Heat inactivated/lyophilized HIV virus for use in proficiency testing programs

Raul Louzao[1], Heidi Register[1], Wes Rountree[1], Clare Morris[2], Sarah Gilbert[2], Bhavna Hora[1], Feng Gao[1] and Thomas N. Denny[1].
Duke Human Vaccine Institute, Duke University, Durham, N.C[1] and National Institute for Biological Standards and Control, Blanche Lane, Potters Bar, Herts, UK.[2]

Background

The Immunology and Virology Quality assessment Center (IVQAC) at the Duke Human Vaccine Institute (DHVI) provides HIV-1 viral load proficiency testing panels to laboratories performing virologic testing for NIAID-sponsored clinical trials with a comprehensive quality assessment program for virologic assays. Typically the program provides participating Labs with PT panels of coded Quality Control materials (QCMs) six times per year. The Panels consist of 1 milliliter of frozen plasma containing a specific HIV-1 viral load concentration.
Proficiency testing of labs performing HIV viral load (VL) is critical to determining that acceptable patient monitoring standards are being established. Current PT programs utilize infectious material which requires a cold chain for shipping and local laboratory storage. We set out to develop a new innovative PT material to reduce the infectious risk of the QCM and overall cold chain requirements by using a heat inactivated/lyophilized HIV virus.

Methods

A Clade C virus from the NIAID EQAPOL virus repository program was selected, spiked into normal human plasma and then heat inactivated (56° for 60 minutes). To confirm viral inactivity, a mini-culture was performed and was determined by p24 Ag and VL testing to be non-replicating. The virus was then lyophilized at a viral load concentration of 50,000 copies/ml. VL testing was performed at four storage temperatures (-20°C, 4°C, 23°C, 30°C) during seven time points. Linear modeling was performed to make descriptive statistics (e.g., estimation of means) and a descriptive evaluation of the means for storage temperatures and time points at the alpha 0.05 level.
Samples were assayed with the Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, v2.0 (Roche™) assay for HIV-1 Viral Load determination and p24 with the Alliance HIV-1 P24 ANTIGEN ELISA Kit (PerkinElmer). To eliminate interlab variation, all assays were performed by one laboratory– DAIDS Virology Quality Assurance [VQA] Laboratory; Duke University Medical Center, Durham, NC.

Discussion

We found statistical evidence of a 0.007 Log^{10} increase per week of storage and that month 6 VL was higher than the average time point VL (**Figure 1**). There was no statistical evidence of storage temperature differences. The mean and 95% confidence interval (CI) of the mean are presented in **Table 1**. . Shown below are VL results for the lyophilized material held at different storage conditions over time and then tested. The model based means for storage temperatures range from 4.26 – 4.42 Log^{10} copies/ml and the model based means for the time points range from 4.28 – 4. 49 Log^{10} copies/ml. Displayed in Figure 1 below are the VL results for the lyophilized material held at different storage conditions over the various time points. The average VL is 4.38 with the standard acceptance criteria of +/- 0.5 Log^{10} copies/ml at 3.88 and 4.88.

Figure 1

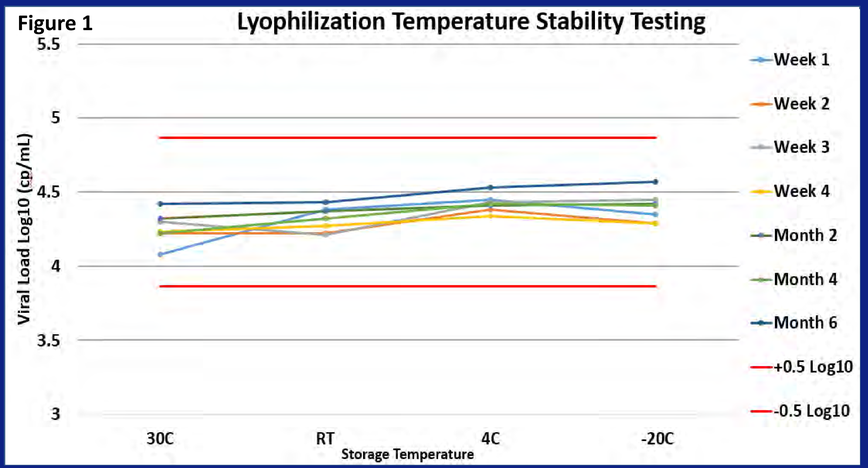


Table 1

Storage Temperatures			
Outcome	Mean	Lower 95% CI	Upper 95% CI
-20°C	4.40	4.37	4.42
4°C	4.31	4.29	4.34
Room Temperature	4.26	4.23	4.28
30°C	4.42	4.40	4.45
Time Points			
Week 1	4.38	4.35	4.41
Week 2	4.34	4.31	4.37
Week 3	4.49	4.46	4.52
Week 4	4.32	4.29	4.35
Month 2	4.28	4.25	4.31
Month 4	4.35	4.32	4.38
Month 6	4.28	4.25	4.31

Preliminary data of on going Sequencing experiments of Lyophilized samples

Lyophilized samples were reconstituted in 1.5ml molecular biology grade water. Viral RNA was extracted from 400µl of each sample using EZ1 Virus Mini Kit v2.0 (Qiagen, Valencia, CA) and subjected to cDNA synthesis using Superscript III Reverse Transcriptase (Invitrogen, Carlsbad, CA) with primers 1.R3.B3R for 3'half and 07Rev9 for 5'half genome. Partial pol gene, integrase gene were amplified with undiluted 5'half cDNA and env gene was amplified from 3'half cDNA from each sample in triplicate using Platinum Taq DNA polymerase High Fidelity (Life Technologies, Carlsbad, CA) using a nested PCR approach. Positive amplification was confirmed on 1% agarose gel.

	Partial pol	Integrase	Env
Lyophilized stored at 30°C			
Lyophilized stored at 23°C			
Lyophilized stored at 4°C		+	+
Lyophilized stored at -20°C		+	
Non heat inactivated control	+	+	+

Conclusion

This Viral Load data collected provides proof that heat inactivated and lyophilized material remains stable and well within the 0.5 Log10 acceptance criteria at all temperature storage conditions for up to six months. Studies are underway to improve the lyophilized material to be more suitable for use in quality assessment of sequencing type assays used for detecting DRM.. Reducing the cold chain requirements, shipping costs and infectious status of QCMs offers significant improvements to current PT approaches. Work supported by NIAID **EQAPOL** HHSN27220170061C.

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