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Introduction and Purpose

Several studies have claimed that HCV RNA levels are stable in samples when stored at 4°C for 3 up to 7 days (Krajden et al., Trabaud et al., Gessoni et al., de Moreau de Gerbehaye et al.). 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) (Cuypers et al., Busch et al., Halfon et al.) whereas others demonstrated that EDTA blood or serum may be stored at 25°C for 4 to 5 days without significant loss in HCV RNA (Grant et al. and de Moreau de Gerbehaye et al.). 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.

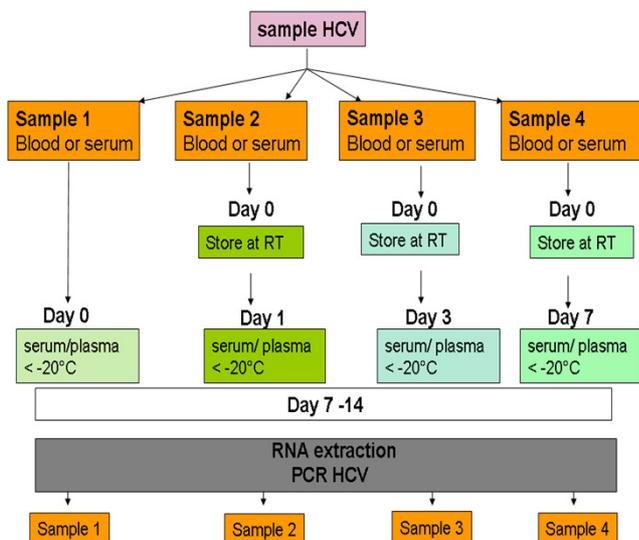


Fig 1: Overview of the protocol

Results (1)

- One sample (Nr.7) was excluded for further analysis because the centre did not determine the RNA level on day 0.
- One result (Nr.4C) on day 1 was excluded because of a statistical outlier.
- The Log₁₀ differences from all samples for the different time points indicate a 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.

Results (2)

Sample	Type	Log10 IU/ml	Log Difference between day 0 and		
			day1	day3	day7
1	serum	4,24	-0,07	-0,31	-0,24
2A	serum	5,44	0,11	0,14	-0,33
2B	serum	5,13	0,02	0,01	0,02
3	plasma	6,65	0,08	0	-0,62
4A	plasma	7,15	-0,12	-0,04	-0,32
4B	plasma	6,11	-0,08	0,05	inhibition
4C	plasma	6,54	-0,8	-0,51	-0,76
5	serum	6,3	-0,17	-0,02	-0,72
6	plasma	3,91	0,04	-0,6	invalid
7	plasma	?	*	*	*
8A	serum	5,75	-0,15	-0,06	-0,78
8B	serum	3,03	-0,02	-0,1	-0,44
9A	serum	4,02	0	-0,15	-1,07
9B	serum	5,72	-0,23	-0,17	-0,06
10	plasma	5,65	-0,24	-0,04	-0,05
Average:			-0,12	-0,13	-0,45

Fig 2: Concentrations HCV RNA on day 0 in Log₁₀ IU/ml and the Log₁₀ difference between different timepoints

* Result excluded for analysis

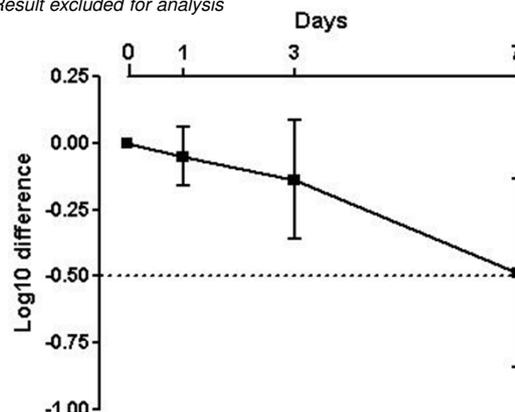


Fig 3: Average and spread of the Log₁₀ differences between different timepoints

Conclusions

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.