Stability of *Entamoeba histolytica* trophozoite DNA in stool samples

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The detection of parasites in stool samples by polymerase chain reaction (PCR) techniques is dependent on the template DNA being sufficiently preserved. We have observed a rapid decline of PCR amplification products from *Entamoeba histolytica* trophozoites dependent on the storage time. The objective of this study was the assessment of the time course of DNA degradation of *E. histolytica* trophozoites in stool. Three hundred, 1000, 3000 and 10,000 HK-9 trophozoites were added to 0.1 g each of stool samples from asymptomatic patients without microscopically detectable parasites. The samples were stored at room temperature for 1 min, 5 min, 10 min, 30 min, 1 h, 2 h and 4 h. The DNA was detected by PCR as described before[1].

Three hundred trophozoites were detectable up to 5 min, 1000 and 3000 trophozoites up to 30 min and 10,000 trophozoites were detectable up to 1 h. The half life of *E. histolytica* trophozoites in stool can therefore be estimated to be approximately 10–15 min.

In conclusion, stool samples may not be stored at room temperature for extensive time periods if a DNA detection of *E. histolytica* trophozoites by PCR is to be successful.

1 Katzwinkel-Wladarsch, S., Löscher T., and Rinder, H., Am. J. trop. Med. Hyg. *51* (1994) 115.

Comparison of different techniques for semiquantitative detection of HBV DNA by PCR

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The polymerase chain reaction (PCR) allows the amplification of minute amounts of nucleic acids. It led to the detection of hepatitis B virus (HBV) DNA in patients with atypical serological constellations not previously associated with the presence of HBV DNA. For the evaluation of clinical subgroups PCR quantitation is needed. To this aim and for laboratory convenience, enzyme linked oligonucleotide-sorbent assays (ELOSA) have become popular and many applications have been published. Different ELOSA systems vary in the binding of the PCR product, the labelling of the probe, its length and detection. For an ELOSA of PCR products of HBV core, hybridization with a 3′-digoxigenin-labelled oligonucleotide probe (30 mer) versus a digoxigenin-labelled PCR product probe (227 mer) and detection with anti⟨Dig⟩POD versus anti⟨Dig⟩AP were compared. The results were compared with nested PCR and Southern blot hybridization.

Methods and results. PCR was performed with established primers, one of which was biotinylated. For the 'short probe' ELOSA and PCR sample was bound to a streptavidin-coated microtitre plate, denaturated by alkali, and hybridized to the probe. This hybrid was visualized by incubation with anti⟨dig⟩antibody, conjugated with POD or AP and detected by ABTS or CSPD. Optical density (OD) or chemiluminescence (CPS) were measured after one hour or 15 min, respectively. The long probe and the PCR sample were separately heat-denatured and then a fluid-phase hybridization was performed. The hybrids were bound to the streptavidin and detected as described above. For external quantitation of the sample a tenfold dilution series (from 10^6 to 10^0 target molecules/PCR sample) of an HBV-containing plasmid was used. We analyzed in this study sero of 37 patients with suspected chronic HBV infection.

Using primers specific for core sequences of HBV we found 24 sera positive by nested PCR and 21 of them positive by Southern blot hybridization. The 'short probe' ELOSA with ABTS resulted in 11 and with CSPD in 9 positive samples. With both substrates the 'long probe' ELOSA gave 21 positive results. For the four ELOSA protocols, quantitation of the PCR products of the dilution series of the standard plasmid gave reproducible linear relations between the initial number of targets and OD or CPS. The lower detection limit of initial copy numbers of target are 100, between 100 and 10, and below 10 molecules for the short probe/ABTS, the short probe/CSPD, and the long probe/ABTS or CSPD, respectively. In 6 of 9 cases the quantitative results were similar for the hybridization with the short probe/ABTS and CSPD (<50% difference). For the long probe this was the case for 15 and 21 samples. All other samples had values near the lower detection limit where quantification is no longer reliable. Five of 6 samples analyzed for intra-assay variability with the short probe/ ABTS and 11 of 11 analyzed with the long probe/ABTS showed reproducible results (+/− 10%). Inter-assay variability was below 10% for 5, below 20% for 1, and below 50% for 1 of 7 samples quantitated with the long probe/ABTS and below 10% for 6 of 7 samples with the short probe/ABTS.

Conclusions. Our results indicate that the choice of the probe for ELOSA is of major concern. In our panel we had seven sera which contained about 100 molecules/ml and further five sera which contained less than 1000