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PATENT DEPARTMENT

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INVENTION DISCLOSURE

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Date:
Patent ID #: CE-90-003
Patent Com: 102

To: REDACTED
From: M. Konrad (for Mark Holodniy)

I. DESCRIPTIVE TITLE: Quantitation of HIV-1 viral RNA in human serum utilizing an in vitro generated internal standard for coamplification and an enzyme linked affinity assay for detection

II. BRIEF SUMMARY What is the invention? What is the problem being solved? How does the invention advance the field beyond what is known in the art? What is surprising? Provide details on process steps, components, results, etc in accompanying pages and use additional pages for the summary if needed.

HIV-1 viral particles in HIV-1 infected human serum undergo a selective extraction procedure for RNA. The resulting viral RNA is coreverse transcribed with an in vitro generated internal standard. The internal standard was constructed utilizing PCR technology and a RsaI site was deleted in a highly conserved sequence of the gag gene. The cDNA undergoes amplification with a biotinylated upstream primer. The resultant PCR products are separated by RsaI restriction enzyme digestion on acrylamide gel, the bands excised and counted for radioactive incorporation. Alternatively products are detected by a nonisotopic enzyme linked affinity assay with a colorimetric reaction. Based on optical density reading the sample copy number can be determined.

III. CETUS SCIENTISTS/CONSULTANTS WHO COULD EVALUATE THIS DISCLOSURE ARE

SIGNATURE OF SUBMITTER(S)		SIGNATURE OF WITNESS (ES)	
[signature]	1/9/90	Michael Konrad	1/9/90
Full First Name Initial Last Name	Date		Date
Full First Name Initial Last Name	Date		Date
Full First Name Initial Last Name	Date		Date
Full First Name Initial Last Name	Date		Date

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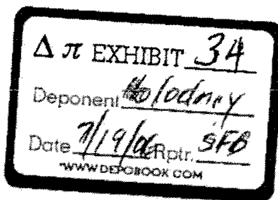
"OUTSIDE COUNSEL'S EYES ONLY"



RMS 00542
CONFIDENTIAL

IV. REFERENCES

A. Lab Notebook(s) Personal lab book

B. Progress Reports (etc.)

C. Relevant Literature and Patent References

V. WHEN DID YOU START WORKING ON THE INVENTION? 9/89

WRITTEN RECORD DATES?

DISCLOSURE TO OTHERS None
(To Whom/Date)

VI. WERE CELL LINES OR PLASMIDS USED IN THE COURSE OF THE INVENTION? IF SO, WHICH? U937 / U1 cell lines pCC2 plasmid

HAVE DEPOSITS BEEN MADE IN CMCC/CTCC? Yes pCC2

VII. **DISCLOSURE TO THIRD PARTIES** (Publications, field test, commercial use or sale, samples sent to others? When? Please keep IPLD informed of potential disclosures after filing this form.) Abstract submitted to UCLA symposium for molecular and cellular biology

THE INVENTION DESCRIBED ABOVE IS SUBMITTED PURSUANT TO MY "EMPLOYMENT INVENTION AND CONFIDENTIAL INFORMATION AGREEMENT".

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

Mark Holodniy, David A. Katzenstein, Sohini Sengupta, Alice Wang*, Clayton Casipit*, David H. Schwartz, Mike Konrad*, Eric Groves* and Thomas C. Merigan, Division of Infectious Diseases, Stanford University School of Medicine, Stanford, CA. 94305. *Cetus Corporation, Emeryville, CA 94608.

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 infectious HIV_{IIIB} virus yielded a log-linear relationship between optical density and 10^2 and 10^3 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 10^2-10^5/200ul 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.

Eric, 12-29-89

Here is the final copy of the abstract submitted with all the names. Here I have given you a copy of the letter I sent to CCHI for the files. I will have the list of patient samples from the IL-2 AZT study, which we can go over next week.

Mark

Copy to
Sninsky
Kwok
Konrad
Wang
Casipit
Shih
Raymond
JSP
Furutani

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