In this issue: Laboratory Medicine Perspectives from Latin America

The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

Quality control in screening for infectious diseases at blood banks. Rationale and methodology

Amadeo Sáez-Alquezar[1], Pedro Albajar-Viñas[2], André Valpassos Guimarães[1], José Abol Corrêa[1]

[1] *Programa Nacional de Controle de Qualidade (PNCQ)/Sociedade Brasileira de Análises Clínicas (SBAC), Rio de Janeiro, Brazil*

[2] *Department of Control of Neglected Tropical Diseases, World Health Organization, Genéva, Switzerland*

ARTICLE INFO

Corresponding author:
Dr. Amadeo Sáez-Alquezar
Programa Nacional de Controle de Qualidade (PNCQ)/Sociedade Brasileira de Análises Clínicas (SBAC)
Rio de Janeiro, Brazil
E-mail: amadeo62@gmail.com

Key words:
internal quality control, external quality control, infectious diseases, screening, blood banks, qualitative tests.

ABSTRACT

Quality control procedures are indispensable to ensure the reliability of the results provided by laboratories responsible for serological screening in blood banks. International recommendations on systems of quality management classify as a top component the inclusion of two types of control: (a) internal quality control (IQC) and (b) external quality control (EQC). In EQC it is essential to have, at least, a monthly frequency of laboratory assessment. On the other hand, IQC involves the daily use of low-reactivity control sera, which should be systematically added in all run, carried out in the laboratory for each parameter. Through the IQC analysis some variations in the criteria of run acceptance and rejection may be revealed, but it is of paramount importance to ensure the previous definition of these criteria and even more importantly, the adherence to them; and that corresponds to the validation of analytical runs of each test. Since 2010 this has been, for instance, the experience of the PNCQ*, developing external quality control programmes on serology for blood banks. These programmes use samples of lyophilized sera well-characterized for the reactivity related to the parameters used for the serological screening of

blood donors. The programmes have used blind panels of six samples for monthly assessments. In the last 50 assessments, which involved 68 blood banks in Brazil, a significant number of instances of non-compliance were observed in all monthly assessments. These results provide strong support to the recommendation of systematic monthly assessments.
(*) *National Quality Control Programme (PNCQ)*

INTRODUCTION

Serological screening for infectious diseases in blood banks currently includes qualitative serological testing for HIV, HTLV, HCV, HBV and syphilis. Anti-*T.cruzi* screening is also conducted in Latin American countries where Chagas disease is endemic. It is recommended that NAT (nucleic acid testing) for HIV, HBV and HCV be used in parallel with serological screening to reduce the risk of transmission during the immunological window period.

Serological screening involves the use of sensitive and specific tests, employing the ELISA (enzyme-linked immunosorbent assay) and CLIA (chemiluminescence immunoassay) methodologies in most cases. The platforms used are automated so that a large volume of samples can be covered in a short timeframe, thereby ensuring easier processing of results[1].

All serological tests used in screening are qualitative and should be accompanied by quality control procedures that are appropriate for this kind of test and guarantee the quality of the end results.

Quality control procedures are necessary to ensure the quality of the results originating from laboratories responsible for serological screening. International and national recommendations indicate that quality management systems must necessarily adopt at least two types of controls: (a) internal quality controls and (b) external quality controls[1-4].

External quality controls entail participation in at least one external quality assessment (EQA) programme using well-characterized panels that contain specimens for all screening parameters and enable assessments to be conducted at least once a month.

Since the late 1990s, with assistance from the Pan American Health Organization (PAHO), reference laboratories at blood banks in most Latin American countries have started participating in serology quality control programmes; many have assumed the role of organizing centres for the internal development of such programmes in their respective countries[5-11]. This tradition of participating in external quality assessment (EQA) programmes is continuing to date, largely in response to the requirements contained in national regulations and recommendations of international institutions. *While not all programmes have the same features, in general they do not feature monthly assessments.*

In so far as the adoption of internal quality control for the use of low-reactivity control sera is concerned, while national regulations do recommend their use, few visible results have been obtained and there appears to be little consistency in the procedures and rules to be adopted.

This report aims to present the most appropriate procedures for the development of external quality control programmes and implementation of internal quality control, with a focus on the qualitative serological testing used to screen blood donors.

INTERNAL QUALITY CONTROL

According to the Clinical & Laboratory Standards Institute (CLSI), an analytical run is the time interval in which a series of measurements are taken in a stable manner in terms of precision and accuracy, taking account of any adverse effects that should be detected appropriately[12].

Internal quality control involves the daily use of low-reactivity control sera, which should be added in all runs, carried out in the laboratory for each parameter.

It is important to remember that each laboratory is responsible for validating its own internal control programme in order to meet basic requirements within its performance parameters. The importance of standardizing the usage and acceptance criteria for the daily use of low-reactivity internal control sera (ICS) must also be taken into account. It must be made very clear that ICS are different to the positive or negative control sera included in diagnostic kits. ICS may be prepared in the laboratories themselves or, preferably, acquired from suppliers specializing in the manufacture of such products.

ICS should be routinely used in the laboratory every day to monitor the performance of each test, as they help to validate analytical runs. The number, frequency and extent of the controls involved will depend on the number and magnitude of the analytical runs. ELISA tests involving microplates use a positive and a negative ICS for each routine microplate. The use of one positive ICS for every 100 or 200 specimens is recommended for continuous flow equipment.

Each ICS should be initially adjusted for use with each specific test. The reactivity criteria shown in Table 1 have been adopted in most laboratories in Latin America[1].

When ICS are prepared in-house, adjustments (dilutions) should be made in order to achieve the reactivity index within the established range. In the case of sourcing from suppliers, most of whom prepare the sera individually for each commercial brand, the values are seldom correct and it is often necessary to make adjustments by diluting the product in order to achieve the desired values. Obviously, this will only be possible when the reactivity values exceed the limits of the chosen range.

After obtaining reactivity values within the chosen range, it is recommended that the ICS be stored at -20º in small volume aliquots for daily use. It is therefore possible to avoid any loss of reactivity that occurs when larger aliquots are stored in refrigerators for several days. Standardization will be performed for each of the positive ICS chosen for each parameter and for each methodology. Twenty tests will be conducted to obtain the mean standard deviation and the coefficient of variation. The transformation of these data into Standard Deviation Units (Z score) makes it possible to discard extreme values (outliers) in the 20 determinations by applying the Grubbs test[13], and facilitates the plotting of the Levey-Jennings graph[14], which will subsequently be used to enter the daily ICS values.

The Levey-Jennings graph yields the successive control values and facilitates daily analysis of the behaviour of ICS within the decision limits: ±1 SD, ±2 SD and ±3 SD. This analysis provides evidence of any deviations from expectations by demonstrating trends (systematic errors) or any dispersion in the results (random errors), in addition to unexpected results which may indicate changes in the reagent batches or significant changes in the equipment.

Daily variations in ICS are best analysed by using some of the Westgard rules[15]. At least three of these, considered "alert" rules (12s, 22s, 41s), facilitate the identification of trends that can be corrected in a timely manner. Two of the rules, considered being "control" rules (1_{3s} and 10x) indicate that the run presented significant changes that are liable to jeopardize the results, which should not therefore be accepted.

Positive as well as negative ICS should be used. The reactivity acceptance criteria for the negative ICS are shown in Table 1. The standardization and application procedures in the Levey-Jennings graph are the same as for positive ICS,

Table 1 Low-reactivity Internal Control Sera (ICS) to monitor daily runs in laboratories carrying out serological screening of blood donors

Positive ICS	
Index: Reading value/cut-off value	**>1.0**
Recommended range	**2.0 – 4.5**
Index for competitive assays	**>1.0**
Recommended range	**0.3 – 0.7**
Negative ICS	
Index: Reading value/cut-off value	**>1.0**
Recommended range	**<0.8**
For competitive assays	**>2.0**

with this sole difference, that the Westgard rules are not used to analyse daily performance. It is important only that the consistency of the (always negative) results around the mean be verified.

As stated above, each laboratory is responsible for validating its own internal control programme in order to meet the basic requirements within its performance parameters. By analysing the behaviour of the ICS, the acceptance and rejection criteria of the runs may exhibit some variation, but the most important consideration is that these criteria should be defined and still more importantly adhered to. *This is the validation of the analytical runs of each test.*

In practice, it is quite common for variations to occur between different batches of the same test from the same manufacturer. If these variations are very significant, they may directly affect the results of the specimens under analysis. In this happens, regulations in some countries recommend that, where batches of diagnostic kits need to be changed, the laboratory should conduct an assessment to ensure that the new batch performs as well as the previous one[3]. Less significant variations are nearly always observed when batches are changed. It must be therefore made clear that when a change occurs, the positive ICS that were previously used must be re-standardized.

Serum panels are used to assess diagnostic kits before they pass into routine use and when changing batches to verify that they perform as well as the previous batch: *Validation of batches of kits before use and batch-by-batch validation[2]. The final recommendations for the use of internal quality control are shown in Table 2.*

EXTERNAL QUALITY CONTROL OR EXTERNAL QUALITY ASSESSMENT (EQA) PROGRAMMES

This refers to an external assessment of laboratory performance to ensure that the procedure is capable of verifying the quality of the results. In general, EQA programmes are developed by leading institutions or suppliers of specific products to ensure quality. These bodies are known as organizing centres (OC). Participation in EQAs may be voluntary or mandatory. Voluntary participation implies individual professional responsibility, since it shows that those in charge of the participating laboratories wish to understand and improve their performance. The

Table 2 Recommendations on internal quality control at laboratories carrying out serological screening of blood donors

Positive ICS should be adjusted for each test within the established reactivity criteria
ICS must be used in all analytical runs and the findings incorporated into the Levey-Jennings graph for daily analysis
Minimum acceptance criteria must be established and applied to validate the daily runs
Standardization of positive ICS must be repeated when diagnostic kits are changed
Whenever possible, it is also recommended that new batches of diagnostic kits be assessed using sera panels with well-characterized reactive and non-reactive specimens (batch-by-batch assessment)

drawback is that only those laboratories that are concerned about their performance are assessed. When participation is obligatory, the data obtained through each programme are more valid because the assessment encompasses all the laboratories in the network (state, country, etc.). Nevertheless, the fact that participation is obligatory is no guarantee that the information will be exploited to the maximum or that services will be improved, because not all participating laboratories will make full use of all the information generated in each programme[1,14].

EQA programmes use blind panels of serum specimens sent by the organizing centre to all participating laboratories (PLs). These specimens must be well characterized by different commercial tests for each parameter, and reactive specimens should be confirmed through supplementary tests. While it is impossible to characterize the specimens using all the tests on the market for assays of each parameter, it is nevertheless recommended that the tests most frequently used in the region be employed, and that the most widespread methodologies are applied, namely ELISA and CLIA.

As for the use of EQA programmes to assess the performance of screening at blood banks, it is advisable that blind panels should contain specimens with variable reactivity for all screening parameters to ensure an exact reproduction of routine screening conditions. It is also possible to use blind sera panels for just one parameter, which frequently occurs in anti-*T. cruzi* screening in countries that are not endemic for Chagas disease.

The best way to obtain the specimens for the panels is to use *plasma units*, which have been discarded because they have demonstrated reactivity to one of the serological screening parameters. These units of plasma are transformed into serum through a process involving recalcification and subsequent dialysis or filtration. Each unit of serum should be characterized before the panels are prepared.

In some situations, dilutions will have to be made to obtain the quantity of specimens needed to carry out the programmes, since all PLs must receive the same specimens to ensure proper analysis and comparison of the end results. Dilution limits exist for each parameter to ensure that the specimens maintain their reactivity properties for all screening tests and for any supplementary tests used to confirm positivity.

The OC of an EQA programme is responsible for the logistics of preparing and delivering the panels to all participating laboratories (PLs) that should process the samples in the same way as for a routine procedure. The idea is to assess the performance of PLs in cooperative spirit, keeping individual results strictly confidential.

Since the serological tests used for screening are qualitative, potential failures in laboratories' internal processes may give rise to two types of non-compliance: (a) False Reactive Results (FRR) or (b) False Non-Reactive Results (FNRR).

The OC should evaluate the performance of the PL and prepare two types of document: (a) an individual assessment for each PL (confidential) and (b) a final report containing strategic information, e.g. about the characterization of the specimens on the panel, the percentage of FRR and FNRR produced during the programme using each of the tests or methodologies adopted by the PL, and the total number of PL. The data should not be confidential; it should be made available to all PLs, as well as persons responsible for the distribution of diagnostic kits and the competent health authorities concerned. This information is put to best use when the assessments and final reports are analysed and discussed internally. All instances of non-compliance (FRR and/or FNRR) should be documented and discussed in order to pinpoint the possible cause. Table 3 lists the principal errors observed in the performance of EQAs.

Final recommendations for optimum implementation of internal quality control and participation in external assessment programmes are shown in Table 4.

In the current context of quality control at laboratories, it is considered essential that EQA assessments should be performed at least once a month. Recent studies show that EQA programmes conducted at serology laboratories screening for infectious diseases in blood banks reported instances of non-compliance (FRR and/or FNRR) in practically all months when an assessment was conducted.

The National Quality Control Programme (PNCQ) is the largest supplier of proficiency tests and internal control sera in Brazil. Since 2010, it has developed external quality control programmes in serology for blood banks.

These programmes make use of specimens of lyophilized serum that are very well-characterized for reactivity in reference to the parameters used for the serological screening of blood donors. The programmes use blind panels of six specimens for monthly assessments.

In the last 50 programmes, which involved 68 blood banks in Brazil, a significant number of instances of non-compliance (FRR and/or FNRR)

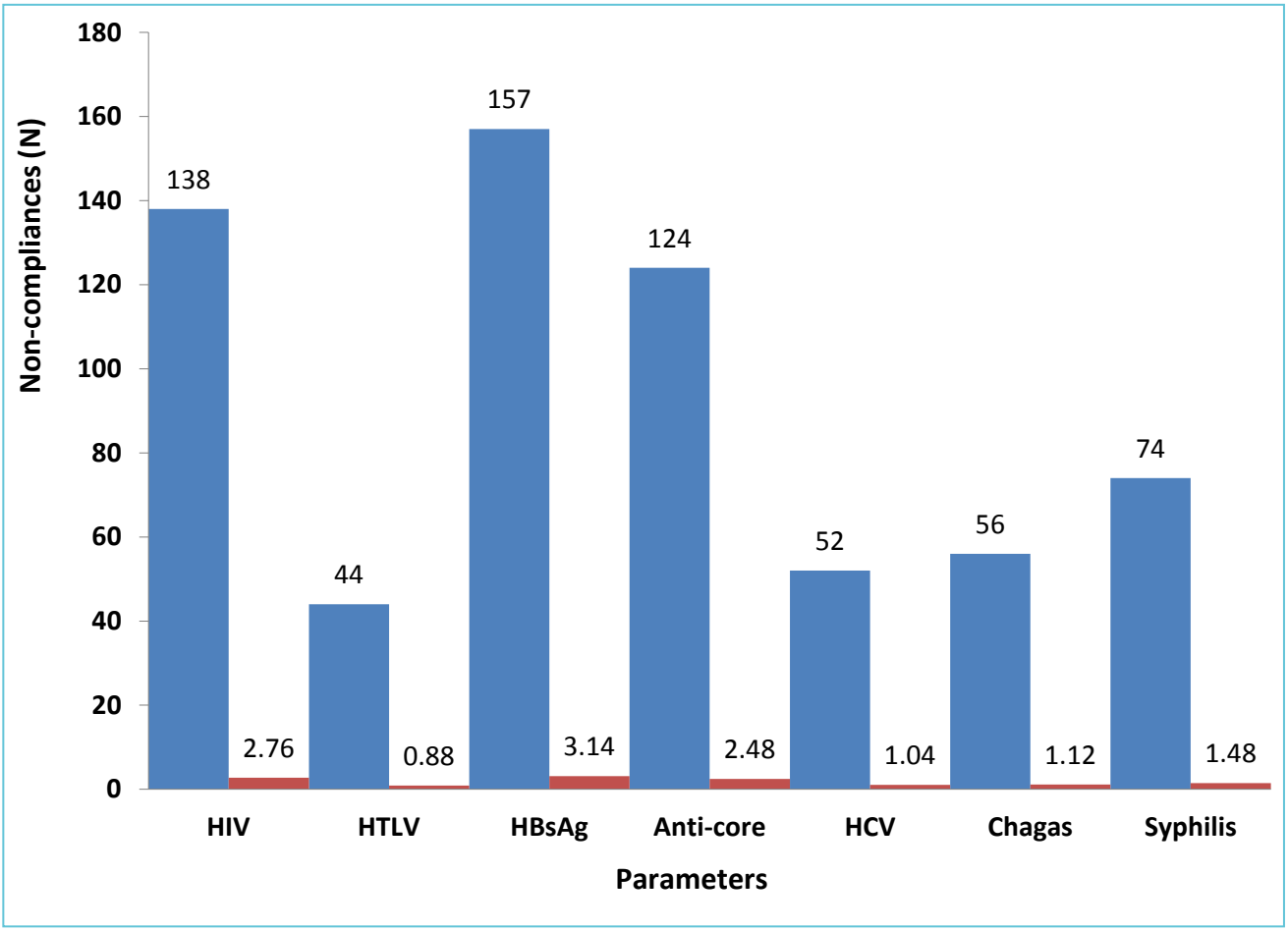
Table 3 The most common problems observed in EQA programmes at serological screening laboratories attached to blood banks

Problem	Phase
Contamination of samples	Pre-analytical/Analytical
Data transcription errors	Analytical/Post-analytical
Kits insufficiently sensitive or specific	Pre-analytical/Analytical
Inadequate internal quality control procedures	Analytical
Inadequate storage of specimens	Analytical/Pre-analytical

Table 4 Aspects of quality control at laboratories conducting serological screening of blood donors

Participation in at least one EQA programme with monthly assessments
Daily use of (+) and (-) ICS to monitor and validate analytical runs
Ongoing training for laboratory technicians
Use of diagnostic kits of proven quality (assessed before use)
Internal quality control of equipment, procedures, diagnostic reagents and comprehensive record of all activities
Regular inspections to ensure compliance with official standards

Figure 1 Total number of instances of non-compliance in monthly evaluation of 50 EQA (blue) and average instances of non-compliance per programme (red)



were observed in all monthly assessments (Figure 1), thus adding weight to the contention that assessments should be performed at least once a month. (Personal observations)

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