infection was proved by detecting B19 DNA in the sera using polymerase chain reaction (PCR) and DNA obtained by proteinase K digestion and phenol-chloroform extraction. However, B19 DNA was also found in 3/69 anti-B19 IgM negative, HIV-infected hemophiliacs (all three patients in CDC [CDC: centers for disease control] stage IV).

The observations suggest that B19 is still transmitted by clotting factors treated for virus inactivation and that reinfection can occur. As far as viremic immunocompromised patients are concerned, persistent infection must be considered. Recently, we introduced a new method for detection of B19 by PCR. Magnetic beads coupled with protein G purified IgG from sera with high levels of anti-B19 antibodies were incubated with the specimen. After magnetic separation the sample was heat denaturated in PCR buffer and the supernatant was used as the substrate in the PCR reaction. This technique proved to be useful because it is time saving, avoids handling of toxic agents and allows the investigation of larger volumes of the specimens.

An improved method for detection and differentiation of fungi in clinical specimens using polymerase chain reaction (PCR)

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Diagnosis of fungal infections is increasingly important in the work of clinical laboratories. Rapid and accurate diagnosis will help the clinician to initiate early and appropriate treatment.

We have developed an improved method for processing and detecting fungi in clinical specimens using the polymerase chain reaction (PCR) methodology. Although there are now approaches to diagnose some specific fungi using different target structures, there are only two which use 18S rRNA as a target[1,2]. We have found that these primers are not specific for fungi.

The rRNA primers designed in our laboratory allowed for the first time the detection of fungi in clinical specimens using broad specificity fungal primers. The primers were tested for sensitivity and specificity. For further differentiation we sequenced a highly variable region of the amplicons.

1 Hopfer, R. L., Walden, P., Setterquist, S., Highsmith, W. E., J. med. vet. Mycol. *31* (1993) 65.

2 Bowman in: Diagnostic Molecular Microbiology, Principles and Applications, pp. 423–430. Eds Persing, D. H., Smith, T. F., Tenover, F. C., White T. J. ASM, Washington DC 1993.

Identification of pathogenic bacteria in fresh and embedded human biopsies by amplification of 16S-rRNA gene fragments

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Sequencing of amplified 16S rRNA genes allows the identification and the phylogenetic classification of bacteria. As living bacteria are not required, this is the method of choice for the analysis of as yet uncultivable or dead bacteria. However, when biopsy specimens were examined, efficient amplification was hampered by the small amount and the poor quality of the DNA extracted from embedded tissues. We have developed a procedure based upon enzymatic lysis, mechanical disruption and phenol-chloroform extraction of deparaffinized tissues, which yielded DNA of sufficient quality for amplification. Nevertheless, only short DNA fragments ($\leq$200 bp) were amplified using genus- or species-specific primers to exclude co-amplification of contaminating bacteria.

By using this method on a series of biopsies, where bacteria have been detected by light microscopy, we were able to identify *Tropheryma whippelii* and *Mycobacterium genavense* in a number of specimens.

Advances in HIV-PCR in respect to the different fields of diagnosis

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We examined 606 HIV-1 antibody-negative and 211 antibody-positive blood samples from hemophiliacs and their relatives, the latter mostly negative for virus isolation from blood. In addition, we investigated blood samples of 10 babies born of HIV-1-positive mothers and 24 sperm samples from 17 HIV-1 seropositive men prepared for artificial insemination. Examinations were usually carried out with DNA prepared from whole blood. When we established our nested PCR we used

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