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Study on the stability of HIV and syphilis reference materials under varied temperature conditions during their transportation

Estudo da estabilidade de materiais de referência para HIV e sífilis sob condições de temperatura variadas durante o transporte

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ABSTRACT

Introduction: The objective of this study was to evaluate the short-term stability of the serum samples used as internal quality control (IQC) of human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (Aids) and syphilis immunodiagnostic assays. These samples were produced at the Center of Immunology-Instituto Adolfo Lutz (IAL), and they were distributed to laboratories participating in the IAL Quality Control Program. **Method:** The sera analyzed by chemiluminescence assay were stored at varied temperature conditions: from 2°C to 8°C (refrigerator), from 15°C to 25°C (room temperature), at 37°C (incubator) and at -20°C (reference temperature) for 12 and 24 hours. **Results:** Comparative analysis of IQC results for anti-HIV and anti-*T. pallidum* (anti-treponemal) showed stability in the reference temperature and at the various simulated temperatures for transporting the samples at the established lengths of time. The data from the simple linear regression analysis of negative serum samples (incubator/24 hours) and in one batch of HIV IQC (room temperature/24 hours) were statistically significant at the level of 5% (p-value < 0.05). **Conclusion:** The sera presented necessary requirements as reference material to be transported to laboratories at refrigeration temperature (2°C to 8°C), at the maximum shipping time of 12 hours.

Key words: reactivity-stability; serum; HIV antibodies; *Treponema pallidum*; analytical quality control.

INTRODUCTION

Laboratory tests enable doctors to minimize doubts and reach diagnoses accurately. Standardization and quality control procedures in clinical laboratories represent indispensable elements for laboratory routine. Quality control of activities to conduct a daily determination of different analytes in several matrices requires the use of reference materials (RM), not only those certified, but also those used in assessing quality of routine testing[1, 2]. Thus, the use of these materials is important to improve laboratory safety and reliability, as well as to favor the acquisition of correct reliable data by professionals[3].

In the past, certification of reference samples was widely understood as a process of assigning values to materials, and little importance was given to their characteristics, such as homogeneity or stability. However, with the growing emphasis upon uncertainty estimates for analytical measurements, there was the clear necessity of estimating imprecision in RM[4]. Nowadays, the employment of stability assays together with homogeneity tests is essential for the process of reference specimen certification because stability is an important parameter indicating the degree of RM conservation during specific periods of time and temperature[2, 5-7].

The behavior of a biological sample during transportation, even under ideal conditions, might differ from that of a sample kept in adequate storage conditions, as during transportation it can be susceptible to possible external influences, such as temperature change. Therefore, it is fundamental to have knowledge of what can happen with the sample if adequate conditions are not kept;

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to this end, the conduction of a short-term stability study is recommended[2, 4, 6, 8].

The quality management plan for good technical practices, about transportation, preservation, and storage of biological samples, has been developed to maintain and ensure stability of these materials[9, 10].

In order to evaluate short-term stability (transportation), RM is stored at different temperatures to elucidate any degradation that may occur during transportation. Based on the results of this evaluation, transportation conditions must be chosen that ensure product stability[4].

The short-term isochronous stability study conducted by Castejon *et al*. (2014)[2] subjected RM to different temperatures simulated for transportation during 24 and 48 hours. Results indicated sera stability in the different tested storage temperatures.

OBJECTIVE

The objective of this study was to evaluate the stability of antibodies against the human immunodeficiency virus (HIV) and against *Treponema pallidum* (anti-treponemal or syphilis antibody) in serum samples under transportation conditions to ensure quality of the produced RM.

METHOD

Plasma bags

The central laboratory of Instituto Adolfo Lutz (IAL) has counted on the cooperation of Hemorrede – Coordenadoria de Ciência, Tecnologia e Insumos Estratégicos da Saúde da Secretaria de Estado da Saúde de São Paulo (CCTIES/SES-SP). By means of a liability agreement signed by the institutions, it became viable for hemotherapy services to provide the Immunology Center (CIM) of IAL with the plasma bags positive for HIV and syphilis, and the negative ones for markers advocated by the law in force on hemotherapic procedures. As a consequence, acquisition of biological samples was assured to prepare specific materials and to meet the demands of control sera for the procedures of internal quality control (IQC) within the subnet of laboratories for diagnosis of HIV infection and syphilis in the state of São Paulo[11, 12].

In this study, we used seven plasma bags with negative serology for the established markers in hemotherapic procedures, two plasma bags with HIV-positive serology, and four bags with syphilis-positive serology, whose results were detected in the screening of blood donors.

Production of reference material

The transformation process of plasma into serum was performed by the recalcification, following the method recommended by the World Health Organization (WHO), with changes[13].

Characterization of sera was done with different sets of diagnostic tests for detection of anti-HIV antibodies and treponemal and non-treponemal antibodies, using the diagnostic routine of CIM-IAL.

In the assessment of transportation stability, a lot of negative serum samples were used, and the following weakly reactive lots (low antibody titers), termed IQC, were prepared: two lots for anti-HIV, and four lots for anti-treponemal antibodies. Thus, the following sera were made: HIV-negative serum – 165, IQC HIV – P174N163 (dilution 1:20,000) and P175N164 (dilution 1:80,000) and anti-treponemal IQC – P01N176 (dilution 1:1,000), P03N177 (dilution 1:100), P04N178 (dilution 1:100) and P06N180 (dilution 1:300).

In the serum samples, the ideal dilution for anti-HIV antibodies was established as that recommended by the Technical Manual[14] about preparation of positive IQC, routinely employed at the HIV/Aids Laboratory of CIM-IAL. In order to establish the ideal dilution of each of the samples, the positive sera 174 (HIV), 175 (HIV), 1 (syphilis), 3 (syphilis), 4 (syphilis), and 6 (syphilis) were diluted in series, respectively, with the negative sera 163, 164, 176, 177, 178 and 180. The dilutions of HIV IQC samples were tested at Advia Centaur – CHIV (Siemens Healthcare Diagnostics, Inc., NY, USA); and the anti-treponemal IQC samples, at Advia Centaur – Syphilis (Siemens Healthcare Diagnostics, Inc., NY, USA). The best dilution to be employed as IQC was that which presented the index in the range of 1.5-4.5 times the cut-off point of the test (equal to 1).

After serum fractionation, to study homogeneity among samples of vials from different lots, a subset of serum tubes was selected by random sampling, using the formula established at the software Microsoft Office Excel (Microsoft Corp., Redmont, WA, USA).

Homogeneity of serum samples was assessed according to Associação Brasileira de Normas Técnicas (ABNT) ISO Guide 35[6], employing the proper model for biological assays developed at CIM-IAL[7].

Short-term stability

In the results of the study carried out by Castejon *et al*. (2014)[2], HIV-positive samples were stable at the 2°C-8°C (refrigerator) temperature, during 48 hours, when assessed in the methods enzyme-linked immunosorbent assay (ELISA)/ immunoenzymatic assay (IEA), indirect immunofluorescence and western blot. Thus, to distribute material to participant laboratories in the Internal Quality Control Program (PCQI) of IAL[12], samples were stored in appropriate boxes, with reusable ice packs, and transported in no more than 12 hours, so that their characteristics were maintained.

In this evaluation, the employed method was chemiluminescence for detection of anti-HIV antibodies and anti-treponemal antibodies, according to the produced RM.

The short-term stability study was conducted using the isochronous model[6]. The RM underwent different temperatures during 24 hours, and each sample was analyzed in repeatability conditions (triplicate). This procedure was aimed at assessing the eventual occurrence of adverse effects in the reactivity of anti-HIV antibodies (two lots) and anti-treponemal antibodies (four lots) during material transportation.

In order to simulate transportation, seven vials of serum samples from each lot were chosen randomly, and one sample from each lot remained stored at -20°C (storage temperature in laboratories after receipt [reference]), and the other six serum vials (test samples) were stored at the different temperature conditions of 2°C-8°C (refrigerator), 15°C-25°C (room temperature) and 37°C (incubator), aimed at simulating RM transport to laboratories, in previously defined time lengths of 12 and 24 hours[2, 6].

After total freezing of serum samples at -20°C (time zero), three vials of each lot were withdrawn from the freezer and one serum vial was stored during 24 hours in each of the temperatures mentioned before. After 12 hours' storage of these samples in different temperatures, the same procedure was carried out with sera from the other three vials that had remained in the freezer[2]. After storage during 24 hours, the test samples of 12 and 24 hours were analyzed in triplicate concomitantly with the reference sample of each lot (kept at -20°C), in the same assay (isochronous model) and in repeatability conditions, to reduce the dispersion of points in relation to time[2, 6, 15].

Data analysis

In the evaluation of short-term stability (transport) results, the simple linear regression analysis was employed[6, 16, 17], using the software Microsoft Office Excel (Microsoft Corp., Redmont, WA, USA) to verify the tendency (slope) of results in relation to storage time under different temperature conditions.

This study was approved by the Human Research Ethics Committee of IAL (CAAE: 57496516.7.0000.0059).

RESULTS

In the homogeneity study, the analysis of variance of data was carried out to assess the occurrence of significant differences in the analyte contents, that is, specific antibodies in sera after vial bottling, and also, to estimate the standard uncertainty associated with homogeneity. The results of this evaluation among the samples contained in vials from each lot and among the vials indicated homogeneity of sera from the respective used lots.

In the stability study of samples stored under different temperatures and the reference serum (at -20°C), results conducted in group in the chemiluminescence assay and in repeatability conditions, are presented in **Table 1**. In this table the measure of variability of triplicates results in each sample, calculated by the coefficient of variation (CV) is also demonstrated.

The result analysis (index value) of weakly positive samples (anti-treponemal and anti-HIV IQC) and of the negative serum, when compared with the data of previous characterization of serum reactivity, demonstrated stability both in the reference temperature (-20°C) and in the different temperatures simulated for transportation. Results of IQC samples presented within acceptable variation ranges, between 1.5-4.5 times the cut-off point (1), and the average values of negative serum were 0.06-0.1.

The variability of average values of triplicates from anti-HIV positive IQC and anti-treponemal positive IQC samples was, respectively, 0.26%-3.85% and 0.18%-3.68%. For negative serum, variability of results was 5.59%-13.86%.

Later, linear regression analysis was applied with 95% confidence to verify stability, demonstrated by the p-value > 0.05 (**Table 2**).

In the simple linear regression analysis with 95% confidence, lots 165 and P175N164, respectively, in incubator and room temperatures (period of 24 hours), presented a p-value < 0.05.

TABLE 1 – Results expressed as index means in chemiluminescence assay and coefficient of variation (%) of sample triplicates detected in the reference sera and test samples

Serum	Reference (-20ºC)		Room temperature (15ºC-25ºC)				Refrigerator (2ºC-8ºC)				Incubator (37ºC)			
	Time zero		12 hours		24 hours		12 hours		24 hours		12 hours		24 hours	
IQC P01N176	2.33	0.5%	2.32	2.28%	2.35	1.3%	2.36	0.88%	2.35	0.49%	2.3	0.25%	2.31	0.9%
IQC P03N177	2.6	1.56%	2.59	2.35%	2.57	0.81%	2.56	1.63%	2.57	3.68%	2.52	2.04%	2.61	0.59%
IQC P04N178	3.1	1.89%	3.16	1.14%	3.15	0.66%	3.17	1.93%	3.16	2.23%	3.16	0.8%	3.14	0.18%
IQC P06N180	2.38	1.21%	2.37	1.46%	2.36	1.47%	2.36	1.07%	2.38	0.64%	2.41	0.24%	2.38	1.21%
IQC P174N163	2.81	1.63%	2.82	1.48%	2.83	1.75%	2.72	2.92%	2.75	1.05%	2.88	1.22%	2.88	0.6%
IQC P175N164	2.27	1.11%	2.38	1.94%	2.41	1.5%	2.26	0.26%	2.31	3.85%	2.45	2.55%	2.4	2.53%
Negative 165	0.07	8.66%	0.08	12.5%	0.08	7.53%	0.06	9.12%	0.07	7.87%	0.08	13.86%	0.1	5.59%

IQC: internal quality control.

TABLE 2 – Results of the *p*-value, obtained by linear regression analysis, attributed to serum lots to verify stability in the different storage temperatures

Serum	Room temperature (15ºC-25ºC)	Refrigerator (2ºC-8ºC)	Incubator (37ºC)
IQC P01N176	0.3807	0.0916	0.3965
IQC P03N177	0.3981	0.5645	0.8384
IQC P04N178	0.2542	0.3356	0.2447
IQC P06N180	0.5235	0.7565	0.7816
IQC P174N163	0.6443	0.2377	0.0596
IQC P175N164	0.0031	0.3439	0.0775
Negative 165	0.1795	0.2443	0.0005

IQC: internal quality control.

DISCUSSION

In the chemiluminescence method, results from serum samples kept in temperatures simulating RM transportation were similar to those found by Castejon *et al*. (2014)[2]. In that study, ELISA/EIE was used for detection of anti-HIV antibodies in the assessment of RM, in specimens stored in thermal boxes with dry ice (solid form of carbon dioxide) to be taken to laboratories. In the present study, results of the evaluation of serum lots in the chemiluminescence assay proved that RM can be transported at the recommended temperature (2ºC-8ºC) and in the time period established (12 hours) to laboratories participating in the Quality Control Program. According to good production practices, during RM transportation, it is important to monitor temperature to keep it in the range indicated by the manufacturer, and to meet the proposed deadline to guarantee that the product characteristics are preserved up to its deliver to the final consumer[18].

Knowledge about the influence of serum sample storage is necessary for each class of antibody and type of assay used[19, 20] to maximize the information of laboratory results in the different applied methods and to ensure reliability. Immunoglobulin measurements and avidity tests were not carried out in the samples analyzed in this study. However, the detected values (indices) showed that the chemiluminescence method applied to evaluate stability of the stored sera in the different transport temperatures did not cause interference in the detection of anti-HIV and anti-treponemal antibodies.

Stability assays, along with homogeneity tests, are essential for the RM certification process because stability is an important parameter indicating the degree of conservation of sera during specific time periods and temperature[5, 6].

According to Gislefoss (2010)[21], the choice of the analysis method may decisively interfere in the results of analytes that tend towards degradation. It is important to comprehend the stability of the material and of the technical choice, because these parameters can be crucial to obtain trustworthy results[2]. The results of this study demonstrated the maintenance of short-term stability of sera, ensuring RM quality to the laboratory. During transportation, samples are subject to the influence of time, temperature, mechanical shocks, among other factors. So, it is fundamental to evaluate the interference of the transport system in the quality of the released laboratory results[22].

In the analysis of samples kept in different conditions of temperature and time of storage, under repeatability conditions (triplicate), there was no alteration regarding sample reactivity. This piece of data shows conformity to the index detected in

the reference sample (-20ºC), besides remaining similar to the value obtained in serum characterization. However, under a statistical point of view, the slope of the results from negative serum (incubator) and IQC P175N164 (room temperature) versus storage time (24 hours) presented 5% significance. Possibly, the evaluation of samples with the greater number of replicates avoids this situation[4]. An alternative is to divide repetitions into two analytical runs carried out in short periods of time to minimize the presence of factors interfering in the analysis, such as electrical current, temperature variation, among others, ensuring, thus, a more robust estimate representing the routine conditions[23].

Precision varies according to the antibody concentration present in the sample, and the lowest the concentration, the highest the coefficient of variation will be[24]. The obtained CV in the triplicates of negative serum (lot 165) in the different storage temperatures showed a percentage between 5.59% and 13.85%, this being the widest variation found in anti-HIV and anti-treponemal samples (weakly reactive samples) IQC. The obtainment of this percentage of variability in the negative sera (absence of specific antibodies) reinforces the relationship of concentration in the analyte to be inversely proportional to CV. The inclusion of an anti-HIV negative serum in short-duration stability tests was aimed at: 1) assessing maintenance of the material in uniform conditions of structure or composition according to the studied property; 2) verifying the eventual occurrence of adverse effects, such as false-positive results caused during the material transportation. The CV of the anti-HIV positive samples IQC (2.72% and 2.26%), stored in the refrigerator for 12 hours, is close to the CV result (2.7%) of the anti-HIV positive sample serum (weakly reactive) obtained in the assessment of intra-assay precision of the kit used in this study (data described in the package insert that accompanies the kit, revision/2016). For the anti-treponemal IQC samples, it was not possible to make the comparison, because this piece of data was not made available by the kit manufacturer.

CONCLUSION

When analyzing the results obtained in the chemiluminescence assay, the sera presented the necessary requisites as RM to be transported to the laboratories participating in the Quality Control Program of IAL in refrigeration temperature (2ºC-8ºC), in the proposed period of 12 hours, since these sera indicated stability.

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RESUMO

Introdução: *O objetivo deste estudo foi avaliar a estabilidade de curta duração de amostras de soro utilizadas como controle de qualidade interno (CQI) de testes imunodiagnósticos de vírus da imunodeficiência humana (HIV)/síndrome da imunodeficiência adquirida (Aids) e sífilis, produzidas no Centro de Imunologia do Instituto Adolfo Lutz (IAL) e distribuídas aos laboratórios participantes do Programa de Controle de Qualidade do IAL.* ***Método:*** *Os soros analisados por meio de ensaio de quimioluminescência foram armazenados em diferentes condições de temperaturas: de 2ºC a 8ºC (geladeira), de 15ºC a 25ºC (ambiente), 37ºC (estufa) e de -20ºC (referência) durante 12 e 24 horas.* ***Resultados:*** *A análise comparativa dos resultados do CQI HIV e* T. pallidum *(antitreponêmico) demonstrou que os materiais permaneceram estáveis, tanto na temperatura de referência quanto nas diferentes temperaturas simuladas para o transporte, no período de tempo estabelecido. No entanto, os resultados da análise de regressão linear simples das amostras de soro negativas (estufa/24 horas) e de um lote de CQI HIV (ambiente/24 horas) foram estatisticamente significativos ao nível de 5% (valor de* $p < 0,05$*).* ***Conclusão:*** *Os soros apresentaram requisitos necessários de material de referência para serem transportados aos laboratórios em temperatura de refrigeração (2ºC a 8ºC) no tempo máximo de 12 horas.*

Unitermos: *reatividade-estabilidade; soro; anticorpos anti-HIV;* Treponema pallidum*; controle de qualidade.*

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