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PATENT DEPARTMENT
PUBLICATION CLEARANCE REQUEST

NAME OF AUTHOR SUBMITTING REQUEST Groves, Eric

NAMES OF CO-AUTHORS M. Kwoh, M Holodniy, D Kurzenstein, Sahini Sengupta, D Schwarz, Thomas Merigan

WE REQUEST CLEARANCE TO PUBLISH THE ATTACHED MATERIAL ENTITLED
Quantition of HIV-1 RNA in Serum and Correlation with Virus isolation using the Polymerase Chain Reaction

AS AN ARTICLE IN THE FOLLOWING JOURNAL/BOOK _____

AS AN ABSTRACT FOR AN ORAL ____ OR POSTER ✓ PRESENTATION
AT Keystone Meeting 3/31 — 4/7
(GIVE NAME, PLACE AND DATE OF MEETING, SEMINAR, ETC.)

IF ORAL PRESENTATION, THE SPEAKER WILL BE _____

DEADLINE FOR SUBMITTING PUBLICATION 12/2/88

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Alice Wang, Michael Kwok

QUANTITATION OF HIV-1 RNA IN SERUM AND CORRELATION WITH DISEASE STATUS USING THE POLYMERASE CHAIN REACTION,

Mark Holodniy, David A. Katzenstein, Sohini Sengupta, David H. Schwartz, Eric Groves* and Thomas C. Merigan, Division of Infectious Diseases, Stanford University School of Medicine, Stanford, CA. 94305, *Cetus Corporation, Emeryville, CA.

The amount of HIV-1 present in serum may be a potential marker in HIV related disease. A method that detects and quantifies HIV-1 viral RNA in serum is presented. To detect HIV-1 RNA, sera was extracted by a guanadinium thiocyanate method, reverse transcribed with MLV reverse transcriptase and amplified by the polymerase chain reaction using a gag gene primer pair(SK38/39) including a biotin labelled upstream primer. The biotinylated PCR product was liquid hybridized to a horseradish peroxidase conjugated probe,bound to avidin, and quantitated from the optical density of a colorimetric reaction.

Reverse transcription and amplification of known amounts of gag gene RNA and known infectious HIV_{IIIB} virus RNA alone yielded a log-linear relationship between 10 and 10^5 copies of gag RNA and $TCID_{50}$ of virus respectively. No HIV viral RNA was detected in the serum of 5 seronegative healthy controls. In HIV infected patients who were not receiving therapy, serum HIV-1 RNA was detected in 0/5 asymptomatic, 4/5 ARC and 4/5 AIDS patients with copy numbers ranging from 10^2-10^5 of serum. Ultracentrifugation of patient sera revealed detectable signal in pellets,but not supernatant, indicating that signal is attributable to viral RNA. In addition, extracted material was directly amplified for the presence of viral DNA and gave no detectable signal.

We have demonstrated that HIV-1 viral RNA can be detected and quantitated in patient serum over a four log range. An RNA gag gene sequence was used to quantitate viral copy number. In addition, a nonisotopic enzyme-linked affinity assay in a microtiter plate system allows easy PCR product detection and quantitation. Quantitation of HIV-1 viral RNA in serum by PCR may be a useful marker for disease progression or monitoring antiviral therapy.

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