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),