SK 38E/SK39E for the first and SK38/SK39 for the second PCR, the following Southern hybridization was carried out with a DIG-labelled ddUTP oligonucleotide (DIG-ddUTP-SK19). DIG-ddUTP-SK19 can be stored over months without loss of sensitivity. Meanwhile we use the primer pair SK431E/SK145 for the first PCR and primer pair SK431P/SK102 for the second PCR. All in all, we found 210 out of 210 antibody-positive specimens to be 'PCR-positive'. One sample did not contain any detectable DNA. 12 patients who were negative in a single PCR turned out to be positive in a nested PCR. Two samples showed a very low DNA-concentration and might have been negative for that reason. All antibody-negative samples proved to be 'PCR-negative', showing that there were no HIV-infected patients without seroconversion in this cohort. 9 patients with equivocal serological results proved to be negative after PCR analysis. These persons had unremarkable clinical data and showed no increased reactivity in repeated ELISAs. 10 babies acquired HIV-antibodies transplacentally from their mothers. Only two symptomatic babies turned out to be PCR-positive and must be regarded as HIV-positive. Virus isolation failed in all 10 cases. 4 of the PCR-negative babies could be followed up, losing their antibodies at least after one and a half years. The preparation of sperm samples includes parallel preparations with proteinase K and GTC-buffer to yield DNA and RNA. Including reverse transcription we observed 16 samples out of 24 to be 'PCR-positive'. The method used before revealed three out of three samples to be negative. All sperm-samples were negative in virus isolation. Two patients, positive for virus isolation from the blood remained negative in the PCR performed with the sperm sample.

HIV-PCR is recommended: 1) in patients with equivocal serological results, 2) in patients suspected to be freshly infected, 3) in newborns born of HIV-seropositive mothers and 4) in sperm samples, when artificial insemination is planned.

Region critical for monocytotropism is concerned in diversification of HIV-1 strains derived from a unique infectious source

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In 1990 eight hemophilia B patients were infected with HIV-1 from one clotting factor lot. In spite of the abundant virus replication soon after infection, the homogeneity of the infecting virus population could be proved by the high homology of the hypervariable regions V1 and V2 in the *env* gene from seven patients at the time of seroconversion[1]. The following sequence analysis (5–13 months after seroconversion) of the V1/V2 region showed that there was only limited variability in the early latent phase of infection[2]. To determine the diversification in the highly functional V3 loop, we sequenced this region in the proviral *env* gene of five patients 16–24 months after seroconversion. Here also the predominant sequences displayed a high degree of homology. The few observed deviations within the V3 loop occurred in a region which is known to be important for monocytotropism. Especially the position 308 displayed multiple variations, i.e. the amino acids His, Asn or Ser in different patients. However, changes in positively charged amino acids critical for the loss of monocytotropism did not occur. In addition, the amino acid at position 305 of the V3 loop was Arg in four patients' (B, D, E, G), but Lys in patient F. In agreement with these results HIV-1 could be cultured from the monocytes of three patients and non- or only weakly-cytopathogenic strains were irregularly isolated from the lymphocytes. Moreover, the viral load in the patients blood was very low as evaluated by quantitative PCR. The results suggest that (1) interpatient variability is still limited two years after infection from a homogeneous source, and (2) sequence variations within the V3 loop occur in a region critical for cell tropism.

1 Kleim, J. P., Ackermann, A., Brackmann, H. H., Gahr, M, Schneweis, K. E., AIDS Res. hum. Retrovir., *7* (1991) 417–427.
2 Kasper, P., Kaiser, R., Kleim, J. P., Oldenburg, J., Brackmann, H. H., Rockstroh, J. Schneweis, K. E., AIDS Res. hum. Retrovir., *9* (1993) 153–153.

Towards routine diagnosis of hepatitis B virus DNA

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Methods for the detection of nucleic acids have added valuable tools to the diagnosis of viral infection. Compared to established methods such as immunoassays or culture techniques nucleic acid detection is either the more direct approach or faster. Since the availability of amplification techniques, the sensitivity of nucleic acid assays has been tremendously enhanced, making the detection of 1 to 10 specific nucleic acid polymers per assay possible. Whereas the amplification reaction itself

can be done within 1 to 3 h, the preparation and extraction of samples and the detection of the amplified products is tedious, allows the throughput of only a few samples and can take two days.
We describe a method for the detection of hepatitis B virus DNA in serum with a homogeneous sample preparation and an ELISA like detection reaction on Boehringer Mannheim ES Systems.

The automated step comprises:
1) Hybridization of denatured DNA labelled with digoxigenin with a biotinylated capture probe.
2) Bound/free separation on a streptavidin solid phase.
3) Binding of peroxidase labelled anti-digoxigenin-antibody.
4) Quantitative detection via ES-substrate colour development.

Turn-round time is 3 h at 37 °C incubation temperature. By using an ES 300 instrument a maximum of 300 samples can be processed per day within 2 instrument runs.

Differentiation of the five serovar groups of the *Mycobacterium avium/intracellulare* complex by hybridization with oligonucleotides specific for subspecies

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In addition to our well adapted method of differentiation of mycobacteria, we introduced a molecular biological protocol via PCR and selective dot blot hybridization in our laboratory. To establish the method, we started analysing amplified 16S rDNA by hybridization with specific oligonucleotides of 7 strains from different serovar groups of the *Mycobacterium avium/intracellulare* complex. We chose a washing buffer that allowed hybridization selective down to one bp difference without elaborative analysis of T_m values. The procedure consists of five steps.

1) Amplification by PCR with a universal 5′ primer and a genus specific 3′ primer yielding a PCR product exclusively from mycobacteria.
2) Dot blot transfer of PCR products on nylon membranes.
3) Hybridization of Dig-11-ddUTP labelled oligonucleotides specific for the mycobacterial species, allowing differentiation down to the subspecies level in some cases.
4) Stringent washing with buffer containing tetramethylammonium chloride, which eliminates the dependence of the T_m values on the base composition of the probe. Additionally, by a given hybridization temperature appropriate for the length of the oligonucleotide, non-specific adsorption of the probes to solid supports is suppressed, resulting in lower non-specific backgrounds.
5) Non-radioactive chemoluminescent detection of hybridized probes using a commercially available detection kit (Boehringer, Mannheim, Germany).

For each of the strains examined, we found a unique hybridization pattern with the specific oligonucleotides used. The corresponding PCR product of a number of strains belonging to a total of 23 other mycobacterial species did not hybridize under the assay conditions outlined above.

Highly sensitive diagnosis of *Bordetella pertussis* DNA

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The 'gold standard' for the detection of *Bordetella pertussis* is the culture of bacteria. Usually a Bordet-Gengou or a Regan-Loewe medium is used, requiring immediate inoculation and incubation. Isolation and identification of *B. pertussis* with this method takes many days and often yields false negative results. Reliable detection of the causal agent is generally possible in the catarrhal stage. We describe a highly sensitive and quick method for detection of *B. pertussis*. With the help of the polymerase chain reaction a specific 183 bp DNA fragment was identified. The area of DNA is a repetitive sequence with 50–100 copies per cell and has a total length of 1.1 kbp. We compared three different methods of detection after PCR amplification: Enzymun-Test (Boehringer, Mannheim, Germany), PAGE with silver staining, and agarose gel with UV detection. For determination of the detection limit a positive control [target sequence cloned in plasmid KS+, (Stratagene)] was diluted from 1 ng to 10^{-8} ng and amplified in a PCR using a reaction mixture containing digoxigenin labelled primer (dig-Per1) and unlabelled primer (Per3rev). After the first amplification, detection was made with the Enzymun-Test. A biotinylated capture probe for hybridization of the denatured DNA frag-