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Abstracts

Isolation of mycobacterial DNA for PCR amplification: Are established methods practicable and efficient?

B. Berger, A. Roth and H. Mauch

Institut für Mikrobiologie und Immunologie, Krankenhaus Zehlendorf, Heckeshorn, Zum Heckeshorn 33, D-14109 Berlin (Germany)

The handling of clinical material in mycobacterial infections in order to obtain DNA for sensitive PCR amplification is known to be a difficult procedure. Conventional extraction methods with organic solvents and precipitation are not suitable for routine laboratory purposes. Quick preparations with simple 'one tube' methods (boiling/thawing, alkaline lysis or chloroform treatment) may contain inhibitory substances in most cases. Although commercially available spin-columns are very suitable for mammalian cells, these methods may be more expensive, include more handling steps, and in our experience release of DNA from mycobacteria is not sufficient. We have explored this issue using following approaches 1) influence of glass beads (100 microns), boiling/thawing, alkaline lysis, treatment with chloroform or phenol alone, and simple proteinase K digestion on the release of DNA from mycobacteria, 2) mixing samples that are difficult to handle (sputum, blood) with minimal amounts of mycobacteria, and evaluation of the sensitivity and performance of PCR (65 kD protein gene, 383 bp) comparing different methods, and 3) estimation of inhibitory effects by spiking the preparations with standard template DNA fragments. Release of mycobacterial DNA, in relation to an expected DNA content of 5 fg/mycobacterium, was poor with 'one tube', methods, e.g. proteinase digestion alone (30%), and best using beads (95%). In our experience, failure of DNA amplification with 'one tube' methods occurs frequently. The aqueous supernatant from a more sophisticated 'one tube' extraction procedure which combined proteinase K digestion, shaking with glass beads, and extraction with chloroform was directly applied to PCR, and produced the best results.
Our data indicate that we should be aware of the possibility that too simple 'one tube' methods are not reliable enough in all situations (inhibitors, optimal DNA-release). Insufficient methods in this regard may be a reason for the disappointing results achieved so far with PCR applied to the diagnosis of mycobacterosis.

Identification of new oral spirochetes in a subgingival plaque sample by 16S rRNA sequence analysis

B.-K. Choi and U. B. Göbel

Institut für Medizinische Mikrobiologie und Hygiene, Universität Freiburg, Hermann-Herder-Str. 11, D-79104 Freiburg (Germany)

Oral spirochetes seem to be associated with periodontal disease and are present in increased numbers in lesions of greater severity. Various morphotypes of spirochetes can be observed, but many of these are as yet uncultivable. In order to identify the diversity of oral spirochetes we have analysed the sequence of the cloned 16S rRNA gene, establishing a DNA and a cDNA clone library. DNA and RNA were isolated from the subgingival plaque sample of a patient with severe destructive periodontitis. cDNA was synthesized from rRNA by reverse transcription. From DNA and cDNA, respectively, the fragments of the 16S rRNA gene (approx. 500 bp) were amplified by PCR. The PCR products were cloned into *E. coli* using the plasmid vector pUC 19. For a reliable analysis a great number of randomly selected recombinants (6418) were screened with a spirochete-specific radiolabelled oligonucleotide probe by colony hybridisation. Of 95 spirochetal clones 6.9% were from the DNA clone library, compared to 0.9% from the cDNA clone library. All the spirochetal clones were sequenced and the sequences were compared with 35 spirochete 16S rRNA sequences. The analysis showed an unexpected diversity of oral spirochetes from a single patient. Seven sequences were chimeras.
Fluorescent labelled, species-specific oligonucleotide probes were designed. The probes allowed direct identification of the new species in a plaque sample by in situ hybridization.

A new, easy and safe method for the purification of nucleic acids for reliable PCR

B. Goergen, P. Feuser[a] and R. Hermann[a]

Medizinische Klinik, Johann-Gutenberg Universität, D-55131 Mainz, and [a]DIAGEN GmbH, Hilden (Germany)

The use of PCR for the detection of nucleic acid sequences in biological samples is becoming increasingly