GCTCAG-3′) and 530r (5′-GA/TATTACCGCGGCG-GCTG-3′). PCR amplification was performed in a total volume of 100 µl, containing 0.5 µg of each primer, 10 µl of 10 × reaction buffer (Boehringer Mannheim), 1 mM (each) dATP, dGTP, dTTP and dCTP, and 100 ng of each bacterial DNA. The reaction mixtures were incubated in a thermal cycler for 3 min at 98 °C. Two units of Taq Polymerase (Boehringer Mannheim, Germany) were added and the reaction was performed for 28 cycles at 55 °C for 1 min, 72 °C for 2 min, and 93 °C for 1 min. The final cycle was an elongation step at 72 °C for 5 min.

Image analysis. 5 µl of the PCR solutions were loaded onto a vertical gel, consisting of 3% Metaphor agarose (FMC, Rockland, Maine). Gels were run at 5 V/cm for 14–16 h at 4 °C, and stained with SYBR green (Molecular Probes, Ca.). Gel images were converted to digitized files and analysed by Image Quant 3.3 (Molecular Dynamics).

Results and conclusion

As determined by image analysis of SYBR green-stained amplification products the experimentally determined ratio corresponded well with the expected ratio calculated from the number of *rrn* genes per equimolar amount of DNA in mixtures containing DNA of *Escherichia coli* and '*Thermus thermophilus*' and DNA of *Pseudomonas aeruginosa* and '*T. thermophilus*'. The values for the pair *Bacillus subtilis* and '*T. thermophilus*' showed higher deviation from the predicted value. The dependence of the amount of 16S rDNA amplification products on these two parameters makes it impossible to quantify the number of species present in 16S rDNA clone library of an environmental sample, as long as these two parameters are unknown for these species.

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Simple 'universal' DNA extraction procedure compatible with direct PCR amplification

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Large scale use of amplification methods in diagnostic laboratories requires efficient but simple DNA extraction procedures compatible with various types of clinical specimens. A combination of SDS and proteinase K has been widely used for solubilizing tissues as well as for the extraction of DNA from microorganisms. However, SDS is inhibitory for *Taq* polymerase at any useful concentration[1] and, therefore, does not allow direct amplification without extraction with organic solvents and ethanol precipitation. We have used Tween 20 to neutralize the activity of SDS on *Taq* polymerase in the amplification mix, resulting in a simple and rapid extraction procedure applicable to a variety of clinical specimens which does not require further purification steps.

Tissues (disintegrated mechanically), sputum (liquefied and centrifuged), liquid specimens (centrifuged) or bacterial cultures were incubated with digestion buffer (50 mM Tris-HCl, pH 8.5, 1 mM EDTA, 0.5% SDS, 200 µg/ml proteinase K) at 55 °C for 3 h or overnight (tissue) followed by heating at 95 °C for 10 min and transferring a maximum of 10 µl directly into the amplification tube. Amplification was in a final volume of 100 µl containing 2% (v/v) Tween 20. This extraction procedure has successfully been used in conjunction with various PCR systems including amplification of *Rochalimaea (Bartonella) henselae* and *R. quintana*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, and *Legionella pneumophila*.

The sensitivity of the procedure was determined using a eubacterial amplification system[2]. Ten µl of the amplified material were analyzed by gel electrophoresis and ethidium bromide staining without hybridization: the equivalent of between 7 (gram-negative bacteria) and 600 (gram-positive bacteria, *Mycobacterium fortuitum*) organisms per amplification was reproducibly visible on the agarose gels. We conclude that DNA extraction with SDS and proteinase K is simple, rapid, applicable to large numbers of specimens, and allows direct amplification without further purification if Tween 20 is included in the amplification mix. It is also compatible with the use of uracil-N-glycosylase (UNG) for the prevention of false positives due to amplicon carry-over (contamination control).

1 Kawasaki, E. S., in: PCR Protocols: A guide to Methods and Applications, pp. 146–152. Eds M. A. Innis, D. H. Gelfand, J. J. Sninsky and Th. J. White. Academic Press, San Diego 1990.
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Detection of *Aspergillus fumigatus* by polymerase chain reaction (PCR)

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Invasive aspergilloses (IA) have a poor prognosis, but it is known that early diagnosis and treatment result in a more favourable outcome. PCR could contribute to a rapid diagnosis. In this study we developed a PCR to detect *Aspergillus fumigatus* and assessed the application to clinical specimens.

The primers were designed based on the published cDNA sequences of the cytotoxin Asp f1, because

Arruda et al. suggest that this protein is the major antigen found in patients with IA and therefore could be a specific virulence factor (see fig.).

AF7	5'ACC	TGG	ACA	TGC	ATC	AA3'	
AF8	5'CAC	AGT	CTC	AAG	TCT	CC3'	
AF9	5'CAA	CAG	CTG	AAT	CCC	AAG	AC3'
AF10	5'CCA	CAA	TGC	CGC	AAA	ACA	CC3'

Figure. Primers 1 and 2 amplify a fragment of 437 bp, 3 and 4 a fragment of 382 bp.

Then isolation, amplification and detection of *A. fumigatus* DNA was optimized using phenol extraction followed by nested PCR or hybridization assays. Both methods are sensitive enough to detect 5 pg of *A. fumigatus* DNA. The authenticity of the amplicon was verified by its correct size on agarose gel analysis after digestion with different restriction enzymes, and direct sequencing performed on both DNA strands. Amplification was specific for *A. fumigatus* since we failed to detect any amplicons of various other fungal species (*A. niger*, *A. flavus*, *P. chrysogenum*, *C. albicans*), of bacteria (*E. coli*, *P. aeruginosa*, *S. epidermidis*) or of human DNA.

All 27 tracheobronchial secretions with positive cultures of *A. fumigatus* from eight patients with proven or probable IA were positive in the PCR. Two patients with a positive antigen test in serum samples and negative cultures in eight tracheobronchial secretions also remained negative in the PCR. These patients had no clinical evidence of IA. One patient who had a positive antigen test but negative cultures showed a positive PCR result in all six tracheobronchial secretions. *A. fumigatus* was then cultured in the last tracheobronchial sample before the patient died. Our technique was also used in 48 serum samples from these patients, but we never got a positive result.

The PCR technique described here appears to be suitable for the detection of *A. fumigatus* in specimens from the respiratory tract.

One of the main limitations to routine diagnostics is that the method itself is laborious and it takes about 2 days to obtain a result. Moreover, trained personnel and experience are necessary to minimize the high risk of contamination with conidia of *Aspergillus* spp., which are widespread in the environment.

Another important consideration is the possibility of colonization of the respiratory tract healthy subjects. This means that the results of a technique as sensitive as the PCR can only be interpreted in the complete clinical context of the patient.

In conclusion, we think that if patients are strongly suspected of having IA but other laboratory methods such as culture, antigen and antibody detection remain negative, it could be advantageous to have another method of diagnosing IA.

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Molecular identification of *Streptomyces albidoflavus* strains

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In order to determine the possibility of discriminating between the phenotypically similar strains of *Streptomyces albidoflavus*, the 16S rDNA of four strains was analyzed. The sequences were found to be identical. The discriminatory potential of the intergenic spacer region between 16S and 23S rRNA has been previously described[1] – these regions show a higher degree of variability than is detected in the 16S rRNA gene. As sequence analysis of the spacer region revealed that there is more than one spacer copy, the intergenic spacer was cloned and sequenced. Regions coding for transfer RNAs were not found within any of the spacer copies of these strains. Due to the small size differences (in some cases, 2–3 base pairs), it was not possible to separate the different spacer fragments by conventional agarose gel electrophoresis. In order to detect possible differences, the intergenic spacer region of each *S. albidoflavus* strain was examined using a novel rapid approach, which allowed high resolution of fragment size. The DNA of the spacer region was amplified by PCR, using a FAM-labelled terminal 16S rRNA primer, and an unlabelled terminal 23S rRNA primer. The PCR products were electrophoresed on a 6% polyacrylamide gel (12 cm long) using an Applied Biosystems 373A DNA sequencer, run set up of 2500 V, 45 mA and 30 W for 4 h. Fragment size was determined by comparison with a ROX-labelled internal standard using GeneScan 672 software (Applied Biosystems). Different numbers and sizes of the intergenic spacer region fragments were observed, showing a characteristic pattern for each strain type. These data indicate that it is possible to discriminate rapidly between these strains using this molecular method. Within the four *S. albidoflavus* strains investigated, three distinct spacer patterns could be determined. In addition, analysis of eight species believed by some workers to be synonyms of *S. albidoflavus* revealed six additional spacer types.

Our investigation was subsequently extended to include more strains of *Streptomyces albidoflavus*, and 47 other species of the genus *Streptomyces*. The results show the uniqueness of spacer patterns at the strain level in *S. albidoflavus*. In order to determine the reproducibility of the results, we selected 12 *S. albidoflavus* strains for spacer amplification, which was performed independently three times. Analysis showed the same composition of the spacer fragments for the three replicates of each strain.

We could also demonstrate that different species of *Streptomyces* have intergenic spacer regions varying