PRUETZ LAW GROUP LLP
Adrian M. Pruetz (Bar No. 118215)
ampruetz@pruetzlaw.com
1600 Rosecrans Avenue, 4th Floor
Manhattan Beach, CA 90266
Telephone: (310) 321-7640
Facsimile: (310) 321-7641

QUINN EMANUEL URQUHART OLIVER & HEDGES, LLP
Brian C. Cannon (Bar No. 193071)
briancannon@quinnemanuel.com
Tun-Jen Chiang (Bar No. 235165)
tjchiang@quinnemanuel.com
555 Twin Dolphin Drive, Suite 560
Redwood Shores, California 94065-2139
Telephone: (650) 801-5000
Facsimile: (650) 801-5100

Attorneys for Defendants and Counterclaimants Roche Molecular Systems, Inc.; Roche Diagnostics Corporation; and Roche Diagnostics Operations, Inc.

UNITED STATES DISTRICT COURT

NORTHERN DISTRICT OF CALIFORNIA

THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY,

Plaintiff,

vs.

ROCHE MOLECULAR SYSTEMS, INC.; ROCHE DIAGNOSTICS CORPORATION; ROCHE DIAGNOSTICS OPERATIONS, INC.,

Defendants.

ROCHE MOLECULAR SYSTEMS, INC. ROCHE DIAGNOSTICS CORPORATION; ROCHE DIAGNOSTICS OPERATIONS, INC.,

Counterclaimants,

vs.

THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY; THOMAS MERIGAN; AND MARK HOLODNIY.

Counterclaim Defendants.

CASE NO. C-05-04158 MHP

DECLARATION OF JEFFREY D. LIFSON, M.D. IN SUPPORT OF ROCHE'S RESPONSIVE CLAIM CONSTRUCTION BRIEF

[PATENT LOCAL RULE 4-5(b)]

Judge: Hon. Marilyn H. Patel
Date: October 3, 2007
Time
Ctrm: 15, 18th Floor

I, Dr. Jeffrey D. Lifson, hereby declare as follows:

1. I am currently the Director of the AIDS Vaccine Program and am also Senior Principal Scientist and Head of the Retroviral and Pathogenesis Laboratory for SAIC Frederick, Inc. at the National Cancer Institute in Frederick, Maryland. I received my BS Med and MD degrees from Northwestern University and Northwestern University Medical School and pursued postgraduate medical and research Fellowship training at Stanford University Medical Center. I have been involved in AIDS research since 1983 while I was at Stanford and a significant part of my research efforts and recognized expertise involve the development and application of assays for monitoring the replication of HIV and related retroviruses. I have published more than 170 scientific papers and serve on multiple professional journal editorial boards, and expert advisory committees. I make this declaration of personal, first hand knowledge and, if called and sworn as a witness, I could and would testify competently thereto. In providing this declaration and any possible subsequent testimony I am acting in an individual capacity, and not as a representative of SAIC Frederick, Inc., or the National Cancer Institute.

2. I attach a copy of my curriculum vitae, which describes in more detail my background and qualifications, as Exhibit A to this declaration.

3. I submit this declaration in support of Roche's responsive claim construction brief to address the interpretation and meaning of certain terms used in the claims of United States Patents Nos. 5,968,730; 6,503,705; and 7,129,041, as well as provide background information about the general subject matter of these patents. Counsel for Roche has retained me to offer expert testimony regarding the level of knowledge of a person of ordinary skill in the art described in these patents around May 1992 in connection with the use of the polymerase chain reaction ("PCR") technique to quantify the copy number of a particular nucleic acid sequence -- in particular use of the PCR technique in AIDS research. I understand that May 1992 is the date on which Stanford filed the original patent application that led to the issuance of all three patents.

4. In connection with the preparation of this declaration, I have reviewed the three patents as well as the prosecution history and references set forth in this declaration and listed in Exhibit B.

BACKGROUND

5. The three patents in this lawsuit list three inventors in common, share a common specification, and are derived from the same original application filed in May 1992. Each patent is entitled: "Polymerase Chain Reaction Assays For Monitoring Antiviral Therapy And Making Therapeutic Decisions In The Treatment Of Acquired Immunodeficiency Syndrome".

- ***PCR***

6. Scientists at Cetus Corporation in the mid 1980s developed PCR, a method to amplify and produce large amounts of specific target DNA sequences out of complex mixtures containing the sequence of interest, admixed with unknown DNA sequences. DNA is a polymer, comprised of four nucleotide subunits. . Each nucleotide is made up of a sugar, a phosphate, and a base (adenine, thymine, guanine or cytosine), with the nucleotides linked together via phosphodiester linkages. In its naturally occurring form, DNA is typically a double stranded molecule that consists of two chains of linked nucleotides, organized in a double helix, with the bases stacked in parallel and oriented to the interior of the helix, like the rungs of a spiral ladder. The bases for the two strands are paired, based on complementary hydrogen bonding, so that the sequence of nucleotides on one strand determines the complementary sequence of the other strand, with adenine bases paired with thymines, and cytosines paired with guanines. It is this property that determines the mechanism for replication of DNA, allowing the DNA sequence to serve as the reproducible encoding of hereditary information for a living cell. This property of base complementarity also provides the basis for accurate copying of target sequences during the PCR.

7. Amplification of select DNA sequences allows scientists to detect the presence of the target sequence in the starting material and/or to accumulate multiple copies for further investigation and testing. PCR is a tool that has had great impact on molecular biology. Cetus scientist Dr. Kary Mullis was awarded the Nobel Prize in Chemistry in 1993 for his PCR work.

8. PCR achieves extreme sensitivity for detection of specific DNA sequences, based on repeated cycling of a multistep reaction process. To conduct the PCR, a scientist

assembles a reaction mixture that includes the input target DNA. The target DNA is the original template from which all copies are derived. Attached to this declaration at Exhibit C is an article from 1990 that sets forth the basic PCR mixture and reaction sequence. Michael A. Innis and David H. Gelfand, "Optimization of PCRs," in PCR PROTOCOLS: A GUIDE TO METHODS AND APPLICATIONS AT 3-12 (1990) (Innis et al, eds.).

9. The mixture also includes synthetic primers; deoxynucleotide triphosphates (dNTPs), the building blocks from which the amplified copies of the target DNA will largely be assembled; and a DNA polymerase enzyme, which synthesizes the new copies of the target DNA sequence from the dNTPs, using the target DNA as a template.

10. *Three step process: one cycle*. The first step of the process ("Denaturation") involves heating a sample containing the DNA sequence of interest to separate the two strands of double stranded target DNA. The specificity of the amplification comes from the second step ("Annealing") in which the reaction mixture is cooled, and short "primer" molecules, designed to be complementary to sequences on one or the other strand of the target DNA sequence are allowed to bind to the target DNA. In the third step ("Extension"), the DNA polymerase enzyme copies the target sequence, extending the sequence starting adjacent to the end of the bound primer molecule. After completion of the Extension step, for each starting copy of target sequence, there should be two copies that are *replicas* of the original target sequence.

11. *Three steps repeated*. The mixture is then heated again in a new Denaturation step that starts the next round of the cyclic process, and the multistep reaction process is repeated.[1] Because the primers and DNA polymerase enzyme create a replica of the original target DNA sequence during each amplification cycle, each new replica sequence can

[1] In the original form of PCR, it was necessary to add fresh DNA polymerase to the reaction for each new cycle, as the activity of the enzyme was destroyed by the heating of the Denaturaton step. By 1990, however, incorporation of DNA polymerase derived from the thermophilic bacterium *T. aquaticus* (Taq polymerase) eliminated this requirement -- the Taq polymerase enzyme has been adapted through evolution to function under conditions of extreme temperatures that characterize its natural habitat, the geothermally heated waters found near geysers, and retains activity through typical PCR thermal cycling conditions.

serve as a template for production of more replica sequences. Thus, each cycle amplifies the amount of the desired DNA sequence in an exponential fashion. The more cycles of amplification that are conducted, the more product is generated. Typically, a scientist will run a given number of cycles and then sample the amplified target sequence product for analysis or other use. See Exhibit C at p. 8-9 ("Cycle Number."). As discussed below, the cycle number in the claimed method of the patents in suit is "about 30 cycles" -- meaning that a scientist conducts PCR for about 30 cycles (each cycle consisting of the three basic steps described above), and then works with the end product of those cycles. This type of PCR method -- running a PCR for a particular number of cycles and then measuring the result -- is known as "end point PCR".

12. *PCR for RNA (Reverse Transciption PCR; "RT-PCR")*. As described above, the PCR amplifies a defined target sequence, starting from a double stranded molecule DNA. However, RNA is another molecule that contains genetic information -- but it is single stranded.[2] To apply PCR to RNA target sequences, the RNA must be converted to DNA. To do this, the scientist must perform a "reverse transcription" reaction on the RNA, using a reverse transcriptase enzyme, primers, and co-factors to convert the RNA to a complementary DNA copy or cDNA. See Ernest S. Kawasaki, "Amplification of RNA" in PCR PROTOCOLS: A GUIDE TO METHODS AND APPLICATIONS at 21-27 (1990) (Innis et al, eds.). Exhibit D.

13. With the development of PCR, scientists sought to apply the new technique to a variety of new targets related to clinically significant conditions and diseases, such as HIV infection. *See* John J. Sninsky and Shirley Kwok, Detection of Human Immunodeficiency Viruses by the Polymerase Chain Reaction, ARCH. PATHOL. LAB. MED., Vol 114: 259 (March 1990), at Exhibit E.

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[2] In a human cell, DNA contains the hereditary genetic information. The genetic information encoded in the DNA is converted to RNA through the process of transcription, and the resulting RNA is used as the coding information to allow production of biologically active proteins, through the process of translation. Some viruses like Human Immunodeficiency Virus ("HIV") carry their genetic information in the form of RNA ("viral genomic RNA").

14. PCR technology has undergone dramatic changes and improvements since it was first developed. What one of skill in the art today would understand by a method claiming a PCR reaction is different now than it would have been in May 1992. For instance, as described in more detail below, since May 1992, scientists have developed "real time PCR" a procedure used to quantify the copy number of a starting target sequence in which the product of the PCR reaction is measured in kinetic fashion, as the reactions are taking place -- as opposed to waiting until the end of a set number of cycles as was used in "end point" PCR. As detailed below, there are fundamental differences between these approaches.

- ***HIV***

15. The three patents in this case relate to using PCR to quantify the amount of HIV in a patient sample. In particular, the patents refer to quantifying HIV RNA copy number in plasma or serum. For instance, the specification states that the invention "is based, in part, on the discovery that plasma HIV copy number, as measured using PCR, may be a sensitive marker of the circulating HIV viral load to assess the therapeutic affect of antiretroviral compounds." '730 patent, col. 2, lines 18-22. As a general matter, "viral load" refers to the amount of HIV in a subject's blood, although the precise use of this term may be modified by the context of the source of the HIV nucleic acids (cells vs. plasma or serum) and the techniques used for its quantification.

16. HIV is a retrovirus, which carries its genetic material in the form of single stranded RNA ("viral genomic RNA"). In the replication cycle of HIV, a virus interacts with a susceptible target cell using molecules on the virus surface to bind to the cell via specific receptors. Following receptor binding, the molecules on the viral surface undergo a rearrangement that results in the membrane of the virus fusing to the cell membrane, allowing the internal part of the virus, the nucleocapsid (which carries the viral RNA), to enter the cell. After entry, the viral genetic material is converted from RNA to DNA, through the multi-step process of reverse transcription, mediated by the viral enzyme reverse transcriptase. The reverse transcribed viral DNA is transported to the nucleus of the cell, where it can be integrated into the DNA of the host cell, through a process mediated by the viral integrase enzyme. Under suitable conditions, the integrated viral DNA can be transcribed by cellular DNA polymerase enzymes to make viral

RNA. Different species of viral RNA are made. Some are used as templates to make viral proteins through the process of translation by which the genetic information encoded in the RNA is used to direct ribosomes to make viral proteins. These viral proteins then provide the material to form a new generation of progeny virions from the infected cell. In addition, copies of the full length viral genomic RNA are made to be packaged into the new generation of progeny virions, and this packaged viral genomic RNA constitutes the genetic material for this next round of progeny viruses. The viral proteins and viral genomic RNA assemble at the surface of the infected cell to form new viral particles, which are released from the cell through a process of budding. After a maturation step mediated by the viral protease enzyme, these new particles are then ready to infect new target cells.

17. As outlined above, the replication cycle of HIV provides several potential RNA and DNA targets that could be detected by PCR or RNA RT-PCR assays as an indicator of the presence and extent of infection.

18. As reflected in my Curriculum Vitae and publication history I have been involved in AIDS related research since 1983, with much of my research involving the development and application of quantitative PCR and RNA RT-PCR methods for studying various aspects of infection with HIV and related retroviruses. See Exhibit A at pages 4-5.

- ***Quantitative PCR***

19. In the early 1990's I, like many others, was involved in research aimed at developing methods for quantitative measurement of HIV to enable monitoring of responses to antiviral treatment. Given the comparatively protracted clinical course of HIV infection, the thinking amongst those involved in HIV research was to develop an assay to measure a virologic response to treatment as an indicator of the pharmacodynamic activity of experimental treatments. Based on extrapolation from other systems, it was reasonable at this time to expect that an effective antiviral treatment would result in a decrease in the amount of viral replication, and it was in principle attractive to try to measure such a decrease in readily accessible blood samples. See Sninsky and Kwok,, 1990 Ex. E at 262 ("experiments on the quantitation of PCR product

suggest that the procedure could be used for the monitoring of viral load in patients receiving therapeutic agents").

20. While PCR can be an extremely sensitive procedure, attempts to measure biologically relevant viral load from in vivo derived patient samples face numerous significant challenges. The efficiency of isolation of RNA from the source specimen may not be quantitative, and may vary from specimen to specimen. In addition, various inhibitors may be present in the source sample and in the extracted RNA that may adversely affect the RT and/or PCR reactions. Small variations in the initial cycles of amplification can have disproportionate impact on the subsequent results of PCR amplifications, and variations from well to well in the precision of temperature control for thermal cycling instruments used for performing PCR amplifications can also impact results. Nevertheless, a number of investigators sought to use basic endpoint PCR methods, in which a PCR (or RT-PCR) procedure was performed for a fixed number of amplification cycles, and the amount of amplified target sequence present after this amplification was measured by a variety of techniques, in an effort to estimate the amount of target sequence present at the start of the amplification. The basic approach is to perform a PCR or RT-PCR reaction, measure the amount of amplified target sequence produced, then estimate the starting target copy number by comparing this amount of product produced to the amounts of product produced from a dilution series of known input copy numbers of a purified control target sequence, which serves for creation of a standard curve.

21. In my own work, my colleagues and I were looking in the early 1990's to develop methods for monitoring HIV replication, in part to assess responses to antiviral treatments. One approach in the art at the time was known as competitive PCR. Gary Gilliland et al., "Competitive PCR for Quantitation of mRNA," in PCR PROTOCOLS: A GUIDE TO METHODS AND APPLICATIONS at 60-69 (1990) (Innis et al, eds.) (Exhibit F).

22. A similar approach was developed by Cetus scientist Alice Wang and her colleagues, also for measurement of mRNA. Alice Wang and David Mark, "Quantitative PCR" in PCR PROTOCOLS: A GUIDE TO METHODS AND APPLICATIONS at 70-75 (1990) (Innis et al, eds.)

(Exhibit G) and Wang, et al., "Quantitation of mRNA by the polymerase chain reaction," PROC. NATL. ACAD. SCI. USA, Vol. 86, pp. 9717-9721 (December 1989) (Exhibit H).

- ***1991 JID Paper***

23. In 1991, Stanford's Mark Holodniy, Thomas Merigan, along with Cetus scientist Alice Wang and others co-authored "Detection and Quantitation of Human Immunodeficiency Virus RNA in Patient Serum by Use of the Polymerase Chain Reaction," THE JOURNAL OF INFECTIOUS DISEASES, 163:862-866 (April 1991) ("JID paper") (Exhibit I).

24. This JID paper uses a five step procedure to quantify HIV copy number from a patient sample, such as a plasma or serum specimen.

i. First, RNA is extracted from the patient sample ("extraction");

ii. Second, RNA is reverse transcribed to DNA with M-MLV reverse transcriptase ("reverse transcription");

iii. Third, PCR amplification is carried out for 30 cycles with primers including SK38, SK39 ("amplification");

iv. Fourth, the PCR product is captured using a biotin tag on one of the primers and captured product is detected using a horseradish peroxidase (HRP) conjugated hybridization probe and a colorimetric enzyme based substrate conversion reaction ("detection"); and

v. Fifth, quantification is accomplished by constructing a standard curve of optical density values from a dilution series of known copy numbers of a RNA standard derived from the HIV *gag* gene ("quantification") and interpolation of the measured optical density values for unknown samples onto this standard curve.

25. In the assay presented in the JID paper, the authors describe an endpoint RT PCR assay, using a non-isotopic readout. RNA is extracted from serum/plasma , reverse transcribed, and the resulting cDNA amplified by PCR using previously described primers specific for HIV sequences. The amplified product is captured onto avidin coated assay wells using a biotin molecule incorporated into one of the primers, and detected, using an enzyme conjugated probe that hybridizes to the interior of the amplified target sequence. The enzyme mediates a reaction that generates a colored reaction product that can be measured spectrophotometrically.

26. Use of this assay to estimate input template copy number depends on the assumption that the amount of amplified product measured at the conclusion of the assay by the colorimetric enzyme readout is a reflection of the input template copy number present prior to

reverse transcription and PCR amplification. The assay also depends on an external standard curve, in which a dilution series of known copy numbers of a synthetic RNA control target template, containing the sequence to be amplified and quantified, are subjected to the same reverse transcription and PCR amplification procedures. A standard curve is then generated based on a regression of known input control standard copy numbers and corresponding measured optical densities from the colorimetric reactions in the assay readout. The measured optical density for an unknown sample is then interpolated onto this standard curve to estimate the corresponding input template copy number. To assess the assay threshold sensitivity, the authors define the average background optical density at 490 nm observed for testing of known HIV negative samples, + 3 standard deviations (OD_{490} =0.135), as the threshold below which the assay cannot reliably distinguish positive samples from negatives. The detection level the authors report for this assay is 40 copies per 200 microliters of extracted plasma or serum.

27. The JID paper concludes that: "virion HIV RNA was detected and quantitated in the serum of HIV-positive patients by PCR" and that: "Serum PCR may provide an additional marker of disease progression and drug efficacy that could improve our ability to monitor the course of HIV infection."

- ***Development of Real Time PCR***

28. A major advance subsequent to May 1992 in the application of PCR methods for quantitative applications involved the development of kinetic procedures for quantitative PCR, designated “real time PCR methods”. The key feature of such methods is the use of non-invasive means of measuring accumulation of amplified product to monitor the ongoing PCR reaction kinetically, in real time. In real time methods, quantification is based not on the *amount of amplified PCR product produced*, but rather *how many amplification cycles are required to first generate a quantifiable (above background) amount of amplified product*. By performing a dilution series of reactions using known copy numbers of input control target template, a standard curve of input target template copy number vs. number of PCR cycles to first quantifiable product is generated. The higher the copy number of starting target template, the fewer cycles required to generate quantifiable amplified product; the lower the copy number of

starting target template, the more cycles required to generate a quantifiable amount of amplified product. Real time PCR, which is based on a kinetic measurement principle and uses different detection probes and reagents than end point PCR, represents a qualitatively different approach to quantitative PCR relative to endpoint PCR based assays.

29. The earliest real time quantitation PCR assay of which I am aware was published in 1993: Russell Higuchi, et al., "Kinetic PCR Analysis: Real Time Monitoring of DNA Amplification Reactions," in BIOTECHNOLOGY, Vol. 11: 1026-1030 (September 1993) (Exhibit J).

30. In 1996, an improved approach for using real time PCR methods for quantitative RT PCR applications for quantitating RNA was described by Gibson et al., "A Novel Method for Real Time Quantitative RT-PCR," in GENOME RESEARCH, Vol. 6:995-1001 (October 1996) (Exhibit K). A version of this approach was later applied for HIV quantification.

LEVEL OF ORDINARY SKILL

31. The patents at issue all rely upon use of a PCR assay in connection with evaluating the effectiveness of anti-HIV therapy. I have been asked to comment on the level of knowledge and skill of a person having ordinary skill in the art of the patents as of May 1992, the date on which the original patent application was filed. In 1992, as is the case today, a physician makes individual decisions about whether and how to recommend a therapeutic decision for a particular patient, including a change in antiretroviral drugs or dosages, considering relevant clinical and laboratory information.

32. With respect to the development and use of PCR techniques, which in the early 1990's were still comparatively new, for measurement of HIV sequences, my best assessment of what would constitute the "level of ordinary skill" in this art would be someone with a medical or graduate degree in molecular biology, virology, biochemistry or a related field and at least two years of relevant laboratory bench experience conducting PCR assays. Over the past 15 years I have successfully supervised and/or trained multiple individuals, including technical staff with BS or MS degrees, in the successful execution of PCR or RT PCR based assays of this level of complexity or greater.

- **Patent Claim Interpretation**

33. Counsel for Roche have informed me that claims are to be interpreted as they would be understood by one of ordinary skill in the art at the time of the filing of the patent application. I have read the patents in their entirety as well as portions of the file histories of the patent applications.

PATENTS AT ISSUE

34. Each of the claims of the three patents is a method of evaluating the effectiveness of anti-HIV therapy of a patient by performing a particular PCR assay. Many of the claims include a step for "measuring" the HIV copy number. For instance, claim 9 of the '730 patent reads (with my emphasis):

> 9. A method of evaluating the effectiveness of anti-HIV therapy of a patient comprising
>
> (i) collecting a plasma sample from an HIV-infected patient who is being treated with an antiretroviral agent;
>
> (ii) amplifying the HIV-encoding nucleic acid in the plasma sample using HIV primers in about 30 cycles of PCR; and
>
> (iii) ***measuring the HIV RNA copy number using the product of the PCR***, in which an HIV RNA copy number greater than about 500 per 200 ul of plasma correlates positively with the conclusion that the antiretroviral agent is therapeutically ineffective.

35. Similarly, Claim 1 of the '705 patent reads with my emphasis:

> 1. A method of evaluating the effectiveness of anti-HIV therapy of an HIV-infected patient comprising:
>
> a) collecting statistically significant data useful for determining whether or not a decline in plasma HIV RNA copy numbers exists after initiating treatment of an HIV-infected patient with an antiretroviral agent by:
>
> (i) collecting more than one plasma sample from the HIV-infected patient at time intervals sufficient to ascertain the existence of a statistically significant decline in plasma HIV RNA copy numbers;
>
> (ii) amplifying the HIV-encoding nucleic acid in the plasma samples using HIV primers via PCR for about 30 cycles;
>
> (iii) ***measuring HIV RNA copy numbers using the products of the PCR of step*** (ii);

(iv) comparing the HIV RNA copy numbers in the plasma samples collected during the treatment; and

b) evaluating whether a statistically significant decline in plasma HIV RNA copy numbers exists in evaluating the effectiveness of anti-HIV therapy of a patient.

36. The '041 patent contains no claims that include the "measuring" step to quantify HIV RNA copy number. In contrast, the '041 patent claims include the phrase "presence or absence of detectable HIV-encoding nucleic acid" and the step of "testing" for such presence or absence. For instance, Claim 1 of the '041 patent reads with my emphasis:

> 1. A method of evaluating the effectiveness of anti-HIV therapy of a patient comprising:
>
> correlating the ***presence or absence of detectable HIV-encoding nucleic acid*** in a plasma sample of an HIV infected patient with an absolute CD4 count, wherein the presence or absence of said detectable HIV-encoding nucleic acid is determined by
>
> (i) collecting a plasma sample from an HIV-infected patient who is being treated with an antiretroviral agent;
>
> (ii) amplifying HIV-encoding nucleic acid that may be present in the plasma sample using HIV primers via PCR and;
>
> (iii) ***testing for the presence of HIV-encoding nucleic acid sequence in the product of the PCR***.

37. Similarly, the '730 patent also includes claims directed to "testing for the presence" or "testing for the absence" of HIV RNA. See '730 Patent, claims 1, 6, 7 and 8. For instance, claim 1 of the '730 patent reads:

> 1. A method of evaluating the effectiveness of anti-HIV therapy of a patient comprising:
>
> (i) collecting a plasma sample from an HIV-infected patient who is being treated with an antiretroviral agent;
>
> (ii) amplifying the HIV-encoding nucleic acid in the plasma sample using HIV primers in about 30 cycles of PCR; and
>
> (iii) ***testing for the presence of HIV-encoding nucleic acid***, in the product of the PCR;
>
> in which the absence of detectable HIV-encoding nucleic acid correlates positively with the conclusion that the antiretroviral agent is therapeutically effective.

- ***Quantification***

38. One of ordinary skill in the art as of May 1992 would interpret the "measuring" steps of the '730 patent and '705 patent to be directed to using PCR to quantify the HIV RNA copy number. The available techniques for HIV quantification at the time, however, were limited.

39. The specification describes in two places a protocol to quantify HIV RNA copy number: column 4 in the "Detailed Description" and columns 9-10 in "Example 6." These descriptions are both essentially the five step endpoint RT-PCR assay set forth in the 1991 JID paper co-authored by the Stanford inventors and Cetus scientists.

The chart on the following page compares the assays:

	1991 JID Paper	*'730 Patent: Cols 4-5*	*'730 Patent: Cols. 9-10*
1. Extraction	"Total RNA from 200 µl of serum was extracted using guanidinium thiocynate"	"RNA may be extracted from plasma using standard techniques . . . for example 200 µl of clarified plasma to which 200 µl of 5M guanidinium thiocynate had previously been added": lines 11-16	"RNA was extracted from plasma using standard techniques . . . for example 200 µl of clarified plasma to which 200 µl of 5M guanidinium thiocynate had previously been added": col. 9, lines 63-66
2. Reverse Transcription	"reverse transcribed with M-MLV reverse transcriptase" [Note: M-MLV refers to Moloney murine leukemia virus]	"From plasma RNA, HIV RNA may be transcribed to cDNA using a suitable reverse transcriptase (for example Moloney murine leukemia virus reverse transciptase)": lines 21-23	"HIV RNA was transcribed to cDNA using Moloney murine leukemia virus reverse transciptase": col. 10, lines 5-6
3. Amplification	"PCR was carried out . . . for 30 cycles of amplification in a DNA thermal cycler (Perkin-Elmer Cetus)"	"tubes containing the reaction may be placed in a DNA thermal cycler (e.g., Perkin-Elmer Cetus) for about 30 cycles of amplification": lines 39-42	"Tubes were placed in a DNA thermal cycler (Perkin-Elmer Cetus) for 30 cycles of amplification": col 10, lines 26-27
4. Detection	SK19 probe labeled with HRP for enzyme linked affinity assay read at 490 nm	In a preferred embodiment, "probe may be labeled with horseradish peroxidase (HRP) and copy number may be evaluated by an enzyme linked affinity assay . . . measured at 490 nm": lines 64-67 and col. 5, lines 27-28	"Enzyme linked affinity assay . . . measured at 490 nm": col 10, lines 33 and 58. The probe used was "SK19" labeled with "horseradish peroxidase (HRP)": col 10, lines 15-16
5. Quantification	External standard curve with cRNA *gag* gene standard of known copy number	"Copy number from subject samples may be determined from the absorbances obtained from a dilution series of an RNA gag gene construct of known copy number. (Holodniy et al., 1991, J. Infect. Dis. 163:862-866)": col. 5, lines 32-36.	"Copy number from subject samples may be determined from the absorbances obtained from a dilution series of an RNA gag gene construct of known copy number. (Holodniy et al., 1991, J. Infect. Dis. 163:862-866)": col. 10, lines 62-66

40. As can be seen, for quantitation -- which is the key step in this assay -- the patent specification directly references the 1991 JID paper.

41. In addition, in the JID paper and the example and description in the patent, the RNA standard used to generate the standard curve is "external." This means the standard is amplified in a separate tube from the unknown test sample.

42. The assay described in the patent is reported to have the same background threshold as that set forth in the 1991 JID paper, based on a the average background absorbance for HIV negative samples + 3 standard deviations of 0.135, corresponding to 40 HIV RNA copies per 200 µl of sample. See Col. 10, lines 59-67; col. 12, lines 51-52.

- **Patent Claim Interpretation**

43. It is my opinion that given the state of the art of PCR quantification for HIV targets as of May 1992, one of skill in the art would have understood the measuring step described in the patent to mean end point PCR.

44. Not only was real time PCR not yet developed, but all of the claims refer to 30 cycles of PCR or about 30 cycles of PCR. Specifying a set number of cycles indicates to one of skill in the art that the scientist is to perform the specified number of cycles and then examine a portion of the amplified PCR product at the completion of the amplification; this is end point PCR. In real time PCR, measurements are performed by non-invasive measurement methods, from cycle to cycle, throughout the amplification process, which is typically carried out for more than 30 cycles to maximize sensitivity.

45. To the extent the "testing for the presence" or "testing for the absence" are interpreted to refer to quantification of HIV RNA, it is also my opinion that those terms would have been understood to mean that HIV RNA was detectably present or absent, based on an end point RT PCR assay of a fixed sensitivity.

46. In addition, it is my opinion that one of skill in the art of PCR techniques would have understood the quantification steps to refer to the skills and techniques available for HIV quantification described in the patent and then available art -- specifically the HIV standards and methods then available and/or described for quantification. Since that time, numerous improvements have been incorporated into quantitative PCR assays for HIV, for example including the use of internal controls, and “armored RNA” standards, in which RNA control standards are packaged within bacteriophage coat proteins and spiked into test samples to provide

internal controls for the efficiency of recovery of HIV RNA from extraction of the source specimen.

I declare under penalty of perjury that the foregoing is true and correct.

Executed at ROCKVILLE, MD, August 28 2007

Jeffrey D. Lifson, M.D.