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Original Research Article

Pilot Study on the Stability of Internal Quality Control Samples For Anti-HCV Immunodiagnostic Assays

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Abstract

Laboratory diagnosis of HCV infection is usually made on the basis of the detection of circulating antibodies. All laboratories carrying out anti-HCV tests, should have a well-functioning quality assurance programme. Quality control (QC) materials are crucial for Internal Quality Control (IQC) and External Quality Assessment Scheme (EQAS). Therefore, preparing IQC samples is cost effective QC material. The present study was carried out to identify the effects of temperature on stability of anti-HCV antibodies in plasma during storage by evaluating anti-HCV antibodies using sensitive and specific ELISA assays intended for detection of anti-HCV antibodies. The stability of anti-HCV positive samples was assessed at three different temperatures 37^0C, 48^0C and 65^0C incubated for a period of 7 days and 14 days by two ELISA Kits. A significant decrease in E-ratios when stored at higher temperatures was observed in the present study.

Keywords: Hepatitis C, HCV, Antibodies, Stability, Internal Quality Control, IQC.

1. Introduction

Current estimates indicate that between 130 and 150 million people worldwide have chronic Hepatitis C Virus (HCV) infection, and that between 350 000 and 500 000 of them die each year [1][2]. Because HIV and HCV share common routes of transmission, it is estimated that 4–5 million people are co-infected with both viruses [3]**.** Yet, most people infected with HCV are not aware they are infected; therefore, HCV has been called the "silent pandemic" [4][5]. Due to the complexity and cost of testing and treatment for HCV, few individuals living in resource-limited settings have access to either. [6].

Serological detection of circulating antibodies is the usual method for laboratory diagnosis of HCV infection. Serological tests for detecting antibodies to HCV are generally classified as screening tests or confirmatory tests. All laboratories carrying out anti-HCV tests, should have a well-functioning quality assurance programme. It is most important that quality assurance procedures be stringently applied so as to maximize the accuracy of the laboratory results. It is recommended that laboratories participate in an external quality assessment at least once a year [7].

Quality control (QC) materials are crucial for Internal Quality Control (IQC) and External Quality Assessment Scheme (EQAS). However, many developing countries are disadvantaged by unavailability and high cost of commercial quality control material [8].

Therefore, preparing IQC Samples is cost effective QC material. The preparation of IQC Samples involves several procedures including processing the biological material, characterizing, splitting into small portions and finally storing them which is challenging in resource-limited settings.

1.1 Objective

The present study was carried out to identify the effects of temperature on stability of anti-HCV antibodies in plasma during storage by evaluating anti-HCV antibodies using sensitive and specific ELISA assays intended for detection of anti-HCV antibodies.

2. Method

The study protocol for collection of samples from various blood banks of Delhi and NCR was approved by Institutional Human Ethics Committee.

The IQC plasma samples used for the present study were part of HCV Evaluation Panel which is used by the laboratory for Quality Evaluation of HCV Immunodiagnostic Assays. The panels were prepared from samples collected from Blood Banks of Delhi and NCR which were not suitable for their use. No personal information of the donor was collected. These samples collected for preparation of panels for evaluation of immunodiagnostic kits were collected, characterized and stored frozen at -20^{0}C in the laboratory. The samples were characterized using screening (Ortho HCV 3.0 ELISA Kit (M/s Ortho Clinical Diagnostics) and Monolisa Anti-HCV ELISA (M/s Bio-Rad)) and confirmatory assays (InnoLia HCV Score Line Immunoassay Kit (M/s Innogenetics)) for the presence of anti-HCV antibodies and also screened for other viral & bacterial markers viz. anti-HIV 1&/2 Ab (Vironostika HIV Ag/Ab ELISA Kit (M/s Biomerieux)), HBsAg (Hepanostika HBsAg ELISA Kit (M/s Biomeriux)) and anti-Syphilis antibodies (Trepanostika TP ELISA Kit (M/s Biomerieux)) to assign the final reactivity status to each panel member. Confirmatory Assay results were analysed by visual classification of intensity in C1, C2, E2, NS3, NS4 and NS5 bands.

The panel characterization details of the samples used for the present study are given in Table 1.

Table 1: Anti-HCV reactivity profile by ELISA, Confirmatory Assay of IQC Plasma samples used for the study.

Plasma Sample	ELISA* (E-ratio: Sample OD/Cut-off)	Confirmatory Assay** Band Pattern Strep/C1/C2/E2/NS3/NS4/NS5	Interpretation
1	7.271	-/4+/4+/2+/4+/2+/1+	Positive
2	7.735	-/2+/3+/-/3+/2+/3+	Positive
3	8.308	-/2+/3+/-/3+/2+/-	Positive
4	7.686	-/3+/3+/2+/3+/±	Positive
5	6.808	-/2+/2+/3+/3+/1+/3+	Positive
6	7.308	-/4+/4+/2+/4+/3+/-	Positive
7	7.302	-/4+/4+/-/4+/2+/-	Positive
8	8.116	-/4+/2+/2+/4+/4+/2+	Positive
9	7.253	-/2+/4+/-/4+/-/-	Positive
10	7.976	-/3+/4+/±/4+/4+/-	Positive
11	8.095	-/4+/4+/2+/4+/4+/1+	Positive
12	7.287	-/3+/2+/-/4+/4+/-	Positive
13	6.79	-/3+/3+/-/2+/1+/-	Positive
14	6.680	-/2+/2+/2+/1+/1+/-	Positive
15	7.015	-/3+/2+/-/4+/3+/-	Positive
16	7.213	-/3+/3+/1+/3+/2+/-	Positive
17	7.448	-/4+/4+/1+/2+/-/-	Positive
18	8.589	-/3+/3+/2+/3+/±/-	Positive
19	9.161	-/3+/2+/±/4+/4+/4+	Positive
20	9.359	-/±/1+/-/3+/-/1+	Positive

*: ELISA Kit used is Monolisa Anti-HCV ELISA manufactured by M/s Bio-Rad.
**: Confirmatory Kit is InnoLia HCV Score manufactured by M/s Innogenetics, Belgium.
Results of ELISA are expressed as E-ratios (Sample OD/Cut-Off), where Positive ≥ 1.0; Negative < 1.0

The serological tests performed with the 20 anti-HCV antibody positive IQC plasma samples used in this study were as per the scope in which they were intended i.e., for detection of anti-HCV antibodies by ELISA Methodology. All testing was performed according to the manufacturer's instructions. The first ELISA Kit used for the study was Genedia HCV ELISA manufactured by M/s Green Cross Medical Science Corporation and second ELISA Kit used was Monolisa Anti-HCV ELISA manufactured by M/s Bio-Rad.

The stability of anti-HCV positive IQC samples was assessed at three different temperatures 37^{0}C, 48^{0}C and 65^{0}C incubated for a period of 7 days and 14 days. In the beginning of the study, 07 aliquots of all the 20 individual samples were brought to room temperature (25^{0}C) and two aliquots of each of 20 samples used for the study were incubated in Incubator set at 37^{0}C; similarly two aliquots each of 20 samples were stored at 48^{0}C and 65^{0}C respectively. One aliquot of each of the 20 samples was tested by both the ELISA Kits to determine the initial anti-HCV E-ratios (sample OD/cut-off) on zero day itself. At the end of each storage period the samples were taken out and analysed by ELISA assays. Each of the sample was analysed in duplicate. The E-ratios recorded at the zero day was compared with the E-ratios recorded at different time periods.

2.1 Statistical Analysis

The results were analysed in Microsoft Office Excel (Microsoft Corp., USA). The data was compared using paired t-test to see if there was any variation in the E-ratios recorded initially at 0 day and different time periods and temperatures.

3. Results

Table 2: Shows the ELISA results (E-ratios) found by simultaneous storing IQC samples (n=20) at three different temperatures 37^{0}C, 48^{0}C and 65^{0}C for a period of 7 and 14 days.

Samples	Baseline		7 Days at 37^0 C		14 Days at 37^0 C		7 Days at 48^0 C		14 Days at 48^0 C		7 Days at 65^0 C		14 Days at 65^0 C	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2
1	10.475	6.688	7.591	6.265	5.917	4.789	4.302	4.458	3.121	1.158	8.332	1.008	0.089	1.236
2	8.703	6.882	5.976	5.979	4.637	5.695	4.102	4.312	2.963	2.589	1.671	2.014	0.02	0.624
3	9.234	7.015	7.36	6.8	4.377	3.899	4.446	5.346	3.33	1.894	3.24	1.987	0.01	1.494
4	8.182	5.756	4.052	6.745	3.64	2.442	2.257	3.217	0.543	3.454	0.039	1.796	0.023	0.2
5	8.218	6.229	4.643	6.27	6.017	2.626	3.251	3.214	4.113	3.214	3.25	2.458	0.02	1.548
6	8.393	5.758	4.183	6.136	4.08	5.432	4.079	2.325	1.24	3.548	0.743	3.258	0.023	1.842
7	8.432	6.232	6.006	5.169	5.083	2.415	4.442	3.588	5.44	4.568	3.385	1.254	0.092	2.111
8	6.568	6.579	4.348	6.422	1.31	2.466	1.805	2.205	0.51	2.345	0.306	2.589	0.052	1.589
9	8.528	6.665	6.872	6.838	3.973	2.825	4.762	5.362	1.777	3.568	1.013	1.458	0.036	1.456
10	10.142	6.826	7.643	6.895	5.577	4.258	3.602	4.587	2.321	4.987	8.803	2.369	2.01	1.458
11	10.089	6.912	7.405	7.251	5.36	4.639	4.36	3.214	3.452	3.688	6.77	2.357	0.023	1.897
12	6.848	6.005	4.747	6.098	2.86	6.321	2.145	2.145	1.157	2.789	0.069	1.258	0.023	0.367
13	5.525	5.504	4.183	5.716	1.557	5.485	1.244	2.321	0.12	4.885	0.016	2.321	0.023	0.245
14	6.564	5.665	5.25	6.031	1.813	2.846	3.251	4.526	1.3	1.235	0.421	2.587	0.023	2.325
15	9.257	5.64	6.625	5.122	3.24	2.555	5.941	1.154	3.387	2.658	0.961	2.358	0.066	0.536
16	5.073	6.141	5.485	5.578	1.97	5.425	4.376	3.897	1.797	1.589	0.832	1.258	0.318	0.673
17	8.653	6.116	8.646	6.06	4.62	2.555	3.925	5.859	1.235	4.589	2.336	1.258	0.485	2.372
18	3.96	6.202	4.046	6.241	0.323	5.489	0.257	4.452	0.1	2.587	0.013	1.002	0.141	2.527
19	9.647	6.393	8.128	6.766	5.003	2.816	2.878	1.458	9.93	2.147	2.654	1.245	9.295	1.236
20	10.591	5.902	7.78	6.232	4.14	5.236	2.271	4.452	7.313	1.458	2.326	1.023	4.108	1.789
Mean	**8.154**	**6.256**	**6.048**	**6.231**	**3.775**	**4.011**	**3.385**	**3.605**	**2.757**	**2.948**	**2.359**	**1.843**	**0.844**	**1.376**

Results are expressed as E-ratios (Sample OD/Cut-Off), where Positive ≥ 1.0; Negative < 1.0
1: Genedia HCV ELISA manufactured by M/s Green Cross Medical Science Corporation, Korea
2: Monolisa Anti-HCV ELISA manufactured by M/s BioRad France

Fig. 1: Graph showing the trend observed in the mean E-ratio of anti-HCV Positive IQC samples stored at 37^{0}C, 48^{0}C and 65^{0}C for a period of 7 and 14 days by ELISA Kit 1.

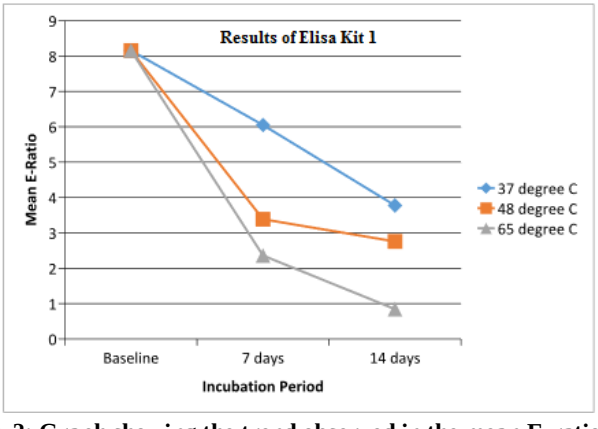
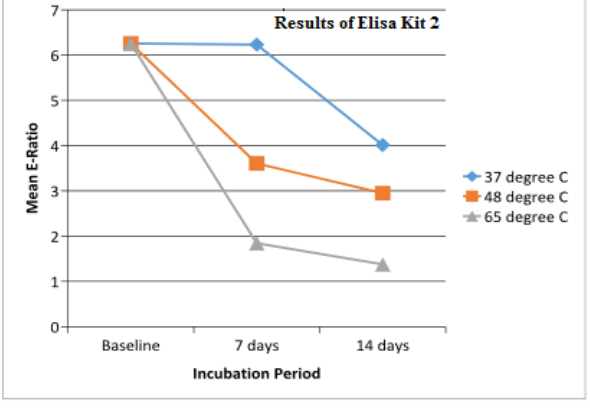


Fig. 2: Graph showing the trend observed in the mean E-ratio of anti-HCV Positive IQC samples stored at 37^{0}C, 48^{0}C and 65^{0}C for a period of 7 and 14 days by ELISA Kit 2.



By analysing the data it has been found that mean E-ratio of IQC samples incubated at 37^{0}C for 7 days decreased significantly ($p < 0.05$) when tested by ELISA Kit 1. Whereas, when tested with ELISA Kit 2 they were comparable with the initial baseline results (tested on day zero) with no significant decrease (Figure 2). After 14 days at 37^{0}C, the mean E-ratio did decrease significantly in comparison to initial values by both ELISA Kits (Figure 1 and 2). Out of 20 samples one sample became non-reactive when tested with ELISA Kit 1 (Table 2).

It was observed that the mean E-ratio of IQC samples incubated at 48^{0}C for 7 and 14 days varied significantly from the initial mean E-ratio ($p < 0.05$) (Figure 1 and 2). Out of 20 samples incubated at 48^{0}C for 7 days one sample became non-reactive when tested by ELISA Kit 1 whereas 04 samples became non-reactive when tested by ELISA Kit 1 when incubated at 48^{0}C for 14 days (Table 2).

There was a significant decrease in the mean E-ratios of IQC samples incubated at 65^{0}C for both time periods i.e., 7 and 14 days in comparison to initial values ($p < 0.05$) (Figure 1 and 2). Out of 20 samples, 09 samples were tested to be non-reactive after 7 days and 17 samples out of 20 tested to be non-reactive after 14 days by ELISA Kit 1. Whereas, 06 samples out of 20 also tested to be non-reactive after being incubated at 65^{0}C for 14 days by ELISA Kit 2 (Table 2). It was observed that the slope at 65^{0}C was more in comparison to 37^{0}C and 48^{0}C which exhibits that with the

increase in temperature the fall in E-ratios is more significant (Figure 1 and 2).

4. Discussion

In resource-limited settings, inadequate prevention and screening strategies is one of the important factor for the prevalence and mortality rates due to chronic viral hepatitis. WHO report describes the current continuum of testing for HCV to be complex and expensive, which means that it is very challenging to implement in resource-limited settings [9].

There is a current challenge to make HCV testing attainable in resource-limited settings. Needless to say, it is important to monitor and regulate the quality of the screening assays ie. Rapid and ELISA assays intended for detection of anti-HCV antibodies and thus the role of Internal Quality Control (IQC) Samples is to monitor the day-day precision and accuracy of a given assay. In the present study we carried out stability tests for IQC samples stored at three different temperatures to assess the significance of elevated temperatures on plasma samples containing anti-HCV antibodies.

From the results it is evident that temperature does affect the stability of anti-HCV antibodies in the plasma samples. The elevated temperature significantly lowers down the E-ratios in comparison to initial values recorded just after bringing samples out of 2-8^{0}C to ambient temperature. Thus, it is important to store IQC samples in ideal conditions.

In resource-limited settings the patient samples requires to be transported from urban, peri-urban and rural settings to the laboratory for processing. This is done using sample transport networks in-country, taking advantage of courier or similar services to take samples to the laboratory and to return results at a later date. But, frequently, these services are not well developed, leading to long delays in returning sample results to patients and loss to follow-up. There is a requirement to have alternate way for preparation and distribution of Proficiency testing Panels to monitor HCV testing practices in Resource Limited Settings. The use of Dried Blood Spot (DBS) samples for HCV testing performed at central laboratories is an important consideration in the implementation of the testing because it greatly simplifies the transport of samples, providing enhanced stability and ease of use for health-care workers. The use of DBS is also cost effective [10]-[13].

5. Conclusion

There was a significant decrease in E-ratios of IQC samples when stored at higher temperatures. Higher the temperature steeper was the fall in E-ratios. Most of the deterioration in E-ratios happened in 7 days itself. The present study shows that the IQC samples need to be stored and transported at 2-8^{0}C which can be challenging in Resource Limited Settings and thus there is a requirement to have alternate way for preparation and distribution of Proficiency Testing Panels.

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References

[1] Lavanchy D. The global burden of hepatitis C. *Liver Int*.; 2009; 29 (Suppl 1):74–81.

[2] Hepatitis Fact Sheet No. 164. (2014) Geneva: World Health Organization; 8 November.

[3] Alter M J. Epidemiology of viral hepatitis and HIV co-infection. *J Hepatol*. 2006; 44 (Suppl. 1):S6–9.

[4] Lemoine M, Nayagam S, Thursz M. Viral hepatitis in resource-limited countries and access to antiviral therapies: current and future challenges. *Future Virol*. 2013; 4: 371–80.

[5] Economist Intelligence Unit (2012) Tackling Hepatitis C with policy innovation: The silent pandemic. London: The Economist.

[6] Hepatitis C medicines and diagnostics in the context of HIV/HCV co-infection: a scoping report (2013). Geneva: UNITAID; October.

[7] Hepatitis C Assays: Operational Characteristics (Phase I) Report 1 January (2001). Blood Safety and Clinical Technology, WHO.

[8] Jamtsho R. Stability of Lyophilized Human Serum for Use as Quality Control Material in Bhutan. *Indian J Clin Biochem.* 2013; Oct; 28(4): 418–421.

[9] Hepatitis C Diagnostics Technology Landscape 1st Edition January (2015) UNITAID Secretariat World Health Organization Technical Report.

[10] Tuaillon E, Mondain AM, Meroueh F, Ottomani L, Picot MC, Nagot N, Van de Perre P, Ducos J. Dried blood spot for hepatitis C virus serology and molecular testing. *Hepatology.* 2010; 51:752–758.

[11] Bennett S, Gunson RN, McAllister GE Hutchinson SJ, Goldberg DJ, Cameron SO, Carman WF. Detection of hepatitis C virus RNA in dried blood spots. *J Clin Virol.* 2012; 54:106–9. 32.

[12] Ross RS, Stambouli O, Grüner N Marcus U, Cai W, Zhang W, Zimmermann R, Roggendorf M. Detection of infectious with hepatitis B virus, hepatitis C virus, and human immunodeficiency virus by analyses of dried blood spots – performance characteristics of the Architect system and two commercial assays for nucleic acid amplification. *Virol J.* 2013;10:72. 33.

[13] Dokubo EK, Evans J, Winkelman V Sherri Cyrus, Leslie H. Tobler, Alice Asher, Alya Briceno, and Kimberly Page. Comparison of Hepatitis C virus RNA and antibody detection in dried blood spots and plasma specimens. *J Clin Virol.* 2014;59: 223–7.