

Multi centre study to evaluate the influence of pre-analytical storage conditions on the RNA concentration of hepatitis C virus

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Objectives: Several studies have claimed that hepatitis C virus (HCV) RNA levels are stable in samples when stored at 4°C for 3 up to 7 days. The stability of HCV RNA levels at room temperature (RT) is less clear. Some studies stated that HCV RNA is unstable at RT (18-25°C) whereas others demonstrated that EDTA blood or serum may be stored at 25°C for 4 up to 5 days without significant loss in HCV RNA. In this multi-centre study (10 laboratories) we tested the HCV RNA stability of 15 samples (8 serum samples and 7 plasma samples) by storing the samples at RT for 0, 1, 3 and 7 days before analysis. **Methods:** Laboratories analysing HCV on serum, divided a fresh serum sample in 4 aliquots, left these aliquots at RT and stored the aliquots below -20°C at day 0, 1, 3 and 7. Laboratories analysing HCV on EDTA plasma, divided an EDTA blood sample in 4 aliquots. The different aliquots were left for 0, 1, 3 and 7 days at RT before centrifugation and immediately stored below -20°C. The RNA extraction and HCV qPCR was done for all aliquots in the same run (avoiding inter-run differences). The concentrations (IU/ml) of the samples at day 0, 1, 3 and 7 were expressed as Log10 values. A Log10 difference > 0.5 was considered as a clinical significant difference. **Results:** One sample was excluded for further analysis because the centre did not determine the RNA level on day 0. One result on day 1 was excluded because of a statistical outlier. The Log10 differences from all samples for the different time points indicate an similar trend: the HCV concentration decreases over time. For 2 out of 14 samples (14%), a Log10 difference > 0.5 was observed at day 3. For 5 out of 12 samples, a Log10 difference > 0.5 was observed at day 7. The mean Log10 difference stayed within the 0.5 limit at all days. However at day 7, the mean Log10 difference was -0.45 ± 0.34 , which demonstrates a significant decrease in HCV concentration for several samples. No statistical difference was observed between serum and plasma samples. **Conclusion:** Although it is clear that it is the best procedure to store samples as soon as possible at <-20°C is, we can conclude that most samples stored at RT for 3 days do not exhibit a significant loss in measured HCV RNA levels. However, storage for more than 3 days at RT will lead to an underestimation of HCV RNA concentrations or false-negative results in the detection of HCV RNA in serum and plasma.

