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

greatly in size (311 to 366 base pairs) and number (1 to 5 bands). Further investigation of a greater number of strains from each species is required in order to determine whether the pattern obtained for a given species is truly characteristic of that species.

The results of our study suggest that fragment analysis of the intergenic spacer region is a powerful tool for the rapid identification of highly related strains. The potential of this method in species differentiation will be further investigated.

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Detection of human cytomegalovirus (HCMV) DNA from cell-free plasma of immunosuppressed patients by nested PCR: influence of nucleic acid extraction method

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Human cytomegalovirus (HCMV) is an important causative organism for life and sight-threatening opportunistic infections in immunocompromised hosts, especially recipients of bone marrow or solid organ transplants and patients with AIDS. Diagnostic methods currently used for HCMV-monitoring include rapid viral isolation (detection of the 72 kD immediate early antigen in cell culture), the antigenemia assay (detection of pp65 in peripheral leukocytes) and HCMV DNA PCR from peripheral blood leukocytes. Detection of HCMV in blood cell samples is an early diagnostic marker of imminent viremia, which is associated with HCMV disease. However, there is growing evidence of the low predictive value of HCMV DNAemia as a marker of symptomatic infection[1,2]. Although detection of HCMV mRNA by RT-PCR for late HCMV proteins holds promise, none of these qualitative methods is able to discriminate HCMV disease from asymptomatic HCMV infection. Next to PCR quantification or RT-PCR, the use of plasma instead of leukocytes as material for HCMV DNA amplification seems to provide better data for the interpretation of positive PCR results.

Methods and results. We compared the results from qualitative HCMV DNA PCR from peripheral mononuclear leukocytes (PBMC) and granulocytes isolated from immunosuppressed patients with results from rapid viral isolation, conventional tube cell culture of these cells or other materials, serology, and HCMV DNA detection from plasma. DNA from blood cell fractions was isolated using a standardized protocol[2] with primer oligonucleotides from the fourth exon of the HCMV-IE1 gene. For the isolation of DNA from cell-free plasma three protocols were chosen. First, HCMV DNA was prepared by a modification of the method of Ishigaki et al.[3]. Secondly, DNA was extracted from thermally inactivated supernatants of proteinase K digestions with phenol/chloroform and precipitation with EtOH. In a third protocol, cell-free DNA was isolated from plasma by precipitation of inactivated proteinase K supernatants with polyethylene glycol (8% w/v PEG 6000) and NaCl (0.7 M) followed by phenol/chloroform extraction. With oligonucleotide primers derived from the DRB1 gene of the major histocompatibility complex (MHC) class II locus we could demonstrate that the DNA isolated from plasma was cell-free. Sensitivity and specificity of DNA amplification was estimated using an DIG-dUTP-dATP-tailed probe for hybridization of amplified products.

Conclusions. Blood cells and plasma preparations from HCMV-seropositive healthy blood donors were all nPCR negative. Detection of HCMV DNA from PBMC and granulocytes (DNAemia) of immunosuppressed patients by nPCR did not correlate with the isolation of infectious virus from these cell populations in cell culture (viremia). However HCMV could be isolated in 60% of cases from other materials of the same patient. HCMV DNA detected in blood cells persisted for up to one year in an asymptomatically infected individual after NTX. The sensitivity of HCMV DNA detection in cell-free plasma (up to 5 fg) depended on the method used for DNA isolation. The rate of HCMV DNA detection in plasma was lower than in leukocytes. In all cases of positive plasma PCR infectious virus could be isolated from any other material of the symptomatically infected patients. Therefore HCMV DNA PCR from plasma of immunosuppressed patients seems to be a suitable and easy alternative to HCMV RT/PCR for routine diagnosis of HCMV disease.

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