were suspended in lysis buffer, DNA was isolated by a standard column procedure and quantified for direct use in PCR. HIV-1-specific sequences were amplified using primers complementary to three different regions of the genome. The amplification of HIV DNA was possible without loss of sensitivity from dried blood samples stored for about 6 months at room temperature. To validate the sensitivity of the method, cells containing a single integrated provirus were titrated with HIV negative donor blood. HIV-1 positive patients were investigated to evaluate the specificity of the method. We could show that our procedure was comparable in sensitivity and specificity to other methods such as phenol/chloroform extraction from isolated peripheral blood mononuclear cells or a whole blood DNA purification procedure. Compared to another procedure of DNA extraction from dried blood spots[2] our method yields more reproducible results with respect to amount and amplification of the isolated DNA.

Detection of PCR products by DNA enzyme immunoassay (DEIA). HIV-1-specific sequences were detected by hybridization with biotin-labelled probes immobilized on streptavidin-coated microtitre wells. Measurement of the amount of hybridized DNA was done by a sandwich ELISA including a monoclonal antibody against double stranded DNA. The absorbancy was read on a spectrophotometer at 450 nm. The results obtained were compared to Southern blot analysis of PCR products using a chemiluminescent detection system. The colorimetric signal obtained by the amplification of increasing numbers of genome copies clearly reveals a positve result for one amplified copy, demonstrating the sensitivity of the assay. The specificity of DEIA was determined by running 26 patient samples in parallel for each of the three different genomic regions. All samples that did not hybridize in Southern blot analysis showed an optical density value below the cutoff. In contrast, the optical density of those samples with a positive signal in blot hybridization varied in general between an OD of 0.3 and 2.1.

Conclusions. The application of PCR and techniques confirming the specificity of PCR products in routine diagnostic laboratories, requires that the procedures are simple and reproducible. The microtitre plate assay we described for detection of HIV-1 is sensitive, simple, rapid and reproducible. The DEIA test is perfectly compatible with standard enzyme linked immunosorbent assay equipment, permitting the processing of a large number of samples. Moreover, the ability to analyze DNA extracted from dried blood specimens, as demonstrated in this study, allows the long-term storage of blood samples even at elevated temperatures and after transport over long distances.

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Detection of hantaviruses by polymerase chain reaction

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Hemorrhagic fever with renal syndrome (HFRS) refers to a group of human diseases with similar clinical symptoms. Members of the genus hantavirus, which belongs to the family Bunyaviridae, were identified as etiologic agents of HFRS[3]. They possess a single stranded, negative sense RNA genome, consisting of three segments, termed small (S), medium (M), and large (L), encoding a nucleocapsid protein, two glycoproteins (G1, G2), and a polymerase, respectively. Four serotypes causing HFRS have been described: Hantaan (HTN), Puumala (PUU), Seoul (SEO), and Dobrava/Belgrade (DOB/BEL). Recently, an additional serotype causing severe disease with high lethality has been found in the 'Four Corners Region' in the USA and described as human pulmonary syndrome (HPS). In contrast to other members of the genera of Bunyaviridae which are transmitted by arthropod vectors, rodents are the reservoir of hantaviruses. In Germany a seroprevalence of anti-hanta antibodies of about 1–2% within the normal population was determined. Although in southern Germany antibodies to both major antigens (PUU/HTN) were detected in patient sera, in western Germany antibodies to the PUU antigen and in Berlin–Brandenburg antibodies against the HTN antigen were predominantly identified[7]. RT-PCR for the detection of hantaviruses was described by several authors[4,5]. We have established RT-PCR for the detection of hantaviruses to allow molecular diagnosis and genetic characterization of new hantaviruses prevalent in eastern Germany and eastern Europe.

From the RNA extraction methods tested, the best results were obtained with a slightly modified acid guani-

dinium isothiocyanate phenol/chloroform (AGPC) method[2]. First-strand cDNA synthesis was performed by use of murine leukemia virus reverse transcriptase (MLV RT) or RNaseH-reverse transcriptase and either random hexamers or an 12 mer oligonucleotide primer (5′-TAGTAGTAGACT-3′) complementary to 3′-ends of all three hantaviral RNA segments[6]. The optimal reaction conditions, annealing temperature and thermocycle profile were determined in sequential experiments using cDNA synthesized from RNA of Hantaan-infected Vero E6 cells. Of numerous S and M segment primers tested one genus-reactive S segment primer pair consistently gave the best results with different hantaviruses. This primer pair was designed to match conserved regions of all hantaviral serotypes, degenerated in 3′ positions to fit to all known hanta sequences (sense primer S1: 5′-GG(AC)CAGACAGCAGA(CT)TGG-3′ antisense primer S2: 5′-AGCTCAGGATCCAT(AG)-TCATC-3′. Two internal primer pairs specific for HTN/SEO and PUU were used for second-round (nested) PCR, resulting in a 376 bp or 304 pb amplification product, respectively. The identity of the products was verified by hybridization, RFLP or sequencing after cloning in appropriate vectors.

Although several patients with suspected HFRS infections were analyzed using this RT-PCR method, only in one case of a patient with acute multi-organ failure of unknown aetiology could a positive PCR reaction in peripheral blood leukocytes (PBL) be determined. This is in general agreement with results of other workers, indicating that hanta RNA in PBL could only be detected early in the course of disease[1].

Recently we were able to amplify hantavirus sequences from a lung tissue specimen of a vole (*Microtus arvalis*) that was also characterized by a positive reaction in hantavirus enzyme immunoassay. The vole was trapped in early 1994 near Malacky, Slovakia, a geographical area where HFRS is endemic. Cloning and subsequent sequence analysis of a 840 bp region in the S segment revealed a novel hantavirus sequence, which led us to conclude that we had found a new hantavirus type.[5]

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Amplification and detection of enterovirus RNA and herpes virus DNA in CSF samples by multiplex polymerase chain reaction

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Herpes simplex virus (HSV) is a common cause of sporadic, acute focal encephalitis. Without early institution of antiviral therapy, death or severe neurologic impairment results in most cases. Enteroviruses are the most commonly identified causes of aseptic meningitis but have also been found in cases of acute encephalitis. Enteroviral CNS infections usually resolve without sequelae. A specific treatment is not yet available. However, herpes simplex virus encephalitis (HSVE) and enteroviral encephalitis are clinically indistinguishable. For a rapid diagnosis of HSVE and of enteroviral CNS infection, the polymerase chain reaction has been employed to detect HSV DNA and enteroviral RNS in CSF samples[1,2,4]. Recently, the simultaneous amplification of HBV and HCV genomic sequences in human serum samples has been described[3]. HCV RNA and HBV DNA were prepared from the samples by two different methods. We tested a simple, commercially available extraction method for the simultaneous isolation of DNA and RNA in one fraction.

Nucleic acids were extracted from 130 µl of human CSF samples and from control samples by the QIAamp HCV Kit (Qiagen) according to the instructions of the manufacturer. Nucleic acids were eluted from the QIAamp column in 50 µl H_2O. Ten microlitres of the nucleic acid eluate were reverse transcribed to cDNA. Enteroviral cDNA and HSV DNA were co-amplified using primer pairs from the 5′-UTR of the enteroviral genome[2,5] and of the HSV glycoprotein B region[1,4]. PCR products were detected by agarose gel electrophoresis and ethidium bromide staining. The specificity of the products was confirmed by a second round of amplification using nested primer pairs.

Positive control samples were spiked with echovirus 6 and with a plasmid containing the HSV glycoprotein B sequence. Nucleic acid extraction and co-amplification yielded two products of the expected sizes. Sensitivity was <100 copies for the HSV plasmid and <0.1 pfu for echovirus 6.

Several clinical samples of patients known to have HSVE or enteroviral CNS infections were tested by the