important. PCR complements traditional serological and other detection methods by allowing discrimination between and detection of specific nucleic acid sequences. In viral hepatitis for example, PCR is used to distinguish between viremic and non-viremic infection, and to monitor viral replication in patients undergoing interferon therapy. However, PCR analysis of biological samples such as blood and other body fluids, is often unreliable due to the presence of amplification inhibitors. These inhibitors are often difficult to separate from the nucleic acids, requiring long and inconvenient purification procedures for their removal. Even then, amplification may be compromised.

The authors have developed a fast, safe and easy protocol to efficiently purify viral nucleic acids from serum for reliable PCR. It is shown, that the sensitivity of the PCR strongly depends on template quality and that our new extraction method provides better quality nucleic acids than standard acid-phenol extractions. Data are presented demonstrating that RNA from as little as 0.01 µl HCV (Hepatitis C virus) positive serum can efficiently be amplified after purification by our procedure. The new procedure combines the handling advantages of spin-column technology with the ability of silica to specifically bind nucleic acids. After sample lysis in a special lysis buffer containing a chaotropic salt, samples are loaded onto a spin-column by microcentrifugation. Nucleic acids are selectively bound to a silica membrane, and contaminants are washed away by two brief washes. Purified nucleic acid is then eluted in water or buffer, ready for direct addition to the PCR reaction. Moreover, this new method requires less hands-on time, uses no organic solvents or alcohol precipitations and minimizes the danger of contamination by infectious agents. It is ideal for simultaneous handling of multiple samples, enabling the preparation of 24 samples within 30 min. So far the method has successfully been tested for samples as different as fresh, frozen or dried whole blood in the presence of all common anti-coagulants, plasma, serum, buffy coat, bone marrow, mucus, cell suspensions, urine and tissue.

Molecular typing and epidemiology of *Borrelia burgdorferi* sensu latu

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Borrelia burgdorferi, the Lyme disease agent, has long been considered a homogeneous species. However, recent evidence based on DNA-DNA hybridization, RFLP-analysis, plasmid profiles, comparative 16S rRNA analysis, and typing with different monoclonal antibodies has led to the separation into three species, namely *B. burgdorferi* sensu strictu, *B. garinii*, and *B. afzelii*.

Direct solid-phase sequencing of in-vitro amplified 16S rRNA gene fragments was used for the typing of 67 *Borrelia* strains isolated from ticks and patients. Biotinylated PCR products were bound on paramagnetic particles, single strands were generated by alkali treatment and isolated by magnetic separation. Single stranded DNA was sequenced by modified Sanger dideoxy nucleotide sequencing.

All isolates showed a species-specific epidemiological distribution. *B. afzelii* was predominantly found in skin biopsies, but was, however, rarely isolated from ticks. To see whether ticks were harboring more than one *Borrelia* species, we have developed several sets of species-specific amplification primers to test for the prevalence within different tick populations.

Detection of parvovirus B19 specific antibodies and DNA in sera of hemophiliacs

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It is known that parvovirus B19 (B19) is transmitted to hemophiliacs by clotting factors prepared from human plasma. However, it is not clear whether B19 is also transmitted by the more recently used inactivated clotting factor preparations. Therefore, we investigated 69 hemophiliacs, mostly children, receiving only clotting factors treated for virus inactivation. 49 of them (71%) were B19 IgG-positive and 18 of the IgG positive hemophiliacs (37%) were also B19 IgM-positive. In contrast, out of 73 age-matched controls only 10 (13%) were IgG-positive, two of them being also IgM-positive. In hemophiliacs treated before 1984 with non-inactivated clotting factors, seroprevalence was very similar: 115/161 (71%) presented B19 IgG and 30/115 (26%) IgM antibodies as compared to their age-matched controls with 16/50 (32%) B19 IgG and no IgM antibodies. In 4 out of 30 IgM positive hemophiliacs, active B19

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