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QUANTITATION OF HIV-1 RNA IN THE SERUM OF ARC AND AIDS PATIENTS USING THE POLYMERASE CHAIN REACTION(PCR)

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Objective: The amount of infectious 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.
Methods: 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.
Results: Reverse transcription and amplification of known amounts of gag gene RNA and infectious HIV_{SF2} virus RNA alone yielded a log-linear relationship between optical density and 10^2 and 10^5 copies of gag RNA and 10^1 and 10^4 $TCID_{50}$ of virus respectively. 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 10^2-10^4/200ul of serum. In a small number of AIDS patient sera, co-cultivation with donor lymphocytes demonstrated a correlation between infectious titer and copy number by PCR. Ultracentrifugation of patient sera revealed detectable signal in pellets, but not supernatant. Similarly, signal from sera could be adsorbed to rCD4 bound to sepharose beads, indicating that signal is attributable to viral RNA in intact viral particles. In addition, extracted material was directly amplified for the presence of viral DNA and gave no detectable signal.
Conclusions: We have demonstrated that HIV-1 viral RNA can be detected and quantitated in patient serum over a four log range by a nonisotopic enzyme-linked affinity assay. The RNA detected in patient sera is from infectious viral particles as evidenced by CD4 binding, sedimentation and culture. 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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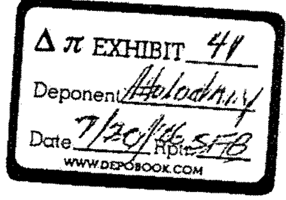

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