

False-Negative Results of PCR Assay with Plasma of Patients with Severe Viral Hemorrhagic Fever

Viral hemorrhagic fevers (VHF) are acute infections with high case fatality rates, associated with the risk of nosocomial transmission (3). A rapid confirmation of the clinical diagnosis is therefore required by methods such as antigen capture enzyme immunoassay and serologic detection of immunoglobulin M. With the development of PCR technology, it has become possible to rapidly test for viruses that cause VHF (4, 5, 10). We have recently confirmed a case of acute yellow fever with fulminating hepatic failure by reverse transcription-PCR (RT-PCR) (2). In spite of the very high viral RNA concentration in the plasma sample, it initially tested negative. A confirmation by PCR would have been missed if we had not tested a duplicate sample, inoculated with *in vitro*-transcribed yellow fever virus RNA, to detect substances that are inhibitory to RT-PCR (5). When this control reaction failed, we diluted the plasma in \log_{10} intervals, reprepared RNA from the diluted material, and tested it by RT-PCR. The virus was clearly detectable in the patient's plasma diluted 1:100, 1:1,000, and 1:10,000 (Fig. 1). Again, no virus was detectable in the undiluted sample, and detection was also partially inhibited in the 1:10 dilution. Quantitative real-time RT-PCR with the diluted material yielded a projected concentration of $>10^6$ copies of viral RNA per ml of the undiluted plasma (Fig. 1). Interestingly, we have observed a similarly strong inhibition phenomenon with plasma from a moribund patient with acute Ebola hemorrhagic fever from Gulu, Uganda (Fig. 2). The viral RNA concentration in this case was 6.9×10^8 copies/ml. No hemolysis, which often causes inhibition of PCR (1), was observed in plasma from the two patients. In both cases, however, the patients were suffering from severe organ

manifestation of their disease (aspartate aminotransferase in yellow fever sample, 15,000 IU; alanine aminotransferase, 6,000 IU).

Inhibition of RT-PCR in plasma samples has been reported to occur at a frequency of 0.34 to 2.1% of tests (patients infected with human immunodeficiency virus type 1 or hepatitis C virus, respectively [6, 9]). It can usually be circumvented by using standard EDTA sampling tubes (as done in both cases described here) and preparation procedures based on silica column purification (7, 8, 11). Furthermore, at least with plasma, inhibition is usually reversible upon repetition of extraction of nucleic acids from the same sample (6, 9). In these cases, however, the concentration in plasma of substances interfering with PCR appeared to be extraordinarily high: amplification was repeatedly inhibited in a second extraction, even though a reliable silica column purification method (viral RNA kit; Qiagen, Hilden, Germany) was applied. Moreover, partial inhibition occurred even in 1:10-diluted material. Two important consequences can be drawn from these observations.

(i) False-negative RT-PCR results are likely to occur for patients with severe viral hemorrhagic fevers, especially in the acute phase of the disease where a rapid confirmation is required. Their plasma may contain large amounts of RT-PCR inhibitors, probably resulting from the decay of tissue. These inhibitors can be detected by control reactions with spiked samples (low copy numbers of control RNA, 1 \log_{10} above detection limit of the PCR) (5). Control reactions to detect inhibitors of RT-PCR are mandatory for a safe diagnosis for patients with suspected VHF.

(ii) When PCR is used for diagnosis of viral hemorrhagic fevers, dilutions of the test sample should be tested in parallel with the original sample. The high viral RNA concentration in samples from acute VHF cases is likely to facilitate the diagnosis in spite of the dilution factor.

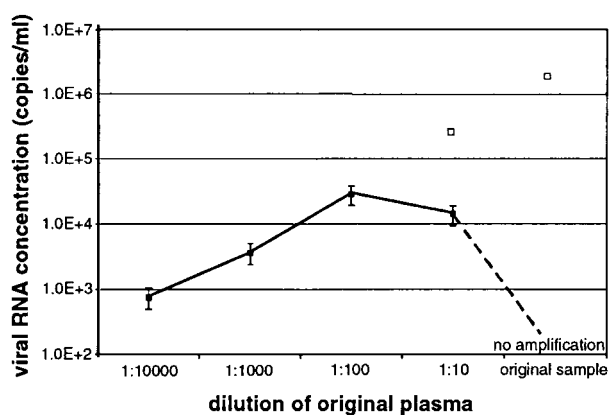


FIG. 1. Results of quantitative real-time yellow fever virus RT-PCR (5) with original plasma and prediluted plasma. Closed data points depict the viral RNA concentration in plasma, as determined by testing of each dilution step of the patient sample (y axis). In the 1:10,000 to 1:100 dilution steps, the \log_{10} of the determined viral RNA concentration correlated with the dilution factor. Open data points represent the extrapolated RNA concentrations in the undiluted and 1:10 diluted samples, indicating that amplification in these samples was inhibited (note the difference between extrapolated and observed RNA concentration).

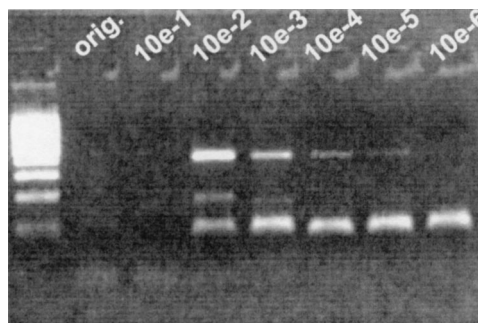


FIG. 2. Result of agarose gel electrophoresis after qualitative Ebola virus RT-PCR (5) with original plasma and prediluted plasma from a patient with acute Ebola fever. The dilution factor is depicted above each gel lane. No amplification was possible when the original plasma (orig.) and the 1:10-diluted plasma (10e-1) were tested. With a dilution of $\geq 1:100$, amplification was possible due to a decreased concentration of inhibitory substances in the analyzed sample.

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