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.

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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.

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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

with the same primer binding sites but a deletion of about 36 bp in the middle of the amplified region. This yielded a 350 bp product using the outer primer and a 290 bp product with the innner primer. The sensitivity of the PCR assay was evaluated by using serial dilutions of *Hind*III-digested cloned VZV DNA (*Kpn*I fragment of the VZV genome cloned into vector pT7/T3a-19). PCR products were detected by agarose gel electrophoresis followed by ethidium bromide staining. Southern blot hybridization using a Digoxigenin-dUTP labelled probe, and enzyme-linked immunoassay. The probe was generated by digestion of the *Kpn* I s fragment with *Mlu*I. A 392 bp fragment, which overlaps with 290 bp of the amplified region, was isolated and DIG labelled.

Results and conclusion. The sensitivity of the PCR assay was determined with cloned VZV DNA. About 200 copies of the target sequence were necessary for detectable amplification by standard PCR and less than 20 copies by nested PCR. Out of 24 human trigeminal ganglia five tested positive for VZV DNA by standard PCR (21%), in eleven cases VZV DNA was detectable using nested PCR (46%). Sequences specific for VZV could be detected in PMBC from children with acute varicella up to six days after the onset of rash by standard (one child) or nested (three children) PCR. This confirms that at the time of haematogenous spread before and during the rash viral DNA can be found within the mononuclear cells. Thus the use of nested primers enhances the sensitivity of the assay and allows the detection of only a few genomic copies of viruses in human tissues.

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Influence of DNA preparation and application on specificity of a 3′-end labelled oligonucleotide probe in a dot blot assay

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The aim of our studies was to establish a method that could detect orthopoxvirus- (OPV) DNA with a oligonucleotide probe within one day.

Methods and results. Purified DNA of five different species of the genus OPV was used. The negative control was parapoxvirus DNA. We chose a nucleotide sequence as probe that contained a conserved region of the OPV species[1,2]: 5′CTTACTTTTTCTTTCTC3′.

The Oligonucleotide Tailing Kit (Boehringer, Mannheim) was used to label the nucleotide sequence[3]. This kit was used to attach a poly-A tail comprising approximately 50 nt to the end 3′-end by means of terminal transferase. Prehybridization (20 min) and hybridization were carried out at 35 °C (Tm 9 °C), as were the stringency washes. Detection was carried out colorimetrically following the standard procedure. For the DNA preparation and application two different procedures were compared:

A) The DNA to be investigated was heated for 10 min in 0.4 N NaOH at 94 °C then cooled on ice. The DNA was applied to a nylonfilter under vacuum with the aid of Hybridot Manifold apparatus (Gibco/BRL) and was then rinsed and simultaneously fixed with NaOH.

B) The DNA was diluted in TE 10/1 buffer (pH 8.0) and denatured by boiling for 10 min prior to application with a pipette onto the membrane. Fixation was carried out by incubation for 20 min at 120 °C.

Method A showed nonspecific reactions especially with NaOH, although an optimization was attempted. Furthermore, the intensity of the signals was very variable. This observation led to the conclusion that NaOH and the powerful suction of the Hybridot Manifold apparatus change the surface of the membrane and therefore the labelled oligonucleotides bind unspecifically. Method B on the other hand yielded reproducible results without background reactions.

Of the three tested nylon membranes, Hybond N, Hybond N+ (both Amersham) and 'Membrane, positively charged' (Boehringer, Mannheim), the latter appeared to be the most suitable with regard to signal development, sensitivity and background. The optimal probe concentration for labelling was found to be 200 ng oligonucleotide per ml hybridization liquid.

After varying the duration of hybridization (0.5 h, 2 h, 6 h), 2 h appeared to be sufficient. In this system it was possible to detect the presence of 100 pg OPV DNA within 8 h. Attempts to improve the sensitivity by using a combination of various labelled oligonucleotides were unsuccessful.

Conclusions. Optimization of the parameters of sample preparation, sample application, probe concentration and also membrane suitability have a considerable influence on the specificity of 3′-end labelled oligonucleotides.

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