas chromatography, mass spectrometry, ID-GC-MS). In lack of definitive or reference methods, consensus mean or median, derived from the data obtained by participants, often reflects true value. There are, however, situations when this does not apply [18] (Fig. 5): in these cases, it is useful to take into consideration the results obtained with different methods separately , in order to avoid false interpretation by the users.

## Conclusions

There are various elements contributing to quality assurance, and we described only some of them. One of the goals for the next years will be to achieve an even and sufficiently high level of quality in laboratories, both large and small, public and private. Also the performance of the laboratories will have to be similar all over the European Union and common programs will have to be developed. This problem is not easy to solve and one of the actions that will have to be undertaken is to spread quality assurance culture in educational rather than punitive terms.

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