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