multiplex PCR protocol and found to be positive for HSV DNA or enterovirus RNA, respectively. Control samples of patients with other CNS infections were found to be negative.

In conclusion, we have described a sensitive and specific procedure to amplify simultaneously enteroviral and HSV genomic sequences in human CSF samples. The multiplex PCR for HSV DNA and enteroviral RNA provides a means to detect HSV or enteroviral genomic sequences without the need for separate extraction or amplification procedures. The rapid differential diagnosis between HSV and enteroviral CNS infection should be of value for therapeutic decisions and prognostic evaluation.

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Evaluation of two RT/PCR assays for HCV by testing blood of intravenous drug abusers

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A highly sensitive and specific RT/double PCR assay with heminested primers was developed to detect hepatitis C virus (HCV) RNA in blood specimens. The performance of this tailored assay and of a commercially available RT-PCR assay (HCM-Amplicor™) were compared by analysis of a coded panel (EUROHEP II) of samples. Both assays proved to be highly reliable with respect to specificity. Although the tailored assay was 1.6 times more sensitive, overall performance of both tests appeared to be similar. To assess the diagnostic utility of the assays, the prevalence of HCV in a population of intravenous drug users (IVDU) was investigated. Both assays appeared to be suitable for testing and showed that IVDU are a group with high prevalence of circulating HCV.

Methods and results. To assure highest technical standards of PCR and to avoid carry-over and cross-contamination the following measures were taken: i) one way flow of technicians and material through four separate laboratory rooms, ii) double testing, iii) random processing of samples, iv) interspersed negative controls for every two clinical samples. Samples with discordant results in one or among the two HCV PCR assays were retested.

Both HCV PCR assays employed oligonucleotide primers directed to similar regions of the highly conserved 5′-non translated region (5′-NTR) of the HCV genome[1]. Oligonucleotides for the tailored assay were the A-oligonucleotides of Bukh et al.[1,2]. Pre-PCR sample extraction for the Amplicor™ was according to the manufacturer. In contrast, plasma was merely denatured by heat in the presence of 0.4% Nonidet-P 40 before being subjected to the tailored PCR. Specificity for HCV of this latter assay had been documented by inclusion of known positive and negative controls and by characterization of amplicons I and II by size, restriction fragment analysis and by amplification with heminested primers. The assay sensitivities of both PCRs were determined by use of a human serum, the infectious titre of which had been measured by inoculation of chimpanzees (H-strain of HCV, kindly donated by Dr. R. H. Purcell, NIH, Bethesda, USA). The direct sensitivity of both assays was in the range of 10 to 100 CID_{50} (50% chimp infectious doses). However, because the tailored assay utilized only 3 µl of plasma instead of a 5 µl equivalent of a 100 µl specimen for the commercial assay, it was 1.6 times more sensitive.

The diagnostic sensitivities of both tests were evaluated on 26 coded samples provided by the EUROHEP study group. Neither test resulted in false positives or false negatives. On one serially diluted sample the tailored PCR was four times more sensitive than the Amplicor™ and than one of two reference assays. However, with a different sample the commercial PCR proved four times more sensitive than the tailored assay. Hence, both tests appeared to be similarly suitable for general testing.

The clinical/diagnostic performances of the two HCV PCRs were assessed by investigation of the virus' prevalence among 114 IVDU who had enrolled in a methadone programme. In addition to prevalence of HCV, the seroprevalences of hepatitis B and C virus were determined (IMX-HBV, HCV-Matrix, Abbott). Among the 114 samples tested, results of only 4 (3.5%) were discordant between the two HCV PCRs and remained so after repeat testing.

Infections by HCV and HBV had been experienced by 72 and 65% of patients, respectively. In 60% of anti-HCV positive cases, circulating virus was detected. This high prevalence of HCV is well in accordance with the high probability (60 to 80%) that infections will progress to chronicity. Furthermore, the investigation showed that IVDU patients present as a high

prevalence group not only for HBV but even more so for HCV.

Conclusion. On the basis of the high prevalence of circulating HCV found with both HCV PCRs, one might speculate that chances for transmission of HCV among IVDU occur more frequently than for transmission of HBV via similar routes. Conversely, instead of transmission through needle sharing or sexual contact, community acquisition of HCV infections might be of particular importance in IVDU.

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Identification of mycobacteria species by 23S ribosomal RNA targeted gene probes

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Conserved molecules like the rRNAs are suitable markers for identification of microorganisms. The most common molecule among the rRNAs is the 16S rRNA, which is used for rapid diagnosis of mycobacteria by direct DNA sequencing techniques[2]. The 23S rRNA is double the size of the 16S rRNA and consists of more high variable regions. The 23S rRNA of the genus *Mycobacterium*, members of the phylogenetic group of Gram-positive bacteria with a high DNA G + C content, contains a characteristic insert of about 100 bases in length. Its primary structure is highly variable. We developed specific oligonucleotide probes, which can rapidly identify and differentiate mycobacteria.

Methods and results. Isolation and purification of genomic mycobacterial DNA was done according to a modified standard procedure[1]. PCR reactions were performed on a Cetus 9600 thermocycler (Perkin-Elmer Cetus Corp., Norwalk, Conn.). For direct sequencing amplicons were purified by spin columns (Pharmacia Biotech, Germany). Sequencing reactions were loaded on a direct blotting electrophoresis (DBE) system GATC 1500 (MWG-Biotech GmbH, Germany) and automatically blotted on a noncharged nylon membrane (direct blotting membrane, GATC GmbH, Germany)[4]. Hybridization with the oligonucleotide probes was performed using a dot format and a modified protocol with tetramethylammonium chloride for washing at a specific temperature[5].

For *M. avium*, *M. nonchromogenicum*, *M. phlei*, *M. bovis*, *M. scrofulaceum*, *M. celatum*, *M. terrae*, *M. flavescens*, *M. xenopi*, *M. fortuitum*, *M. tuberculosis*, *M. gastri*, *M. gordonae*, *M. kansasii*, and *M. malmoense*, the 23S rRNA insert of helix 54 was amplified and the primary structure determined by direct sequencing. The primary structures were aligned and completed using published results[3]. Probes were designed for specific detection of *M gastri*, *M. fortuitum*, *M. nonchromogenicum*, *M. phlei*, *M. celatum*, *M. malmoense*, *M. scrofulaceum*, *M. kansasii*, *M. terrae* and *M. xenopi*. Only *M. gastri* and *M. kansasii* could not be distinguished on the level of the helix 54 insert.

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Use of 16S rDNA targeted oligonucleotide probe to detect phenotypic heterogeneity of *Bacillus mycoides*

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Many *Bacillus* species exhibit a high degree of similarity both in phenotye and 16S rDNA sequence, making their identification somewhat difficult. Examples of such species are those of the *B. cereus* group composed of *B. cereus*, *B. mycoides*, *B. thuringiensis* and *B. anthracis* which demonstrate only small differences in phenotype and high 16S rDNA sequence similarity (>99.4%)[1].

Thirty-four *Bacillus* species isolated from a peat bog sample were identified on the basis of fatty acid analysis as strains of the species *Bacillus mycoides*. The degree of similarity to the fatty acid profile of the *B. mycoides* strain in the fatty acid profile database was in the range 20–80% and *B. mycoides* was considered the best match. In some cases these results were in conflict with the phenotypic data, which showed many of these strains to be motile and all to have a non-rhizoid colony morphology, characteristics different to those described for the species *B. mycoides*[2].

Full 16S rDNA sequence was determined for five repre-