0014-4754/94/090790-10$1.50 + 0.20 © Birkhäuser Verlag Basel, 1994

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

important. PCR complements traditional serological and other detection methods by allowing discrimination between and detection of specific nucleic acid sequences. In viral hepatitis for example, PCR is used to distinguish between viremic and non-viremic infection, and to monitor viral replication in patients undergoing interferon therapy. However, PCR analysis of biological samples such as blood and other body fluids, is often unreliable due to the presence of amplification inhibitors. These inhibitors are often difficult to separate from the nucleic acids, requiring long and inconvenient purification procedures for their removal. Even then, amplification may be compromised.

The authors have developed a fast, safe and easy protocol to efficiently purify viral nucleic acids from serum for reliable PCR. It is shown, that the sensitivity of the PCR strongly depends on template quality and that our new extraction method provides better quality nucleic acids than standard acid-phenol extractions. Data are presented demonstrating that RNA from as little as 0.01 μl HCV (Hepatitis C virus) positive serum can efficiently be amplified after purification by our procedure. The new procedure combines the handling advantages of spin-column technology with the ability of silica to specifically bind nucleic acids. After sample lysis in a special lysis buffer containing a chaotropic salt, samples are loaded onto a spin-column by microcentrifugation. Nucleic acids are selectively bound to a silica membrane, and contaminants are washed away by two brief washes. Purified nucleic acid is then eluted in water or buffer, ready for direct addition to the PCR reaction. Moreover, this new method requires less hands-on time, uses no organic solvents or alcohol precipitations and minimizes the danger of contamination by infectious agents. It is ideal for simultaneous handling of multiple samples, enabling the preparation of 24 samples within 30 min. So far the method has successfully been tested for samples as different as fresh, frozen or dried whole blood in the presence of all common anti-coagulants, plasma, serum, buffy coat, bone marrow, mucus, cell suspensions, urine and tissue.

Molecular typing and epidemiology of *Borrelia burgdorferi* sensu latu

B. Graf, M. Schuppler and U. Göbel

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

Borrelia burgdorferi, the Lyme disease agent, has long been considered a homogeneous species. However, recent evidence based on DNA-DNA hybridization, RFLP-analysis, plasmid profiles, comparative 16S rRNA analysis, and typing with different monoclonal antibodies has led to the separation into three species, namely *B. burgdorferi* sensu strictu, *B. garinii*, and *B. afzelii*.
Direct solid-phase sequencing of in-vitro amplified 16S rRNA gene fragments was used for the typing of 67 *Borrelia* strains isolated from ticks and patients. Biotinylated PCR products were bound on paramagnetic particles, single strands were generated by alkali treatment and isolated by magnetic separation. Single stranded DNA was sequenced by modified Sanger dideoxy nucleotide sequencing.
All isolates showed a species-specific epidemiological distribution. *B. afzelii* was predominantly found in skin biopsies, but was, however, rarely isolated from ticks. To see whether ticks were harboring more than one *Borrelia* species, we have developed several sets of species-specific amplification primers to test for the prevalence within different tick populations.

Detection of parvovirus B19 specific antibodies and DNA in sera of hemophiliacs

A. Große-Bley, A. M. Eis-Hübinger, R. Kaiser, J. Oldenburg[a], H. H. Brackmann[a], T. F. Schwarz[b] and K. E. Schneweis

Institut für Medizinische Mikrobiologie und Immunologie und [a]Institut für Experimentelle Hämatologie und Transfusionsmedizin, Universität Bonn, Sigmund-Freud-Str., D-53127 Bonn, and [b]Max von Pettenkofer Institut für Hygiene und Medizinische Mikrobiologie, Universität München, München (Germany)

It is known that parvovirus B19 (B19) is transmitted to hemophiliacs by clotting factors prepared from human plasma. However, it is not clear whether B19 is also transmitted by the more recently used inactivated clotting factor preparations. Therefore, we investigated 69 hemophiliacs, mostly children, receiving only clotting factors treated for virus inactivation. 49 of them (71%) were B19 IgG-positive and 18 of the IgG positive hemophiliacs (37%) were also B19 IgM-positive. In contrast, out of 73 age-matched controls only 10 (13%) were IgG-positive, two of them being also IgM-positive. In hemophiliacs treated before 1984 with non-inactivated clotting factors, seroprevalence was very similar: 115/161 (71%) presented B19 IgG and 30/115 (26%) IgM antibodies as compared to their age-matched controls with 16/50 (32%) B19 IgG and no IgM antibodies. In 4 out of 30 IgM positive hemophiliacs, active B19