

SHORT COMMUNICATION

Stability of HIV RNA in plasma specimens stored at different temperatures

B Amellal,^{1,2,3} R Murphy,^{3,4} A Maiga,^{1,2,3} G Brucker,^{2,4} C Katlama,^{2,3,4} V Calvez^{1,2,3} and AG Marcelin^{1,2,3}

¹Department of Virology, Pitié-Salpêtrière Hospital, Paris, France, ²Solidarité Thérapeutique et Initiatives contre le SIDA (Solthis), Paris, France, ³Université Pierre et Marie Curie, Paris, France and ⁴Department of Infectious Diseases, Pitié-Salpêtrière Hospital, Paris, France

Objectives

In resource-limited countries, HIV-1 RNA quantification is usually performed in reference laboratories. Samples from remote areas are transported under suboptimal conditions. Here we evaluated HIV-1 RNA stability in plasma stored at different temperatures for 1 week.

Methods

Blood samples collected in ethylenediaminetetraacetic acid (EDTA) and processed within 6 h of collection were tested by HIV-1 RNA quantification using Roche Cobas Ampliprep-Cobas TaqMan[®] (Roche Diagnostics). The results were compared with matched HIV-1 RNA concentrations determined from plasma stored for 1 week at 4, 22, 30 or 37 °C.

Results

A total of 51 samples were evaluated: 10 stored at 4 °C, 15 at 22 °C, 16 at 30 °C and 10 at 37 °C. Keeping plasma at 4, 22 or 30 °C for 1 week did not affect HIV RNA measurement. Compared with HIV-1 RNA concentrations determined from fresh plasma, the correlation was significant for each of the three temperatures with no RNA decay. In contrast, HIV-1 RNA levels decreased significantly when plasma was stored at 37 °C. The 10 samples submitted at this temperature showed a weaker correlation ($\rho = 0.84$; $P = 0.012$) and a significantly reduced median HIV-1 RNA concentration ($-0.92 \log_{10}$ HIV-1 RNA copies/mL; $P = 0.005$).

Conclusion

Plasma can be saved for up to 1 week at 30 °C before shipping to a reference laboratory for HIV-1 RNA quantification.

Keywords: HIV-1, quantification, RNA stability, temperatures

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Introduction

The need for reliable HIV-1 RNA quantification for monitoring of therapy is now even greater in developing countries as access to antiretroviral drugs is rapidly increasing. In these countries, reference laboratories that perform HIV-1 RNA quantification are often physically distant from many of the remote clinical centres that are unable to perform such testing on site. Shipping of infectious samples from the clinic to the reference laboratories is thus necessary.

Under optimal conditions, blood samples collected in ethylenediaminetetraacetic acid (EDTA) are processed within 6 h of collection and plasma samples are stored at either -20 or at $2-8$ °C for up to 5 days. This procedure is recommended by the manufacturer of the Cobas Monitor[®] and Cobas TaqMan HIV-1[®] tests (Roche Diagnostics, Basel, Switzerland), who performed quality assurance evaluations with both these tests. Although it is always advantageous to process specimens as soon as possible and to store them under optimal conditions, this is not always possible in some settings where local laboratory conditions are inadequate or nonexistent and specimens must be transported to a central laboratory.

In order to find a practical solution for these suboptimally equipped laboratories and clinics, we evaluated the stability of plasma HIV-1 RNA in different temperature

Correspondence: Dr Bahia Amellal, Laboratoire de Virologie, Hôpital Pitié-Salpêtrière, CERVI, 83 boulevard de l'Hôpital, 75651 Paris, Cedex 13, France. Tel: +33 1 42 17 74 31; fax: +33 1 42 17 74 11; e-mail: bahia.amellal@psl.aphp.fr

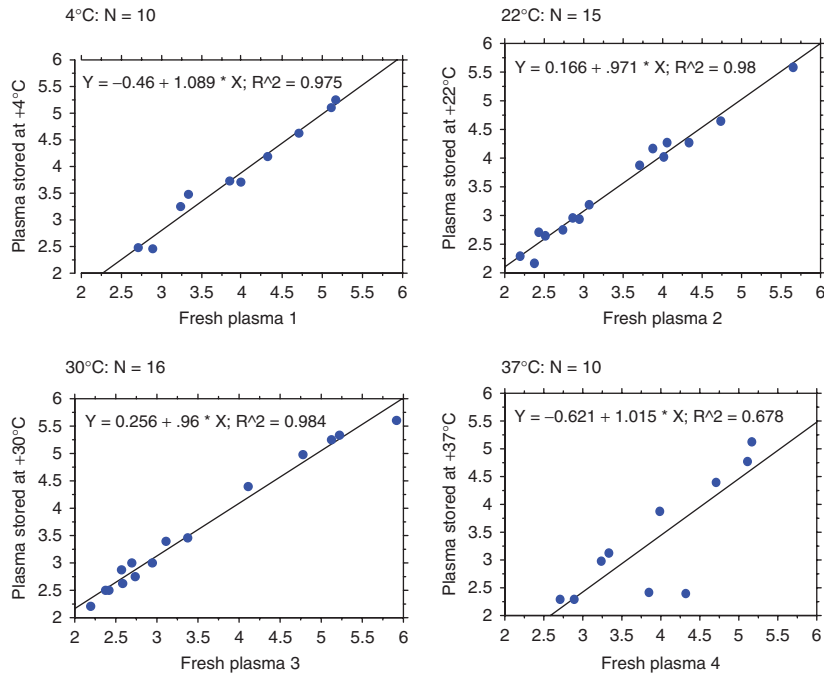


Figure 1. HIV-1 RNA loads (log₁₀ copies/mL) determined from fresh plasma were correlated with those determined from the matched plasma, saved 1 week at one of the four temperatures: + 4°C ($\rho = 0.98$, $P = 0.003$); + 22°C ($\rho = 0.99$, $P < 0.001$); + 30°C ($\rho = 0.99$, $P < 0.001$) and + 37°C ($\rho = 0.84$, $P = 0.012$).

conditions by testing specimens following storage for 1 week at 4, 22, 30 or 37 °C. The plasma HIV-1 RNA concentration was then determined using Roche Cobas Ampliprep-Cobas TaqMan (RCAP-CTM).

Materials and methods

The study population consisted of adult patients receiving combined antiretroviral therapy (ART) enrolled in a study of the natural history of viral burden at La Pitié-Salpêtrière Hospital, Paris, France. HIV-1 disease in these treated patients ranged from asymptomatic early-stage infection to clinical AIDS. Each patient had a whole blood sample drawn by venipuncture and collected in standard EDTA-containing tubes. Within 6 h of collection, blood samples were processed and the liquid plasma was subjected to HIV-1 RNA quantification using RCAP-CTM. An aliquot of each of these plasma specimens was stored at -20 °C. Samples with known HIV-1 RNA measurements were selected for this evaluation. We used plasma samples saved for <1 week at -20 °C so that the HIV-1 RNA determined in our experiments would reflect the effects of temperature exposure rather than the effects of long-term freezing.

The plasma samples were split into four batches and submitted to four different temperature storage conditions: 4, 22, 30 and 37 °C. After 1 week, plasma samples were processed for RNA extraction and quantification using

RCAP-CTM with a limit of detection of 50 HIV-1 RNA copies/mL. A total volume of 1050 μ L of liquid plasma was needed for this determination. The results of HIV-1 RNA quantification obtained from the fresh plasma were compared with those determined for the matched plasma subjected to the specific storage temperature conditions.

Using STATVIEW[®] software version 5.0 (SAS Institute Inc., Cary, NC, USA), both parametric and nonparametric tests were performed. Median values of plasma HIV-1 concentrations were compared using the Wilcoxon test for paired observations and correlated using Spearman's correlation coefficient. A linear regression test was also performed. A P -value of <0.05 was considered significant.

Previous studies found the intra-assay variability for a given sample assessed using the Roche assay to be $\leq 0.2 \log_{10}$ copies/mL [1], whereas biological variability within an individual receiving stable ART has been estimated to be $0.3 \log_{10}$ copies/mL [2]. Therefore, we considered changes $>0.5 \log_{10}$ copies/mL (the sum of these two factors) to be significant [3,4].

Results

Fifty-one plasma samples were assayed: 10 at 4 °C, 15 at 22 °C, 16 at 30 °C and 10 at 37 °C. HIV-1 RNA quantification carried out on plasma specimens stored at 4,

22 or 30 °C for up to 1 week showed no effect of any of these temperatures on HIV-1 RNA quantification when compared with the matched value determined from fresh plasma. The correlation was highly significant for each of the three temperatures and, correspondingly, there was no significant HIV-1 RNA decay. In contrast, keeping plasma at 37 °C for 1 week had a negative effect on HIV-1 RNA measurement. The 10 liquid plasma samples stored at this temperature showed a weaker correlation ($\rho = 0.84$; $P = 0.012$) and had a significantly reduced median HIV-1 RNA concentration ($-0.92 \log_{10}$ copies/mL; $P = 0.005$) (Fig. 1).

The variation in plasma HIV-1 RNA measurement was well within the accepted $\pm 0.5 \log_{10}$ interval for all plasma samples maintained at 4, 22 or 30 °C for 1 week. The median value for the specimens stored at 37 °C was lower than the matched fresh value by $>0.5 \log_{10}$ copies/mL. Three of 10 samples had values $>0.5 \log_{10}$ copies/mL lower than those of freshly tested plasma; specific values were -0.59 , -1.42 and $-1.94 \log_{10}$ copies/mL. These three discrepancies, particularly the two highest ones, made the correlation weaker at 37 °C compared with the three other temperatures.

Discussion

Transportation of whole blood or plasma samples must be carried out under local conditions which comply with local regulations [5]. In this study, plasma specimens were processed within 6 h of collection and HIV-1 RNA quantification was carried out using RCAP-CTM.

From the data obtained here, we conclude that anti-coagulated blood samples collected in EDTA, and processed under optimal conditions for plasma preparation, can be saved at 4, 22 or 30 °C for up to 1 week without affecting HIV-1 RNA measurement. With this relaxed storage option, consistent and reliable results can be obtained from plasma specimens stored for up to 1 week at 30 °C before being shipped to a central laboratory and processed.

Our results showed a significantly weaker correlation of paired HIV-1 RNA measurements for plasma saved at 37 °C compared with other temperatures. The 10 values obtained at 37 °C were all lower than the matched values. The three outlying values, -0.59 , -1.42 and $-1.94 \log_{10}$ copies/mL, particularly the two most discordant ones, made the correlation weaker than those obtained in the experiments carried out at the other temperatures. Because of the low number of samples tested at 37 °C and because the measurements were made only once, we cannot speculate on the percentage of discordant values we would expect to find when samples are stored at this temperature.

Regarding other studies performed to assess HIV-1 RNA stability in liquid plasma processed and stored at different temperatures [3,5–7], plasma specimens saved for up to 3 days

at either room temperature or 4 °C showed good stability of HIV-1 RNA under these conditions [3,5,6,8]. In another investigation, the duration of the experiment was extended and it was shown that plasma samples can be maintained for up to 14 days at 5 °C for HIV-1 RNA quantification [7]. This was confirmed in another experiment where the addition of lysis buffer to the plasma did not improve stability [9]. At 25 °C, the HIV-1 RNA half-life was determined to be nearly 7 days [7]. All these findings are consistent with our results. It was reported elsewhere, however, that at 30 °C HIV-1 RNA levels declined significantly after 2 days when quantification was carried out using the Nucleic Acid Sequence Based Amplification (NASBA) technology [9].

Reliable HIV-1 RNA quantification in plasma is dependent upon the processing conditions and also the technology used to extract RNA and amplify it. We suggest processing plasma within 6 h, as currently recommended, and storing it for up to 1 week at temperatures not exceeding 30 °C before transportation to a central laboratory.

Our results cannot yet be extrapolated to whole blood samples. In some resource-limited settings, plasma separation might be difficult to perform under suitable conditions before shipping of samples to the reference laboratory. Evaluations on whole blood samples need to be performed in order to determine whether plasma processing is needed and under what conditions.

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