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International Clinical Pathology Journal

Research Article

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Quality analytical planning in blood bank serological screening qualitative assays

Abstract

Background: Transfusion Medicine safety is held by regulations and government legislation inside a total quality frame. In order to develop a quantitative quality planning in the serological screening of seven infectious markers in blood donations, a cross-sectional, study was conducted.

Methods: We performed the quality design in Bio-Rad's xMark microplate spectrophotometer by ELISA for HBsAG, anti-HIV, HTLV-1/2, SYPHILIS and HBcAb; and CHAGAS, and HCV with Bio-Rad and Biokit antibodies, respectively. The guide CLSI EP-12 was used to evaluate the imprecision for each marker. The minimal concentration for each marker from a seropositive asymptomatic donor registered on e-Delphyn® was used to determine the quality requirements, the sigma metric, and the ΔSE.

Results: The values found by the sigma metric calculi were 7, 7, 4, 4, 5 and 4 for anti-HIV, HBcAb, HBsAg, HCV, HTLV-1/2, SYPHILIS and CHAGAS, respectively. All Ped near 0.90 had a Pfr 0.03 or less. The quality control procedures for the qualitative ELISA guarantee a good sensitivity. With the statistic control charts, it was possible to select rules with high Ped and low Pfr for each serological marker.

Conclusion: we showed a quantitative quality planning during the serological infectious screening of blood donations demonstrating different sigma levels at Peruvian Hospital. The development of strategies to diminish the uncertainty by donation and will secure the safe transfusion of units that will save lives.

Keywords: quality control, serological screening, ELISA, Transfusion-Transmissible Infections

Volume 7 Issue 1 - 2019

Jeel Moya-Salazar,[1,2] Rommel F Díaz-Rosado[3]
[1]Hospital Nacional Docente Madre Niño San Bartolomé, Peru
[2]Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia Lima, Peru
[3]Servicio de Banco de Sangre y Medicina Transfusional, Hospital Nacional Guillermo Almenara Irigoyen, Peru

Correspondence: Jeel Moya-Salazar, Hospital Nacional Docente Madre Niño San Bartolomé, 957 Pacific street, Urb. Sn Felipe, Lima 07, Peru, Email jeel.moya.s@upch.pe

Received: January 21, 2019 | **Published:** February 28, 2019

Introduction

Blood is a public resource and it is a component of therapeutic use which certainly requires sufficiency and safety that strengthen the transfusion guaranties. In 1984, the World Health Organization established that transfusion safety would only be achieved through the design of quality guarantee procedures.[1] Since then, the insurance of the collected product's biological quality has grown exponentially. Nowadays, laboratory tests are available with enough reach for the blood banks to have reliable serological sieve procedures for the principal infectious agents and the quality monitoring.[2] Nonetheless, nowadays the infectious disease transmission risk via blood persists. It is certainly little, but significant.

The global outlook regarding these infections added to the growing necessity of blood products entails to limit to the maximum the possibility of Transfusion-Transmitted Infections (TTI), improving the blood-products quality, diminishing the economic cost and the uncertainty for donation, and, therefore, assuring the safe blood transfusion. Among the screening techniques, and based on its reproducibility, automation, cost-benefit, long caducity, and acceptable sensitivity and specificity, it is of great choice and use the enzyme-linked immunosorbent assay (ELISA) for the serological screening of blood donations.[3]

The detection of transmissible diseases with ELISA requires a quality insurance system to guarantee the maximum suitability for the obtained results. As the blood products are never exempt of infectious transmission risk, but can be significantly diminished applying quality control processes (QC).[4] The usual ELISA's QC is based on strongly positive control tests included in the reagents kit, provided by the manufacturer, or control serum prepared from patients, which are potentially contaminant. Acceptable traits for absorbance values (Abs) are often wide, so they can be widely applied. QC procedures operating with these wide interval controls could not be able to detect a significant clinical lost in the assay, it is, they cannot distinguish the progressive reduction of analytical sensibility in the assay that could interfere in the degree of medical decisions. CLIA'88 points to the necessity of separate positive control utilization in laboratories from the controls used to calculate the cut-off point, internal controls, and some require an additional intern control from the kit provided by the manufacturer.[5,6] Undoubtedly, there is not guarantee that the test QC (ELISA) could be improved with these regulations. Sadly, in several developing countries, like to Peru, Blood Banks don't know why they run controls, not even which ones, how many, or how to assure the total quality with the use of the cutoff from the reagents provided by the manufacturer, in asymptomatic patients with detectable levels of serum-positivity. Since a time ago schemes of quality evaluation for qualitative tests have been being developed, like the serological screening. Green et al.,[7] developed an scheme that allow us to evaluate and design an optimized QC procedure for ELISA, and to know the expected yield level in a laboratory that uses clinical analysis.[7] Here, the quality requirements for the essay were defined, and adequate characteristic control rules based on the information about the quality requirements, the methods yield characteristics, and the functioning of the candidate QC processes.[8,9] While this scheme was elaborated for the quality control of HBsAg with ELISA, it could be applicable to other infectious markers inside the serological screening of Blood Banks. In this way, we aimed to develop a quantitative quality control

planning in the serological screening for the seven infectious markers in donations in Blood Bank.

Materials and methods

We performed an analytic, cross-sectional, non-experimental, one-single-center study during 2014 in the Blood Bank and Hemotherapy area of the "Hospital Nacional Guillermo Almenara Irigoyen" of the "Seguro Social de Salud" from Lima, Peru (EsSalud).

TTI Markers

For the determination of HBsAg, HIV, HTLV-1/2, SIFILIS and HBcAb for ELISA, Bio–Rad antibodies (Hercules, CA) were used, and for the determination of CHAGAS and HCV, Biokit antibodies (Lliça d'Amunt, Barcelona) both with their respective positive controls. The serological screening was automatized with the sample manager system Bio-Rad x Mark microplate spectrophotometer (Hercules, CA) with an incubator with programmable temperature control, operated with Microplate Manager® 6 software. All plates were washed in a Bio-Rad Immunowash Microplate Washer model 1575 (Hercules, CA).

Data collection and sample processing techniques

All donations, the processing, and the transfusion mechanics fulfilled the national and international requirements, regulations and legislations.[10-12] The positive results were codified for the seven infectious markers from the Data Management System for Blood Banks e–Delphyn® (Hemasoft, Singapore) to the quality matrix. In this matrix, we selected the minimal seropositive reported concentrations for each marker in asymptomatic blood-donors during the period of study.

Quality analytic requirements

The calibration was analyzed through linearity based on what was described by the manufacturer of the kits, resulting in all essays being linear, with which the minimal acceptable optical density δ was determined (Abs). The quality data was evaluated expressed as a correlation between the sample Abs and the cutoff. The cutoff was derived from the media of three negative calibrators plus a fixed value, as in most qualitative ELISAS's. The minimal positive reported concentration (Abs) was used to determine the clinical Quality Requirements for each infectious marker.

Quality planning

With the percentages of quality requirements the Quality Planning could be developed, here the medically important errors in immunoenzymatic assays that can affect the levels of medical decision were described. The bias was not evaluated and was considered with a value of 0 for all the infectious markers.[13,14] *A contrario sensu* the imprecision (%CV) for each marker was determined in accomplishment with the procedures indicated in the CLSI EP-12 guide,[15] with the exception of HBsAg by ELISA that was previously determined.[7] We evaluated the methodology yield with these acceptable values using the Sigma Metrics, power-charts and OPSpecs charts, evidenced by graphics inside the statistical control charts that point to the amount of controls that are needed per analytic run and the control rules of clinical assay. The QC candidate selection procedure was performed under the rules: $1_{2.5s}$, 1_{3s}, $1_{3.5s}$, $2_{2.5s}$, $1_{3.5s}/2_{2.5s}$y $1_{3.5s}/2_{2.5s}/R_{4s}$.[16] Likewise, they were evaluate with the statistical control strategy tables based on the sigma metric.

Data analysis techniques

The data analysis was performed in the statistical analyzer IBM SPSS v20.0 () and MS–Excel 2010 for Windows. The data from e–Delphyn® register system was tabulated into the matrix were it was codified and the criteria for the verification of methods was evaluated. Finally, the statistical revision and the construction of graphics were developed. The principal limitation was not performing all the analysis of the assay: as the optimal concentration of covering; the detectability; the intra, inter-assay and total imprecision, etc. because it is costly and they were not included within the experimental design.

Ethics

Ethic and legal principles are secured with the care taken by the researchers for the obtained information, which was used only for this research. Additionally, based on the international ethics code for blood transfusion, we used the blood-donations correctly in all the stages in order to avoid social, sanitary and political implicances.[17] This study had the approval of the Ethics Committee of the Hospital and the agreement of the Heads of each Department

Results

The precision results in concentrations near the cutoff for the evaluation of the ELISA qualitative assay performance for each serological marker are shown in Table 1, being the mean 9.38 of imprecision. As well, Quality requirements are detailed, calculated from the cutoff and the minimal acceptable concentration in Abs., and the values found by the calculi of the sigma metrics oscillated between 4 and 7 for the infectious markers. The values found by the sigma metrics calculi oscillated between 4-7, being the methodic and the high performance the markers for Hepatitis B, and the lowest were HIV, HTLV-1/2, CHAGAS and HCV (Table 1). This points to a poor performance, but it can be controlled under traditional QC schemes and maintain itself in the quality with the application of a statistical control ($p<0.05$). Sigma metrics is a methodology that enhances processes and whose goal is to get less than a defect per million (99,9997% hits), it evaluates the ability of the analytes, even though the quality requirements are different for each magnitude they standardize with the six sigma to perform an inter-methodic comparison with only one value. In this sense, the values can be expressed and the strategies of statistical control based on sigma can be known as it is shown in Figure 1. Besides of sigma metrics, the quality control design of the expressed data in terms of Abs. was evaluated. The ΔSE (systematic error–SE_{crti}–), was calculated from the difference between the sigma value and 1,65 standard deviations (SD), that in clinical quality is equivalent to the maximum permissible error that does not overcome the quality requirement up to 90% of quality insurance (statistical value equivalent to 1,65 SD), for each serological marker (Table 1).

Table 1 Summary of the characteristics of the quality analytical planning during serological infectious screening in blood donations.

	Infectious markers						
	HIV	**HBcAb**	**HBsAg**	**HCV**	**HTLV-1/2**	**SIFILIS**	**CHAGAS**
Abs	0.285	0.343	0.598	0.989	0.376	0.76	0.416
Cuttoff	0.129	0.025	0.066	0.697	0.251	0.395	0.329

Citation: Moya-Salazar J, Díaz-Rosado RF. Quality analytical planning in blood bank serological screening qualitative assays. *Int Clin Pathol J.* 2019;7(1):22–26. DOI: 10.15406/icpjl.2019.07.00194

Table Continued....

	Infectious markers						
	HIV	**HBcAb**	**HBsAg**	**HCV**	**HTLV-1/2**	**SIFILIS**	**CHAGAS**
Quality requirements	54.74	92.71	88.96	29.53	33.25	48.03	20.91
Precision data (%CV)	11.33	11.77	12.4	7.19	8.2	9.59	5.15
Sigma	4	7	7	4	4	5	4
ΔSE*	3.18	6.23	5.52	2.46	2.4	3.36	2.41

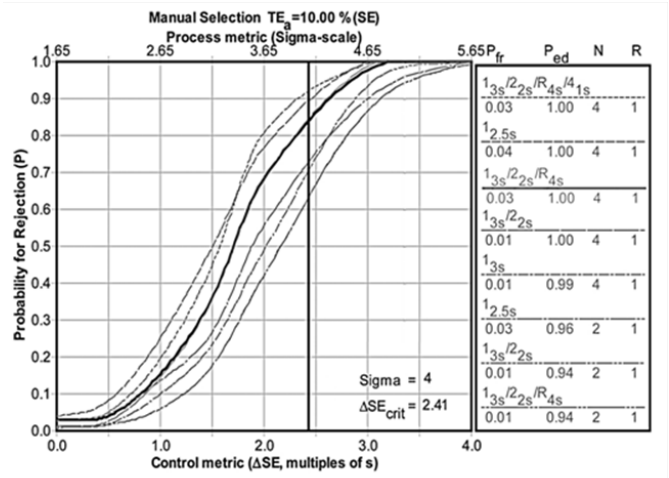

Figure 1 Sigma metricsfor Chagas. For Chagas point to the election of the multirule13s/2.2s/R.4s/41swith 4 positive controls that have a Pfrof 0,03 and a Ped of 100% (AQA).

Another control chart is the operative specifications (OPSpecs) and the power charts. The power charts represent the most powerful graphics because of the quantity of data that can be obtained from it during the quality planning. Also, this requires the calculus of ΔSE.[18] These power functions consists in presenting the information about the performance of a graphic control rule of reject probability against the analytical error measure as shown in Figure 2.

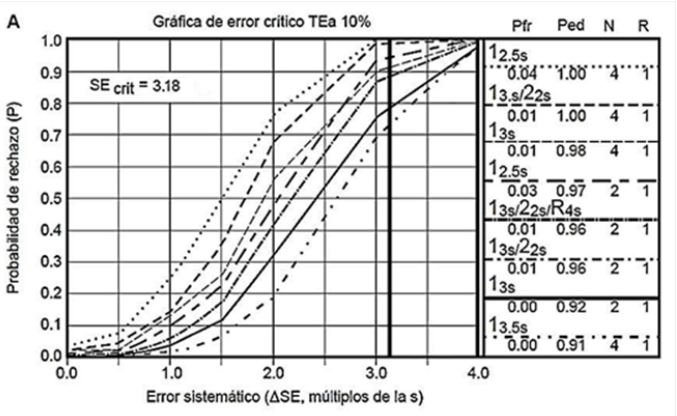

Figure 2 Power charts for the systematic error of HIV. For HIV it was obtained a Ped between 0,98 and 1,00 with a Pfr of 0,01 or higher, where the best option as probable statistic quality control strategy is the multirule $1_{3.s}/2._{2s}$ with four positive controls that have a Ped 1,00 and Pfr near 0,00.

As shown in Figure 3, OPSpecs charts are proven in the quality evaluation of immunoenzymatic assays for the serological screening in blood banks. These operative specification charts describe the imprecision and inexactitude that are permissible for a method and the QC needed to monitor the method performance and be able to detect medically important errors. There exist eight normalized graphics according to the number of controls that are run, being necessary in all cases the normalization of the data obtained from the imprecision and bias comparing their percentages with the analytical quality requirement. On average the sigma metrics determined for the viral infectious markers was of 6, in comparison with the markers for the bacterial marker (5 sigma) and parasite (4 sigma), (Table 1). It is not serious that our method works with certain error, what is serious is that this error was higher than the maximum permissible according to the method quality specifications.

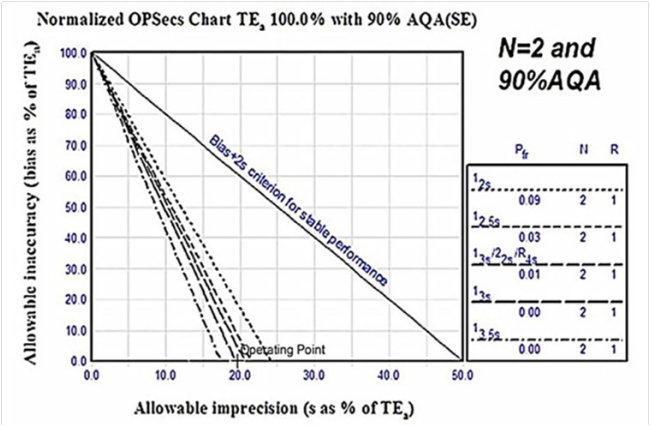

Figure 3 OPSpecs chart for HTLV–I/2.The expression of control data in the OPSpecs charts for HTLV-1/2 point to the election of the multirule13s/2.2s/R.4s with 2 positive controls that have a Pfr near 0,01 and a Ped of 90% (AQA), which allows the systematic and random complete evaluation of the immunoenzymatic assay.

Discussion

This quality analytical planning study for qualitative ELISA tests showed a high level for Hepatitis B and HIV markers and poor performance for HCV, HTLV-1/2 and CHAGAS. The Quality Management system in the Blood Bank must increase the satisfaction and expectative of the users fulfilling the governmental and non-governmental regulative requirements. This system requires more efficient processes, error prevention, cost reduction, user satisfaction, and improvement of competitiveness, among others.[3,19] I While the processes in Transfusion Medicine are a system of high complexity, nowadays there exist, rentable evaluation criteria for establishing an optimal performance, and for prevent and correct problems that detracts the quality of the assays. Qualitative validation of immunoezymatic assays with the CLSI EP-12 guide consists of an established set of experimental requirements in order to evaluate the yield on its own test surroundings. The evaluation of the assay performance qualitative features are of high clinical utility, as they have a wide spectrum of clinical purposes, and a basic example of the QSEs (Quality system essentials) quality management system, basic for every organization, in all the operations of the flow of work of the medical attention service.[15]

The CLSI EP12 guide addresses the QSEs, for this reason it must considered a consensus guide in qualitative assays, where the parameters of performance, acceptability criteria, and others are well defined.[15,20] The evaluation of methods is a key for the fulfillment of the international quality requirements, accreditation organisms and for a continuous improvement. Although there exists every time more qualitative methods, there is confusion on its classification and nomenclature, there is not a consensus about the performance of validations and ignorance persists in the blood banks. The imprecision determined with the EP-12 guide was heterogeneous for each marker, showing the precision between the cutoff and the medical decision levels, securing an optimal yield with the use of low positive controls (expressed in S/C –ratio between sample absorbance and the cutoff absorbance) and moderately positive (expressed as ranges).[21,22]

The EP-12 evaluation protocol presented metrological strength allowing a reliable and statistical evaluation of the assay performance (Table 1).[15] Even though the limitations over sensibility assays on markers as HIV, HTLV–1/2 and VHC with ELISA have been previously described.[7] In this study, we demonstrate that the evaluation methodology is applicable to other infective tests as SIFILIS or CHAGAS, giving a linear response for all cases. Our findings disagree with the study by Roble et al. (2016) who obtained sigma deficient for HIV (σ=2.7), HBsAg (σ=2.6) and HCV (=4).[23] This could be due to the use of other methods to obtain these values and the use of other analysis systems such as VITROS ECi / ECiQ 3600 (Ortho Clinical Diagnostics, Raritan, US). Assays were grouped in two ranges based on the sigma methodology from the practical evaluation with the statistical control strategies table. Optimal performance (sigma ≥6), and the controllable methodology under traditional quality schemes (sigma 3 to 6), being the performance features dissimilar for each marker (sigma ≥6 for HBsAg and HBcAb, and sigma between 3 and 6 for HTLV-1/2, SIFILIS, CHAGAS, HVC and HIV).

These results point to different quality levels in the general processes for establishing and maintaining a statistical quality control procedure. For the HBV markers simple statistical control tests are applied (mono-rule: 1_{3s}) with two positive controls, it has a Ped (Probability of error detection) of 0.91, and basically, without false rejection. This results are concordant with previous evaluations where the optimal yield was demonstrated and in the quality evaluation.[24,25] For HTLV-1/2, CHAGAS and HVC, in general the $1_{3s}/2_{2s}$ QC strategy with 4 positive controls, Ped=1.0 and Pfr (Probability of false rejection) of 0.03 controls was chosen. The Quality Planning determine for SIFILIS the election of a traditional statistical control (multirule: $1_{3s}/2_{2s}/R_{4s}$) with two positive controls, Pfr=0.01 and Ped=0.96. In the same way, we preferred for HIV the multirule $1_{3.s}/2_{2s}$ with four levels of control selection, a Ped=1.0 and Pfr=0.01, as these are better for evaluating the critical loss of sensibility in the evaluated assay.[26,27]

These rules can be selected in order to give a high Ped and maintaining low Pfr. Thus, user laboratories can enhance the assay (ELISA) yield through the determination of their own quality requisites, the assay precision evaluation in their own milieu, and the development of personalized QC norms, as most of the hematological and biochemistry assays are. This research can be performed using the Sigma metrics, the power charts and/or the OPSpecs charts, which have shown their usefulness in this kind of study.[18,28]

Conclusion and recommendation

In summary, we shown a quantitative quality planning during the serological infectious screening of blood donations demonstrating different sigma levels at Peruvian Hospital The transmission risk for infectious diseases through transfusions is a mayor difficulty for blood security and availability. Particularly in low-and-middle-income countries, where strategies and commitment of innovative resources, as the application of good practices in Transfusion Medicine, the promotion and increase of voluntary donations, the implementation of new screening techniques and the development of strategies to reduce insecure donations are required. In this sense, and as an applied example of clinical chemistry laboratories, it is necessary the development of a Quality Assurance program for Hemotherapy centers. This program should include a responsible of the internal quality control, of the participation in an external quality control program, and of the development of a research to solve the quality problems in the results, inside a better sanitary management.[29]

Acknowledgments

We acknowledge the assistance given by Yeffrid Yalta Iraola, M.T., from SimeCorp; the scientific advice of Jorgelina L. Blejer, PhD, from Hemocentro de Buenos Aires, Argentina; and the help translating this work of the biologist Roberto Ubidia-Incio from Mars Society Peru

Conflicts of interest

The author declare no conflicts of interest.

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Citation: Moya-Salazar J, Díaz-Rosado RF. Quality analytical planning in blood bank serological screening qualitative assays. *Int Clin Pathol J.* 2019;7(1):22–26. DOI: 10.15406/icpjl.2019.07.00194