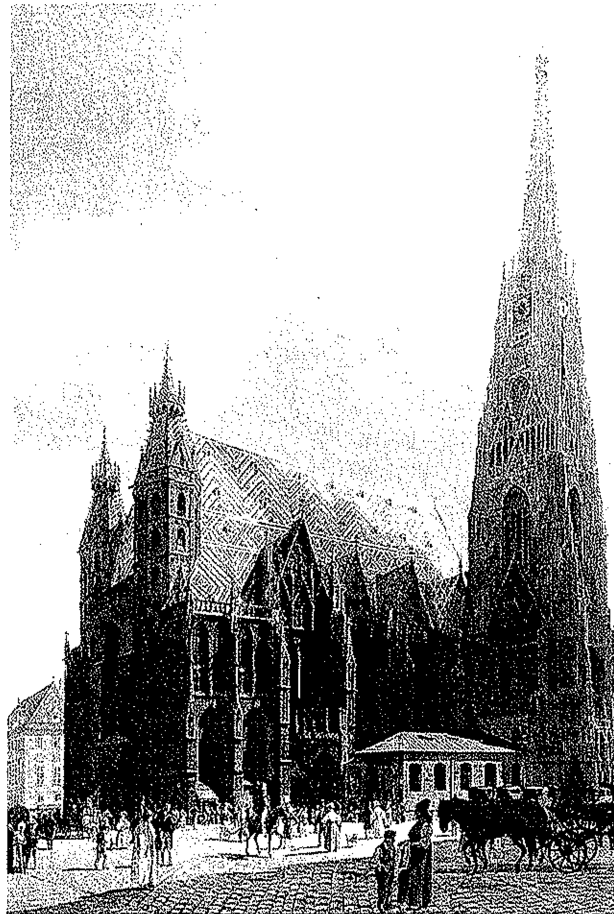
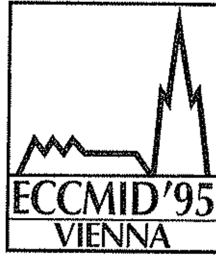
7th EUROPEAN CONGRESS OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

Vienna / Austria, March 26 – 30, 1995



ABSTRACTS



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Under the auspices of

European Society
of Clinical Microbiology
and Infectious Diseases

products generated were visualized by ethidium bromide staining after electrophoresis in a 2% agarose gel.

Four groups of strains were studied: 1) Five consecutive isolates from the same patient; 2) Eight strains involved in two different nosocomial outbreaks; 3) Eight strains from three different clusters; 4) Seventeen non-related strains.

In groups 1, 2 and 3 the analysis of the AP-PCR profiles revealed identical patterns among the strains of each group, but poor discriminatory power when used with unrelated strains.

We conclude that this method could be used with small numbers of isolates from outbreaks as a rapid preliminary test before restriction fragment length polymorphism and Southern blot hybridization can be done.

452 Rapid, Simple Method for Detection of *Mycobacterium tuberculosis* in Cerebrospinal Fluid Samples by Polymerase Chain Reaction

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The purpose of this study was to evaluate the clinical utility of simple DNA isolation technique from cerebrospinal fluids (CSF) for using polymerase chain reaction (PCR) in the diagnosis of tuberculosis meningitis. An insertion element IS 6110, a DNA sequence unique to the Mycobacterium tuberculosis complex of organism, was amplified by polymerase chain reaction (PCR) from cerebrospinal fluid (CSF), for the diagnosis of tuberculosis meningitis. Three DNA extraction methods including heating the sample in a boiling water bath (first method), protein salting out after boiling (second method), enzymatic lysis and phenol chloroform extraction (third method) were compared with each other. Non specific bands and DNA smears were obtained after PCR when we isolate DNA by direct boiling. We did not have such problems in the second and third methods. According to this result, a total of 35 CSF obtained from 35 patients with or without tuberculosis meningitis, was prepared by second DNA extraction method. The PCR for M. tuberculosis was positive in 13 patient and negative in 22. M. tuberculosis was detected all culture positive cases, and also culture negative but clinically suspected tuberculosis meningitis. When correlated with clinical histories, cultures and other laboratory results, the sensitivity and the specificity of this method for diagnosis of tuberculosis meningitis were 100% and 91% respectively. We conclude that this simplified DNA extraction procedure can be used in routine clinical practice.

453 Direct Detection and Identification of *Mycobacterium tuberculosis* and Mycobacteria from the *M. Tuberculosis* Complex, from Sputum, by rRNA Amplification Test

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The aim of the present study was to evaluate the sensitivity and specificity of a new direct rRNA amplification test for screening the presence of mycobacteria from the *M. tuberculosis* complex (MTD, Gen-Probe), in sputum, by comparison to culture.

Eighty three sputum specimens collected from 52 patients (39 men and 13 women) in 1993 and 1994 where checked for the biological diagnosis of pulmonary tuberculosis.

All specimens were treated by classical methods applied to culture (i.e. homogenization, decontamination with N. acetyl cystein sodium hydroxyde). Each decontaminated sediment was screened for the presence of acid-fast bacilli by fluorescence microscopy after auramin-rhodamin staining, inoculated in Middlebrook 7H12 medium (Bactec 12, Becton Dickinson) and on Löwenstein-Jensen supplemented with sodium pyruvate (0.5 ml), and stored at −20° C for further studies. Mycobacteria were identified by classical phenotypical determination tests (colony morphology, nitrate reductase and catalase at 20 and 70°C) plus specific DNA hybridization (Accuprobe, Gen-Probe) for *M. tuberculosis* complex, *M. avium intracellulare* and *M. gordonae*.

Detection of *M. tuberculosis* complex rRNA was applied to 37 *M. tuberculosis* positive specimens, 7 specimens positive for atypical mycobacteria (*M. xenopi, M. kansasii, M. avium intracellulare, M. chelone* and *M. gordonae*) and to 39 culture negative specimens.

Direct microscopy was positive for 18 out of the 37 specimens positive for *M. tuberculosis*, for 2 out of the 7 specimens positive for atypical mycobacteria and for 2 of the 39 culture negative specimens.

The rRNA amplification test MTD was positive for the 18 *M. tuberculosis* positive specimens (from 9 different patients), for 16 out of the 19 *M. tuberculosis* microscopy negative, culture positive specimens and for the 2 specimens which were positive at microscopy but negative in culture (both from the same patient under antituberculosis chemotherapy).

The rRNA amplification test was negative for the 7 specimens positive for atypical mycobacteria. The rRNA amplification test was positive for 13 out of the 37 specimens negative by microscopy and culture. They corresponded to 6 different cases, and in 4 cases the test was positive for several specimens collected simultaneously.

In conclusion, direct screening of mycobacteria of the *M. tuberculosis* complex can be done with good sensitivity-specificity by using the rRNA amplification test MTD. However, due to the doubtful positive results obtained with microscopy negative, culture negative sputum specimens, further informations on the clinical status of the patients are mandatory.

454 Rapid Detection of *Mycobacteria* Utilizing Strand Displacement DNA Amplification and Uracil DNA Glycosylase Amplicon Decontamination in a Simple Disposable Device

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In order to provide for more rapid diagnosis of *Mycobacteria*, we have coupled a sensitive, isothermal DNA amplification system, SDA, with UDG to eliminate sample carryover of previously amplified products. Since both of these methods do not require thermal cycling, a simple disposable device, known as DAD (Decontamination/Amplification Device) has been developed, that allows for these two steps to be carried out consecutively. Reagents for these reactions are housed in the disposable in a stable dried form, which allows for facile automation. Also this allows for flexibility when analytes want to be switched. With this system, we have been able to co-amplify sequences that identify *Mycobacteria* genus, *Mycobacterium tuberculosis*, and an internal amplification control. The system allows for reliable detection of 10 input genomes of M.tb DNA. Also, with the use of this DAD, amplicon carryover has been nonexistent in our lab, eliminating the need for multiple rooms to perform the reactions. The time required to perform decontamination and amplification with this device is less than 3 hours. Most recent data will be presented, discussing SDA performance, UDG effectiveness, and comparisons of amplifications performed on clinical samples in the DAD versus microcentrifuge tubes.

455 Multiplex Detection of *Mycobacterium tuberculosis* and Differentiation from Mycobacterium Genus Using Strand Displacement Amplification

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A sensitive and specific multiplex system has been developed for the detection of *Mycobacterium tuberculosis (M. tb)* and differentiation from other members of the genus Mycobacteria. The system is based upon the simultaneous amplification of three DNA sequences using target generation strand displacement amplification (SDA). The SDA system was designed to simultaneously amplify a region from the 16S ribosomal RNA gene sequence for Mycobacteria genus, the insertion element IS6110 for *Mycobacterium tuberculosis* and an internal positive control sequence. The SDA products were detected using a microwell chemiluminescent assay that employed simultaneous hybridization and capture of the amplified product. The potential problem of contaminating SDA products generating false positive results was eliminated through the use of Uracil DNA glycosylase (UDG). The multiplex SDA system was optimized for incorporation of UDG as a routine decontamination control. Following multiplex SDA, specific hybridization reactions were performed to detect Mycobacteria genus, *Mycobacterium tuberculosis* and the internal signature control. The SDA system was shown to be highly specific for the differentiation between *M. tb* and other members of the genus. No other bacteria were detected (38 species) when tested at 10^6 genome copies. Amplification efficiencies (greater than 10^8) were verified from each SDA reaction through the internal signature control. Sensitivity allowed the detection of less than 5 organisms of Mycobacteria with no false positives. This SDA system will allow the rapid, sensitive and specific detection of *Mycobacterium tuberculosis* and should facilitate the development of improved diagnosis for this pathogenic organism.

456 *Mycobacterium tuberculosis* Strains Unidentified by Using the IS*6110* Probe can be Detected by Oligonucleotides Derived from MT308 Sequence

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Tuberculosis (TB) is still a problem world-wide. During the past 5 years, new diagnostic technologies based on DNA methods including PCR and non radioactive hybridisation were developed to aid tuberculosis control. An insertion sequence-like element, IS*6110* was extensively used in PCR tests for the direct identification of *M. tuberculosis* in uncultured clinical specimens. However, some *M. tuberculosis* strains that failed to hybridise with the IS*6110* probe were recently found. We evaluated the frequency of such IS*6110* negative strains among a series of strains isolated in Vietnam and Burkina-Faso and