including the serovars 1–6 and 8–9 tested, proved to be positive with this PCR method. Further clinical studies are warranted to evaluate the applicability of this assay for the direct and early detection of this mycobacterium directly from clinical specimens.

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Rapid PCR detection of *Mycobacterium tuberculosis* in sputum samples

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The diagnosis of *Mycobacterium tuberculois* (MT) using conventional microbiological methods is still a time-consuming process which, particularly in regard to the rise of cases of drug-resistant mycobacteria, needs to be shortened. PCR is a suitable method, because of its independence of the growth of the organism. The aim of this study was to develop a PCR method feasible for a routine laboratory. Sputum samples were liquefied, decontaminated and concentrated by standard methods. The mycobacteria were lysed by using a combined treatment of sonication and heat incubation in presence of a detergent. The lysate containing the genomic DNA was added directly to the PCR (without prior phenol/chloroform extraction), and the PCR performed in two steps. First, for screening, we performed a genus specific PCR on 49 sputum samples (10 MT culture-positive, 39 MT culture negative) using primers published by Hance et al.[1] The 383 bp PCR products were separated on agarose gel followed by a dot-blot hybridization with a digoxigenin labelled probe. Compared to the culture, the sensitivity of this PCR was 90% (one false-negative) and the specificity 89.7%, whereby the four culture-negative but PCR-positive samples were from confirmed MT-infected patients undergoing chemotherapy. Secondly, the same samples were tested in a PCR using primers specific for MT-complex described by Eisenach et al.[2]. The 123 bp PCR products were separated on agarose gel. The sensitivity of this PCR was 60% and the specificity 97.4%. The detection level of both PCRs was 10^2 cells/ml.
These results indicate that this two-step-PCR can be a useful adjunct to conventional methods for the rapid diagnosis of MT in sputum.

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Comparative 16S rRNA analysis for the identification of slow-growing and uncultivable bacteria from activated sludge

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So far, analysis of complex microbial ecosystems has been based on culture and subsequent biochemical identification. However, cultivable microorganisms represent only 10% of the entire microbiota. Molecular genetic analysis has been applied to identify and phylogenetically classify all members of a microbial community without prior cultivation.
Activated sludge samples from a wastewater treatment plant (Berlin-Marienfelde) were analyzed by culture and molecular genetic tests for the presence of nocardiform actinomycetes and mycobacteria. First we isolated bulk DNA from which 16S rRNA gene fragments were selectively amplified by using well established 16S rRNA amplification primers. Amplicons were ligated into plasmid pUC 19 and used to transform competent *E. coli* cells. About 3000 clones of the entire 16S rRNA clone library were analyzed by colony hybridization using a probe specific for 16S rRNA from mycobacteria and nocardiform actinomycetes. Positive clones were subjected to comparative sequence analysis and were classified phylogenetically. Only one sequence corresponded to known, cultivable bacteria. By using genus-specific primers for nested-PCR it was possible to amplify mycobacterial 16S rRNA genes directly from the original bulk-DNA.

Determination of the individual efficiency of amplification by means of PCR-MIMIC's

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PCR-results may be false positive, caused by contaminations, or false negative. In the latter case inhibitors diminish an optimal amplification. False negative results can be detected using probe-inherent single-copy genes or by spiking with template DNA. Negative PCR-results are only valid in combination with proper controls. Such controls give no information about the amplification efficiency. By the use of internal standards

in the same reaction vial together with the template, amplified copy numbers can be quantified. We have constructed a standard for the PCR-detection of the insertion element IS 6110 of *Mycobacterium tuberculosis*. The PCR-MIMIC construction kit of Clontech was used. In this reaction the same primers amplify a template sequence of 123 bp and the standard of 360 bp. The standard is added in 3 different concentrations (6, 60, 600 molecules per sample), the probe is amplified and the products are separated, blotted and detected with enhanced chemiluminiscence.
The results show that the lower detection limit is not constant. In some experiments only 600 molecules of the standard were detectable. This varying efficiency may be caused by inhibitors within the individual sample. Especially in the case of negative results, one has to consider this phenomenon. The use of internal standards gives information concerning the lower detection limit and prevents false negative results.

Identification and characterization of medically important *Candida* species by using PCR-fingerprinting

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A PCR fingerprinting assay was applied to identify and to characterize the genetic relatedness among medically important *Candida* species. By using different primers of arbitrary nucleotide sequence in the polymerase chain reaction, distinctive and reproducible sets of amplification products were observed for reference strains of all *Candida* species tested. Primers were chosen which generated only little if any intraspecies DNA polymorphisms. DNA was extracted from yeasts by a minipreparation method. PCR protocols used for different primers were optimized with regard to primer concentration, amount of template DNA, annealing temperature and cycling program. Amplified products were separated by electrophoresis in agarose gels and detected after straining with ethidium bromide. The PCR patterns were evaluated by a laser densitometer using an appropriate computer software.
Comparing the PCR profiles obtained from clinical isolates with those yielded from reference strains it was possible to identify different *Candida* species even if they were not typable by the conventional biochemotyping. Furthermore, this PCR technique allowed to establish the genetic relatedness of distinct *Candida* species and might therefore be, useful for phylogenetic and taxonomic studies within this genus.

Detection of DNA polymorphisms in *Leishmania* species by using single primers in the polymerase chain reaction

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DNA polymorphisms were assessed in different *Leishmania* species by amplifying genomic DNA with single primers of arbitrary nucleotide sequence. The method is simple and fast. It requires only small amounts of DNA and can be applied to any species from which DNA can be prepared. No prior sequence information is required.
Primers used as hybridization probes in conventional DNA fingerprinting or reported by other authors were tested for their applicability in this PCR fingerprinting technique. Reaction conditions were optimized for each primer. The number, reproducibility and intensity of bands in a fingerprint should be a function of several parameters, including the concentration of salts, primer, annealing temperature, template concentration, primer length and primer sequence. PCR products that are not shared among all strains act as polymorphic markers. As each primer gave a different pattern of AP-PCR products, the data allow the differentiation of closely related strains even of the same species.
The amplification products were electrophoresed in an agarose gel and photographed after staining with ethidium bromide.
Several primers were found which enabled the detection of variable DNA sequences in different *Leishmania* species in this PCR approach. The PCR patterns could be used for species identification in *Leishmania* isolates. Using an appropriate computer software this method is well suited for the determination of genetic relatedness among *Leishmania* species and can be applied to phylogenetic and taxonomic studies.