(ATI-gene) was developed to distinguish cowpox virus from other orthopoxvirus species. For this purpose, two primer pairs were designed, with one primer of each pair radioactively labelled. The discriminating bases are located at the 3'-end of the upper upstream primer (TT), at the 5'-end of the lower downstream primer (A), and at the 3'-end of the lower upstream primer (A), forming a one base overhang at the ligation site. Two mismatching bases (GG) were added at the 3'-ends of the upstream primers to prevent ligation at this end. Detection of the cowpox ligation product was achieved by autoradiography after denaturing polyacrylamide gel electrophoresis. To increase the sensitivity, the corresponding region of the ATI-gene was initially amplified by consensus primer detected polymerase chain reaction (PCR) prior to LCR.

All 9 cowpox viruses examined could be clearly discriminated from reference strains of camelpox virus, mousepox virus and monkeypox virus, as well as seven vaccinia virus isolates. The PCR-coupled LCR was shown to be a reliable tool for the screening of cowpox virus infections, which is of particular importance with respect to the increasing number of unusual hosts.

To our knowledge, this is the first report of a LCR that uses a double nucleotide deletion. This LCR does not require any fill-in reaction (substitution of bases) and is not limited to the detection of base pair changes from A-T/T-A to G-C/C-G or vice versa.

Identification of Epstein-Barr virus transactivator BZLF1 mRNA in uncultured peripheral blood lymphocytes by RT-polymerase chain reaction

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The cascade of gene expression during the lytic replication of *Epstein-Barr* virus (EBV) is initiated by three immediate early regulatory proteins. Based on a nucleic acid detection method for one of these factors, the proof of active EBV replication is possible even before the onset of serological markers and will therefore be of great value for diagnosis and treatment. For early detection of lytic replication in the case of EBV associated diseases, we developed a reverse transcription polymerase chain reaction (RT-PCR), followed by a second 'nested' PCR with primers overlapping the first and second exon coding for BZLF1.

We report detection of mRNA for the immediate early transactivator BZLF1 in peripheral blood lymphocytes (PBL) of donors with reactivation of EBV and chronic infection (CAEBV). No transcripts were detected in PBL of normally latently infected individuals of EBV-negative BJAB cells.

The significance of this observation for pathobiology, diagnosis and treatment of clinical forms of EBV infections like infectious mononucleocleosis (IM), CAEBV, chronic fatigue syndrome (CFS) or reactivation of latent EBV and rejection of organ transplants needs further evaluation and will be discussed.

Dependence on the taxon composition of clone libraries for PCR amplified, naturally occurring 16S rDNA, on the primer pair and the cloning system used

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Recent advances in molecular techniques have allowed the determination of the genetic variety of microbial inhabitants in the environment. Encouraging progress has been made in the elucidation of as yet undetectable biodiversity in different natural samples, such as marine environments, forest soil, acidothermal soil, hot spring environments and the environment of a continuous bioleach bioreactor. Despite the novelty of the results, certain problems associated with the methods applied to quantitatively describe the microbial populations have been recognized. Unsolved problems in the cloning strategy include: 1) the recovery of cells and/or nucleic acids from the environment, 2) the conditions under which PCR amplification of the target genes is performed, including selection and specificity of primers, the dependence of PCR amplification on the quality of DNA and the possibility of capture of *Taq* polymerase by abundantly occurring DNA, 3) the influence of the cloning strategy and cloning vectors, such as shotgun cloning (minute percentage of DNA are rrn operons), sticky end cloning (presence of restriction sites within the rrn operons may prevent cloning of the amplified product), blunt end cloning (low transformation efficiency), and clonability of heterologous rDNA, 4) the detection of clones by sequence analysis

and/or by colony-, dot or PCR multiplex hybridization, and 5) the lack of reference sequences that may erroneously suggest the taxonomic novelty of a clone. Elucidation of the composition of a bacterial community occurring in a natural sample was attempted by dot blot hybridization of 16S rDNA clone libraries with taxon-specific oligonucleotide probes. The composition changed significantly when the same batch of isolated DNA and the same cloning vector, but two different pairs of amplification primers were used. The distribution of taxon-specific clones was also different from that obtained previously using one of the same primer pairs but a different cloning system. The results indicate that our present knowledge of this approach allows neither the complete qualitative nor the accurate quantitative determination of microbial community compositions.

PCR-based detection of *Mycobacterium tuberculosis* in sputum samples using a simple and reliable DNA extraction protocol

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Several procedures to release DNA from acid-fast mycobacteria for polymerase chain reaction-based amplification have been reported. Nevertheless, up to now there is no extraction method available which is simple and reliable enough to allow its application in routine clinical practice.

We present a rapid, simple and reliable protocol for the extraction of mycobacterial nucleic acids as template molecules for a subsequent polymerase chain reaction. Samples were suspended in extraction buffer and subjected to several cycles of freezing in liquid nitrogen and heating in a boiling water bath. After the treatment, the rigid cell wall of the mycobacteria was cracked and DNA could be reliably amplified from the supernatant. For the evaluation of this procedure we used serial dilutions of liquid culture. Additionally, PCR was capable of detecting mycobacteria in sputum samples from 13 out of 13 patients with clinically suspected tuberculosis which were positive by smear and culture. Amplified DNA products were characterized both by length and direct sequencing. Using PCR primers which hybridize to a conserved sequence that flanks a hypervariable region in the 16S rRNA gene of mycobacteria, we were able to distinguish even between distinct mycobacterial species by determining the nucleotide sequence of the amplification products.

In 15 smear- and culture-negative cases without suspected tuberculosis, PCR led to negative results. The routine applicability of this new extraction protocol for nucleic acid from mycobacteria will be further evaluated.

Specific detection of *Mycobacterium avium* using DNA amplification

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Infections caused by the *Mycobacterium avium*complex (MAC) contribute substantially to morbidity and mortality in patients with AIDS, and the prevalence is increasing. There is an urgent need for methods that can detect this pathogen more rapidly and directly in body fluids.

We have evaluated a PCR amplification method based on the DNA probe sequence by Fries et al. (insert pMAv22)[1]. To improve specificity of PCR, a new primer was selected (bases 37–56) and used together with the described primer Mav 22B in order to amplify a 148 bp sequence specific for *M. avium*. A 94 bp peroxidase labelled probe produced with the primers Mav22A and B was used for Southern blot hybridization and assayed by means of enhanced chemiluminescence. The sensitivity of the assay was determined by using *M. avium* DNA in the presence of 0.5 µg human genomic DNA or blood spiked with different amounts of bacteria. While the detection limit was 5–10 fg, 10 bacteria/ml blood were detectable in spiked specimens. No bands were visible if the PCR assay was tested with 1) 23 of the most common mycobacteria other than *M. avium* (including 3 clinical isolates of *M. intracellulare*, determined by direct sequencing of a hypervariable gene region for the 16S rRNA and the strains *M. intracellulare* serovar 7, 13, 14 and 17 so far tested), 2) DNA from 10 common pathogens of the human respiratory tract and 3) human genomic DNA from 30 various clinical specimens, all culture negative for mycobacteria. We routinely use this method for the identification of *M. avium* in mycobacterial cultures, because it is rapid, specific, easy to perform and very sensitive. All cultures ($n = 30$) diagnosed as *M. avium* by conventional methods and the above-mentioned sequencing thus far (except for the above mentioned *M. intracellulare* isolates),