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