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**Detection of HIV-1 RNA sequences by *in vitro* DNA amplification**


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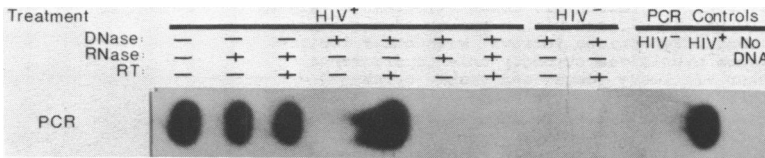
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Efficient *in vitro* amplification of DNA sequences is now possible using a thermostable DNA polymerase *Tag* from *Thermus aquaticus* in a polymerase chain reaction, PCR (1). Proviral DNA copies of human immunodeficiency virus (HIV) have been detected from cell culture materials and directly from HIV<sup>+</sup> patient samples with increased sensitivity over earlier methods (2,3). This polymerase is completely specific to a DNA substrate; *Tag*-mediated PCR therefore detects only proviral DNA, an indication of at least latent retroviral infection. We have extended PCR into a specific assay for RNA sequences, a measure of active infection in detecting retroviral genomes or virus-specific mRNA.

We used 3% of the nucleic acid extracted from 10<sup>7</sup> HUT78/HIV<sub>AAV</sub> (HIV<sup>+</sup>) cells or equivalent from uninfected line HUT78 (HIV<sup>-</sup>) for each PCR reaction, treating some preparations with RNase-free DNase or RNase. After thirty cycles of *Tag*-PCR, using SK38/39 primers within *gag* of HIV-1 (3), we detected amplified product using a <sup>32</sup>P end-labelled oligomer, SK19.



DNase digestion of proviral sequences was sufficiently complete so that no proviral sequence was detected by exclusive use of *Tag*-mediated PCR of the HIV<sup>+</sup> RNA sample (slot 4). As expected, RNase digestion did not prevent HIV-1 amplification (slot 2). Using Moloney murine leukemia virus reverse transcriptase (RT) and primers specific to the *gag* region of HIV-1, the RNA preparation from HIV-infected material was successfully amplified (slot 5), a result apparently dependent upon the synthesis of a HIV-specific cDNA intermediate.

RT/*Tag*-PCR detection of HIV RNA was 10<sup>4</sup> times more sensitive than detection of unamplified material using a slot blot and a <sup>32</sup>P primer-extended probe. RT/*Tag*-PCR of RNA will be an important measure of HIV status and more generally an assay for the presence of low copy number RNA species.

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