Volume 16 Number 9 1988 Nucleic Acids Research

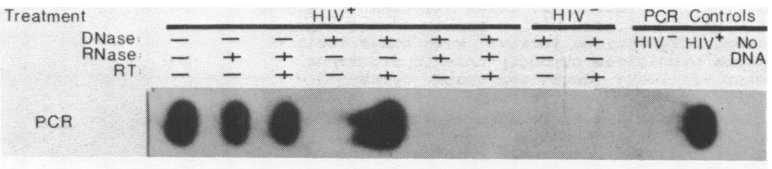
Detection of HIV-1 RNA sequences by *in vitro* DNA amplification

Bruce C.Byrne, Jian Jun Li, John Sninsky and Bernard J.Poiesz

Department of Medicine, State University of New York Health Science Center at Syracuse, 750 E. Adams St., Syracuse, NY 13210 and Department of Diagnostics Research, Cetus Corporation, Emeryville, CA 94608, USA
Submitted March 9, 1988

Efficient in vitro amplification of DNA sequences is now possible using a thermostable DNA polymerase Taq 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^+ patient samples with increased sensitivity over earlier methods (2,3). This polymerase is completely specific to a DNA substrate; Taq-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 10^7 $HUT78/HIV_{AAV}$ (HIV^+) cells or equivalent from uninfected line HUT78 (HIV^-) for each PCR reaction, treating some preparations with RNase-free DNase or RNase. After thirty cycles of Taq-PCR, using SK38/39 primers within gag of HIV-1 (3), we detected amplified product using a ^{32}P end-labelled oligomer, SK19.



DNase digestion of proviral sequences was sufficiently complete so that no proviral sequence was detected by exclusive use of Taq-mediated PCR of the HIV^+ 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/Taq-PCR detection of HIV RNA was 10^4 times more sensitive than detection of unamplified material using a slot blot and a ^{32}P primer-extended probe. RT/Taq-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.

Acknowledgements:
Supported by NIH N01HB67021 to BJP and NIH N01AI72654 To D. Duggan. We thank G. Ehrlich, S. Kwok, M. Abbott and L. Zaumetzer for their assistance and fruitful discussions.

References:

1. R.K. Saiki et al. (1988) Science 239, 487-491.
2. S. Kwok et al. (1987) J. Virology 61, 1690-1694.
3. C-Y Ou et al. (1988) Science 239, 295-297.