tribute to removing inhibitors. Although the method produces a crude DNA preparation, inclusion of a chloroform extraction step combined with the above mentioned pretreatment makes further purification unnecessary.

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## Direct sequencing versus cloned amplicon sequencing in HIV-1 diagnosis

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It is now well accepted that the HIV-1 phenotype changes during the course of infection. These changes are due to mutations occurring in the retroviral genome. Mutations in the *env* gene are observed most frequently and are selected for under the pressure of the patient's immune system. The combination of PCR and subsequent sequence analysis of the amplicons give the opportunity to define the viral genotype at each stage of infection. An exact knowledge of the viral phenotype might be a prerequisite for successful prevention and treatment strategies. We have compared two sequencing methods for determining the genotype present in several patients. On the one hand sequences were obtained by the rapid and inexpensive method of direct amplicon sequencing, and on the other hand by the more time consuming and expensive method of cloning the amplicons prior to sequencing. By direct sequencing the major type of virus present in the patients at a given point in time could be identified whereas sequencing of cloned products gave an estimate of the diversity in the whole sequence swarm of the viruses. As target sequences for amplification we selected the relatively conserved gene for the retroviral polymerase (*pol*) as well as the conserved and hypervariable regions of the gene for the outer surface glycoprotein (*env*) detectable in the PBMC DNA of HIV-1 infected patients. Both genes are important for recent therapy studies[1,2].

**Methods and results.** The *pol* genes of several patients were amplified by nested PCR and the amplicons were sequenced directly. After the PCR, the amplicons were separated on an agarose gel and subsequently extracted from the gel matrix. The amplicons were then sequenced. An evaluation of the X-ray films obtained after sequencing revealed defined *pol* genotypes from each patient investigated. For the majority of nucleotide positions a signal was obtained on the X-ray films (fig. 1A). This strongly indicates a uniform virus population with regard to these positions. Only for a few nucleotide positions was more than one single nucleotide present on the gel (fig. 1A). Hence, for these positions the virus was heterogenous. This was substantiated by sequencing amplicons cloned independently. However, the method of direct sequencing was sufficient for defining the major viral genotype present in each patient.

Similar results were obtained for the conserved regions of the *env* gene, such as the region coding for the CD4 binding. The major genotype could easily be determined by direct sequencing. As mentioned for the *pol* gene regions investigated, the conserved regions were amplified by nested PCR prior to direct sequencing. The same strategy was employed to determine the major genotype of the hypervariable regions of the *env* gene. But in contrast to the conserved regions an unequivocal determination of the major genotype was not possible. As shown in figure 1B, X-ray films obtained from the hypervariable regions could not be evaluated. Therefore the amplicons were cloned and then sequenced (fig. 1C). All clones differed from each other. Insertions and deletions led to overlapping sequences which could not be resolved by direct sequencing.

**Conclusions.** HIV-1-specific sequences could easily be amplified from the DNA of peripheral blood lymphocytes or from the viral RNA present in the plasma. A detailed characterization of the resulting amplicons requires sequencing. Here we have shown that the sequencing strategy depends on the variability of the region of interest. The higher the variability of a distinct region the more a cloning step is necessary prior to sequencing to obtain reliable information. Conversely, direct sequencing analysis is usually sufficient to record changes of conserved viral sequences,

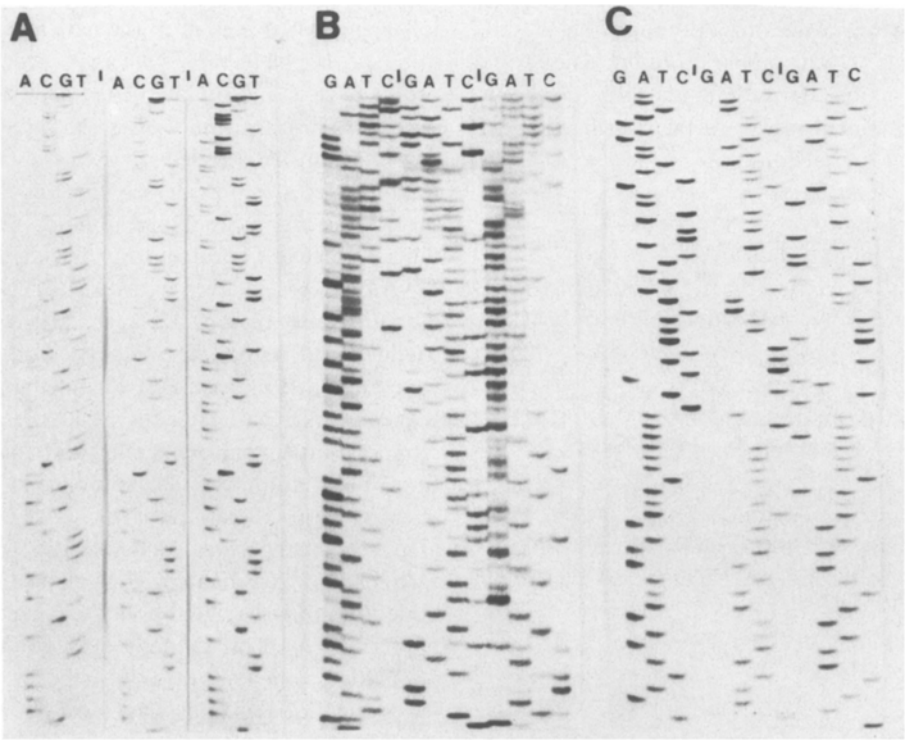


Figure 1. Amplicons from the *pol* gene (*A*) and a hypervariable region of the *env* gene (*B*) were sequenced directly. (*C*) shows the same hypervariable region of the *env* gene, but the amplicons were cloned prior sequencing.

e.g. during therapy with reverse transcriptase inhibitors.

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## Diagnostic value of human cytomegalovirus DNA PCR regarding different clinical specimens

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Human cytomegalovirus (HCMV) is a major pathogen in immunocompromised individuals, especially organ transplant recipients or HIV-infected patients, and a common infectious cause of congenital abnormalities. Furthermore, HCMV is associated with neurological disorders, e.g. encephalitis or polyradiculopathy in AIDS patients.

After primary infection HCMV, like other herpesviruses, develops a life-long persistence, possibly in monocytic blood cells[4]. In immunosuppressed patients reactivation of the virus or superinfection leads to active infections with often fatal prognosis. A rapid, sensitive, and specific diagnosis is a prerequisite for the successful treatment of HCMV infections[2].

Efforts in HCMV diagnosis have been made by replacement of the time-consuming tissue culture methods by the more rapid 'centrifugation culture' coupled with indirect immunofluorescence assay. Furthermore, PCR techniques have been established to detect viral DNA (or RNA) in several different clinical specimens. Comparing the results of 'centrifugation culture' and PCR for the monitoring of transplant recipients, especially bone marrow recipients, DNA detection has been shown to be a sensitive and early marker of active HCMV infections[3].

In our laboratory, PCR techniques have been established to detect HCMV DNA in different clinical specimens like PBLs, urine, bronchial lavage and cerebrospinal fluid (CSF). For HCMV PCR it has been