Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 11, pp.22336-22338, November, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

STABILITY OF PLASMA SAMPLES POSITIVE FOR ANTI-HIV ANTIBODIES STORED OVER A DECADE

***Charu Mehra Kamal, Arun Kumar, Pankaj Kumar Sharma, Sanjeev Kumar Sharma, Brij Bhushan, Rajeev Kumar, Mohit Sharma and Reba Chhabra**

National Institute of Biologicals, Plot No. A-32, Sector-62, Institutional Area, Noida-201 309 (U.P.), India

ARTICLE INFO

Article History:
Received 28th August, 2015
Received in revised form
16th September, 2015
Accepted 07th October, 2015
Published online 30th November, 2015

Key words:

HIV,
Antibodies,
Stability

ABSTRACT

In order to ensure the quality of diagnostic kits for safeguarding public health in the country the evaluation of immunodiagnostic kits is done with in-house reference plasma panels. In this context, the maintenance of plasma samples stored frozen is extremely important. The objective of the present study was to evaluate the stability of plasma samples positive for anti-HIV antibodies stored over a decade. In this study, the 104 stored anti-HIV antibody positive samples were tested by ELISA methodology and the E-ratios were compared with the earlier recorded E-Ratios to check if there was any significant decrease in the values. The results were statistically analysed by paired t-test methodology and no significant difference was found in the E-ratios. Hence, the anti- HIV positive plasma samples stored at -20^{0}C were stable for as long as twelve years by ELISA methodology.

***Citation*:** Charu Mehra Kamal, Arun Kumar, Pankaj Kumar Sharma, Sanjeev Kumar Sharma *et al.,* 2015. "Stability of plasma samples positive for anti-HIV antibodies stored over a decade" *International Journal of Current Research*, 7, (11), 22336-22338.

INTRODUCTION

Host and viral markers that occur during HIV infection are used to identify infection, and monitor viral infection, and monitor viral replication, disease progression, and immune status (Constantine and Zink, 2005). HIV diagnostic testing has come a long way since its inception in the early 1980s. HIV infection is identified either by the detection of HIV-specific antibodies, p24 antigen testing in serum or plasma or by demonstrating the presence of the virus by nucleic acid detection using polymerase chain reaction (PCR), or rarely these days, by growing virus in cell culture. Antibody testing is the method most commonly used to diagnose HIV infection (Fearon, 2005). Given its specific affinity for the antigen, the integrity of the three dimensional chemical structure of antibody molecules is crucial for this interaction. Structural changes or molecular aggregation, depending on storage conditions, lead to a decrease in the antibodies activity, which may lead to false negative results (Castejon, *et al.,* 2014). The degradation of stored biological material is an important factor required to be investigated.

****Corresponding author: Charu Mehra Kamal,***
National Institute of Biologicals, Plot No. A-32, Sector-62, Institutional Area, Noida-201 309 (U.P.), India.

When samples are tested after being stored for a long time this evaluation becomes complex. When results are not reproduced, it is difficult to verify whether material degradation occurred in fact, or the difference in results is due to use of more sensitive assays developed over time (Pinsky *et al.,* 2003). In order to ensure the quality of diagnostic kits for safeguarding public health in the country the evaluation of immunodiagnostic kits- Rapid, ELISA, ELFA, CLIA, Confirmatory and Ag/Ab, Combo diagnostic kits is done with in-house reference plasma panels. In this context, the maintenance of plasma samples stored frozen is extremely important.

Due to scarcity of available data on which the adverse effect of long term storage may cause denaturation of antibodies, it is relevant to conduct research through implementation of sensitive and specific assays. The concern with the storage time of the plasma is related to various factors, such as temperature and samples freeze thawing cycles, which may cause occasional changes in the results of a particular test due to physical decay and consequently, the biochemical modifications of antibodies of interest (Pinsky *et al.,* 2003; Cao, *et al.,* 2003; Fipps, *et al*., 1988 and Franks, 2002).

Objective

There is a lack of an established norm in relation to the storage time of biological samples and plasma samples are available with known reactivity status of anti-HIV antibodies. This study was proposed to investigate stability of plasma stored for periods of two to twelve years. In the present study, the stability of specific antibodies was evaluated using sensitive and specific assays used routinely in the laboratory to check the reproducibility of anti-HIV antibodies.

MATERIALS AND METHODS

The study protocol for collection of samples from various blood banks of Delhi and NCR was approved by Institutional Human Ethics Committee. The plasma samples used were from plasma bags which were not suitable for use by the Blood Banks. No personal information of the donor was collected. These samples were collected for preparation of panels for evaluation of immunodiagnostic kits from 2002 to 2014, which were stored and frozen at -20^{0}C in the laboratory. The in-house prepared panels were well characterized using an array of ELISA and Western Blot Methodologies.

The serological tests performed with the plasma samples in this study were as per the scope in which they were intended i.e., for detection of anti HIV antibodies by ELISA and Western Blot Methodologies. Plasma samples used in this study which were collected over a decade were analysed in 2014. A total of 104 samples were included in the study and their years of storage are as given in Table 1.

Table 1. No. of samples and years of storage

Year of storage	No. of Samples
1-4 yrs.	22
5-8 yrs.	57
9-12 yrs.	25
Total	104

In order to ascertain the presence of adverse effects on stored plasma samples over the years, the reproducibility of results was determined by ELISA/EIA. Plasma samples were tested using Vironostika HIV Uni-Form (Biomeriux SA, France) and Vironostika HIV Ag-Ab ELISA and the E-ratios (sample OD/cut-off OD obtained by ELISA) were recorded for analysis. The E-ratios recorded at the time of collection of samples were compared with the E-ratios recorded at present.

Statistical Analysis

The results were analysed in Microsoft Office Excel (Microsoft Corp., USA). The two sets of data were compared using paired t-test to see if there was any significant level of reduction in the E-ratios.

RESULTS

Previous values of E-ratios recorded for 104 samples were compared with present E-ratios respectively. The null and alternate hypotheses considered were as follows:

H_0 (null hypothesis): $\mu_1 - \mu_2 = 0$
H_1 (alternate hypothesis): $\mu_1 - \mu_2 > 0$

where μ_1 is mean of previous/baseline anti-HIV E-ratios and μ_2 is mean of present anti-HIV E-ratios

In the present study, the level of significance (α) which was considered to check the hypothesis was 0.05. The analysis showed mean difference = 0.91 and p = 0.073 and since p-value > α, the null hypothesis is accepted which means the difference between the E-ratios of samples (previous vs. present) did not vary significantly. The results showed that there was no statistical difference in antibody levels in stored samples which corroborates that the anti HIV antibody plasma samples were stable for as long as twelve years.

DISCUSSION

Frozen plasma banks are an important source required for preparation of in-house reference panels for quality evaluation of critical immunodiagnostic kits for HIV-1&/2 Antibody. Despite the widespread use of banked serum/plasma specimens that have undergone multiple freeze-thaw cycles, there is a paucity of data available regarding the effect of numerous freeze-thaw cycles on the measured antibody result. This information is particularly relevant for sensitive assays, such as enzyme-linked immunoassays (EIAs), which measure protein structures (antibodies) prone to denaturation. Because these biological specimens may be used for multiple investigations over a period of time, concern may exist that repeated freeze-thaw cycles might affect the results of a particular assay by physically damaging the antibody of interest (Pinsky *et al.,* 2003). Stability studies of a range of biomolecules over long periods under controlled conditions are rare and restricted to a limited number of pre-defined proteins and analytes (Elliott P. and Peakman T C. 2008). This study is of great significance as the data available in the literature is limited and numerous queries are received from researchers and technicians regarding reactivity and stability of plasma containing anti-HIV antibodies.

In the present study, it was observed that stored plasma samples showed reproducible results for 12 years by ELISA/EIA method. Although this study did not include measurement of immunoglobulins and avidity tests for the analysed samples the difference between the previously and presently analysed samples was not statistically significant. Direct comparison of baseline and final assay values showed no evidence for change in actual antibody level. It is crucial to plan the storage of biological sample in ideal conditions which allows the use of this material in future studies. Since the moment of the material collection, it is necessary to define the sampling, transport, and storage procedures, as well as monitoring equipment to ensure sample integrity. Strict control of these conditions provides an added value to the samples, allowing ensuring quality, traceability, and reproducibility of results (Somoza and Tora, 2009). There are several parameters that should be taken into account to ensure stability of plasma. The sample volume should not be very less as evaporation process and antibodies adsorption on the surface of storage vial can modify sample concentrations. Scarcity of HIV positive

samples in low HIV prevalence areas/countries is of prime concern where these samples are required to be used for future studies/evaluations etc. There is a requirement to plan to store samples in ideal conditions. The statistical analysis in this study exhibits that the samples remained stable even after twelve years when stored under ideal conditions.

Conclusion

The present study showed that the anti HIV positive plasma samples stored at -20^0 C were stable for as long as twelve years by ELISA methodology.

Acknowledgement

The authors thank the blood banks of Delhi NCR for providing the samples for the study.

REFERENCES

Cao, E., Chen, Y., Cui, Z. and Foster, P.R. 2003 Effect of freezing and thawing rates on denaturation of proteins in aqueous solutions. *Biotechnol. Bioeng.,* 83(6):684-90.

Castejon, M.J., Yamashiro, R., Camila, C.O., Oliveira, C.A. and Ueda, M. 2014. Stability of anti-HIV antibodies in serum samples stored for two to eighteen years periods *J. Bras. Patol., Med.* Lab. Vol. 50 No.4 Rio de Janeiro July/Aug.

Constantine, N.T. and Zink, H. 2005. HIV testing technologies after two decades of evolution. *Indian J. Med Res.,* Apr; 121(4); 519-38

Elliott, P. and Peakman, T.C. 2008. The UK Biobank samples handling and storage protocol for the collection, processing and archiving of human blood and urine. *International Journal of Epidemiology,* 37; 234-244

Fearon, M. 2005. The laboratory diagnosis of HIV infections. *Can. J. Infect Dis. Med Microbiol.,* Jan-Feb; 16(1): 26–30.

Fipps, D.R., Damato, J.J., Brandt, B. and Burke, D.S. 1988. Effects of multiple freeze thaws and various temperatures on the reactivity of human immunodeficiency virus antibody using three detection asssays. *J. Virol. Methods,* v 20, n 2, p. 127-32,.

Franks, F. 2002. Protein stability: the value of 'old literature'. *Biophys. Chem.,* V 96, n.2-3,p. 117-27.

Pinsky, N.A., Huddleston, J.M., Jacobson, R.M. and Wollan, P.C. 2003. Effect of Multiple Freeze-Thaw Cycles on Detection of Measles, Mumps, and Rubella Virus Antibodies *Clin. Diagn Lab Immunol.,* Jan; 10(1): 19–21.

Somoza, N. and Tora, M. 2009. Biological safety in the storage and transport of biological specimens from patients with respiratory diseases used in research settings. *Arch. Bronchoneumol.,* V 45, n.4 p. 187-95.

****