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

molecules/ml. Most of them were detected by the long probe but not by the short probe. When PCR for the S- or PreS-gene was included it was possible to detect all 24 HBV-positive sera (not shown) by ELOSA. The reliable lower quantification limit for the long probe is 250 molecules/ml and for the short probe 2500 molecules/ml. Surprisingly, chemiluminescence did not produce better qualitative or quantitative results. The data suggest that the usage of several replicates allows relative quantification[1] in most cases. One possible drawback we see is the hybridization efficiency. Six of our positive samples showed great differences between the number of target molecules suggested by agarose gel electrophoreses or by hybridization (Southern blot or ELOSA). All of them contained more than 10^6 molecules/ml. For these cases and for the samples where the short probe and the long probe gave discordant result (2 cases) we think that competitive PCR will be the method of choice, but in most cases ELOSA with the long probe gives reliable results and is highly sensitive.

1 Ferre, F., PCR Methods Appl. *2* (1992) 1.

Monitoring of antiretroviral chemotherapy with the NASBA™; an isothermal method for enzymatic amplification of HIV-1 RNA

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The quantitation of HIV-1 RNA is probably one of the most important and reliable markers for the evaluation of the efficiency of antiretroviral therapy in HIV-infected patients. Recently, the nucleic acid sequence based amplification (NASBA) technique has been introduced on the international market. The quantitative HIV-1 RNA detection by NASBA HIV-1 RNA QT (Organon Technica, Eppelheim, Germany) is based on the co-amplification of the HIV-1 sample RNA together with internal calibrators. The quantity of amplified RNA is measured by means of electrochemiluminescence (ECL).

In the present study, the monitoring of antiretroviral therapy was performed in consecutive samples from six HIV-infected patients using the NASBA HIV-1 RNA QT and quantitative p24 antigen detection (Abbott, Wiesbaden, Germany). In order to evaluate the reproducibility of the NASBA, different samples were tested in duplicate in each run and on consecutive days. As a supplementary quality control for quantitation of HIV RNA, a dilution panel of HIV-1 RNA standard corresponding to expected target copy numbers (5000, 500 and 50 copies/reaction) was employed.

The NASBA showed a high reproducibility; the intra- and inter-assay coefficients of variation were $<10\%$. The results obtained by the amplification of the dilution panel corresponded to the quantification of NASBA QT using the undiluted (5000 copies) HIV-1 RNA standard.

So far, the NASBA HIV-1 RNA QT is the only commercially available amplification assay which permits a quantitative detection of HIV-1 RNA. Our results, although preliminary, show that the NASBA allows an accurate and reproducible quantitation of HIV RNA combined with a simple nucleic acid isolation procedure and non-radioactive detection of the amplificates.

1 Compton, J., Nature (Lond.) *350* (1991) 91.
2 van Gemen, B., Kievits, T., Nara, P., Huisman, H., Jurriaans, S., Goudsmit, J., and Lens, P., AIDS *7* (suppl. 2) (1993) 107.
3 Kievits, T., van Gemen, B., van Strijp, D., Schukkink, R., Dircks, M., Adriaanse, H., Malek, L., Sooknanan, R., and Lens, P., J. virol. Methods *35* (1991) 273.

Detection of varicella zoster virus DNA in human tissue by standard and nested polymerase chain reaction

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After primary infection, which results in chickenpox, varicella zoster virus (VZV) establishes latency in sensory ganglia. In cases of immunosuppression, reactivations known as zoster can occur. The molecular mechanism of maintaining latency and reactivation are unknown. Latent VZV cannot be reactivated by cocultivation. Thus PCR can be used to detect small amounts of viral DNA in different kinds of tissue.

In this study we analyzed single trigeminal ganglia from deceased patients, as well as peripheral mononuclear blood cells (PMBC) from immunocompetent patients suffering from chickenpox, for DNA sequences specific for VZV immediate early gene 63 by PCR.

Methods. The DNA from ganglia and PMBC was extracted using proteinase K digestion followed by ethanol or isopropanol precipitation[1]. To achieve maximal sensitivity we used standard as well as nested PCR[2]. Amplification with the outer primer yielded a 386 bp product. The size of the nested PCR product was 326 bp. In the case of nested PCR the first and second amplification (30 cycles each) were carried out in the same tube to minimize the contamination risk. To prevent a carry over from samples containing positive control DNA we constructed a competitive fragment