EXHIBIT 2

Dockets.Justia.com

UNITED STATES DISTRICT COURT

DISTRICT OF MASSACHUSETTS

AMGEN INC.,	)	
Plaintiff,	)	Civil Action No.: 05-12237 WGY
v.	)	
F. HOFFMANN-LA ROCHE LTD., a Swiss Company, ROCHE DIAGNOSTICS GmbH, a German Company and HOFFMANN-LA ROCHE INC., a New Jersey Corporation,	)	
Defendants.	)	

EXPERT REPORT OF DON H. CATLIN, M.D.

SUBJECT TO PROTECTIVE ORDER

CONTAINS AMGEN CONFIDENTIAL MATERIAL

TABLE OF CONTENTS

PAGE NO.

I. INTRODUCTION 1

II. SUMMARY OF OPINIONS 7

III. TESTING FOR EPO DOPING USING ISOELECTRIC FOCUSING 8

A. The Doping Control Test Procedure 8

1. Sample preparation 10

2. Isoelectric focusing 11

3. Immuno-blotting 14

4. Visualization 15

B. The Doping Control Test in Practice 17

C. The Validity, Accuracy, and Reliability of the EPO Doping Test 18

IV. ORIGIN OF SAMPLES 20

V. METHODS 22

VI. RESULTS 24

VII. INTREPRETATION 25

I. INTRODUCTION

1. I am President of Anti-Doping Research ("ADR"). ADR is a recently formed research institute with a mission to create innovative solutions to problems in detecting the use of sports doping agents.

2. Unfortunately, at the pinnacle of sport, such as the Olympic Games, there are extreme pressures to succeed through cheating by taking performance-enhancing drugs. My goal throughout my career has been to protect and develop the integrity of sport by keeping it unsullied by drug use. Drug use is unfair to honorable, rule-abiding competitors and to the sports-loving public.

3. Thus, for more than 25 years, I have devoted my practice and research to detecting illicit doping by athletes. Doping is defined by the Court of Arbitration for Sport (CAS) as "the presence in the athlete's body of a prohibited substance."[1]

4. In 1960, I graduated from Yale University with a B.A. degree. In 1965 I graduated from the University of Rochester with a M.D. From 1965-1969, I served as an intern and resident in Medicine at the University of California, Los Angeles (UCLA). From 1969-1972, I served a tour in the U.S. Army as a major stationed at Walter Reed Army Institute of Research. From 1972 - 2007, I served on the faculty at UCLA as an assistant, associate, and full Professor of Molecular and Medical Pharmacology. In March 2007, I resigned from my position to focus on my research at ADR full-time. I remain a professor emeritus at UCLA.

5. In the course of my academic career, I have trained many students in my

---

[1] Olympic Movement. Anti-doping code. *http://www.gnoc.com/code.htm*. *See USADA v. Fuentes*, AAA No. 30 190 0075904 at 2. *See also* the World Antidoping Code http://www.wada-ama.org/rtecontent/document/code_v3.pdf.

discipline. Moreover, I have tried to instill in them both exacting scientific standards and a high sense of ethics and fairness. My former students are now involved in all aspects of the medicine and chemistry. I have trained hundreds of medical students, some of whom specialize in the disciplines of sports medicine and toxicology. Chemists I have trained are employed throughout the analytical chemistry industry, doping laboratories and pharmaceutical companies.

6. In 1982, I founded the first sport testing laboratory in the United States (the UCLA Olympic Analytical Laboratory) and directed this laboratory for 25 years while it became the largest and most successful such laboratory in the world. It was accredited by the International Olympic Committee (IOC) and later by the World Anti-Doping Agency (WADA). It is also ISO (International Organization for Standardization) certified. The laboratory routinely tests for over 200 different illicit compounds. Among the laboratory's accomplishments are performing the testing for the 1984 Olympic Games of Los Angeles, the 1996 Olympic Games of Atlanta, and the 2002 Olympic Games of Salt Lake City. Its clients include the United States Anti-Doping Agency ("USADA," which oversees drug testing for the U.S. Olympic Committee), the National Collegiate Athletic Association, Major League Baseball, the National Football League, the U.S. Department of Justice, the U.S. Department of Defense, and many smaller clients.

7. Over the course of my career my laboratory has developed at least 8 new tests for performance-enhancing drugs that were previously undetectable. I have also been responsible for placing a number of drugs on the WADA and before that the IOC's list of prohibited substances. I am probably best known for developing tests to catch users of "designer steroids."[2] For example, I developed the first test to detect the steroid tetrahydrogestrine "THG"

---

[2] Catlin, D.H., *et al.*, "Tetrahydrogestrine: discovery, synthesis, and detection in urine," *Rapid*

which was at the center of the well known (and still ongoing) BALCO scandal involving many professional athletes. My laboratory worked with the U.S. Department of Justice on the BALCO case. I also developed the carbon isotope ratio (CIR) test which differentiates between testosterone produced naturally and pharmaceutical testosterone introduced externally. This is the test that caught Floyd Landis, who came in first in last year's Tour de France cycling competition.

8. Over the years the Olympic Analytical Laboratory has caught literally thousands of athletes using performance enhancing drugs. Many of these athletes have suffered serious punishments, such as suspension from competition for extended periods of time, based on the results of our testing.

9. Many sporting bodies, including both the Olympic organizations and professional sports leagues have relied solely upon my testing results to make decisions both about individual athletes and their drug abuse policies. I have presented evidence to the national and international Courts of Arbitration for Sport on numerous occasions, and they have also relied on my work to identify and punish illicit performance enhancing drug use.

10. Because of the potentially career-ending implications of a positive drug test, those of us who administer such tests must do so with scrupulous and meticulous care. Over the years, I have been involved, as a member of the international drug testing community, in designing and implementing a series of checks and balances to avoid incorrectly accusing honest athletes at all costs. For example, I introduced to the International Olympic Committee that testing labs be required to obtain ISO certification before they are eligible for IOC (now WADA) accreditation.

Commun. Mass. Spectrom. 18:1245-49 (2004).

11. Our laboratory has very specifically designed procedures for testing and documentation to avoid mistakes. Our procedures are constantly scrutinized by the accrediting organizations and, importantly, by the accused athletes themselves. For example, each sample of athletes' urine is divided into two portions. If the first, or "A" sample is found to be positive for a prohibited substance, the "B" sample is tested. This second test is done in the presence of the athlete and/or any representatives or experts the athlete chooses. Only if the "B" sample is also positive is the athlete at risk of any penalties. Needless to say, the athletes and their experts study every aspect of our procedures to avoid punishment.

12. My professional interests include the detection of drugs on the IOC/WADA list of prohibited substances, and in particular the effects and the detection of anabolic steroids and of erythropoietin ("EPO"). I am the author of more than one hundred scientific articles and book chapters.

13. I have also been deeply involved in popularizing and supporting the acceptance of the test for recombinant human erythropoietin ("EPO") doping. After this test was originally developed in France by Francoise Lasne and her colleagues, I adopted it in my laboratory and I was instrumental in its adoption as the recognized test for EPO doping by the international sporting bodies. I am an author, along with Drs. Lasne, Howe, Nissen-Lie, Pascual, and Saugy of the WADA Technical Document[3] that sets forth the current international standard procedure for EPO testing. I have authored three peer-reviewed publications on the detection of recombinant human EPO and darbepoetin alfa. The UCLA Olympic Analytical Laboratory now has five years of experience with the EPO doping test and is a WADA-accredited testing center for the drug.

---

[3] http://www.wada-ama.org/rtecontent/document/td2004epo_en.pdf.

14. In my work on the detection of EPO and darbepoetin alfa, I extensively studied and used the Lasne method, which is known as isoelectric focusing ("IEF"), to accurately and reliably detect the presence of recombinant human EPO and darbepoetin alfa separate and apart from any naturally occurring EPO that may have been present in the blood or urine of a test subject. For instance, during the 2002 Winter Olympics, I detected and reported on the first instances of darbepoietin alfa use in athletes and successfully defended the results in cases before the Court of Arbitration for Sport ("CAS").

15. A copy of my curriculum vitae, reflecting my professional experience, affiliations, and work is attached to this report as Exhibit A. I intend to testify fully as to my credentials and experience relevant to the matters addressed in this Report.

16. Within the last four years, I have testified in the following matters:

- Deeja Youngquist: American Arbitration Association, Arbitration Tribunal. United States Anti-doping Agency, claimant and Deeja Youngquist, respondent, February 2005. Decision: Respondent committed a doping violation [recombinant human EPO found in urine of athlete].

- Adam Bergman: Court of Arbitration for Sport, Arbitration between United States Anti-doping Agency, appellant and Adam Bergman, respondent, February 2005. Decision: The Respondent was guilty of a doping offence [recombinant human EPO found in urine of athlete]. The respondent was declared ineligible for a period of two years.

- Genevieve Jeanson: while waiting for a date before the Court of Arbitration for Sport, USADA and Ms. Jeanson reached an agreement whereby Ms. Jeanson acknowledged that the positive test report [for use of recombinant human erythropoietin] performed by the UCLA Olympic Analytical Laboratory is a violation of the applicable rules, including the USADA Protocol and the UCI Anti-Doping Rules, (both of which have adopted the WADA Code), and accepted punishment of:
  - A two-year period of ineligibility under Article 10

of the WADA Code beginning on July 25, 2005; and,

- Pursuant to Article 10 of the WADA Code, disqualification of all competitive results obtained on or subsequent to July 25, 2005, including forfeiture of any medals, points and prizes.

- Eddy Hellebuyck: Court of Arbitration for Sport, Arbitration between International Association of Athletics Federation, appellant and Eddy Hellebuyck, respondent, May 2006. Decision: The Respondent has committed an anti-doping rule violation[recombinant human EPO found in urine of athlete]. The respondent was declared ineligible for a period of two years.

- Adham Sbith: American Arbitration Association, Arbitration Tribunal. United States Anti-doping Agency, claimant and Adham Sbeih, respondent, March 2004. Decision: Respondent committed a doping violation [recombinant human EPO found in urine of athlete].

- Nina Kraft public declaration that she used recombinant human EPO, no arbitration.

- Athlete A, name not known, accepted a two-year penalty thereby avoiding arbitration.

- Arbitral Award: CAS 2002/A/370 Lazutina v. IOC. Decision: Respondent committed a doping violation. Darbepoetin found in urine of athlete.

- Arbitral Award: CAS 2002/A/374 Muehlegg v. IOC. Decision: Respondent committed a doping violation. Darbepoetin found in urine of athlete.

Cases not involving EPOs (partial list)

- Arbitral Award: CAS 2002/A/376 Baxter v. IOC. Athlete found guilty of a doping offense involving a stimulant.

- Arbitral Award: CAS 2002/A/389-393 (Austrian Ski Team) v. IOC. Team found to have committed a doping offense.

17. I am being compensated for my expert consulting work at the rate of $300 per

hour. ADR is also receiving payment for the materials and labor required to perform the experiments described below. Moreover, ADR received a grant from Amgen for $100,136.00 for research that might lead to a new test for EPO doping. I am very proud of our association with Amgen. Amgen is the only major pharmaceutical company I know of that has taken appropriate responsibility for the potential abuse of its products by providing funding and its expertise to the anti-doping effort.

18. I reserve the right to supplement my report if further relevant information comes to my attention. I may also prepare and present further graphics, demonstratives, or other presentation aids consistent with the contents of this report.

II. SUMMARY OF OPINIONS

19. I am submitting this Expert Report in support of the validity of U.S. Patent Nos. 5,447,933; 5,618,698; 5,756,349; 5,955,422; 5,441,868; and 5,621,080.

20. I have been engaged to perform the technique of isoelectric focusing (IEF) that I have used in my work on blood doping to test whether or not naturally-occurring human EPO and recombinant human EPO differ in structure, whether in purified form or as excreted in urine, or as present in the cell conditioned media of EPO-producing recombinant cells.

21. Shortly following receipt of samples of EPO from Amgen and obtaining samples of urine, scientists at ADR, operating under my direction and supervision, performed an IEF analysis according to our standard procedures (Exhibit H and I). As described in detail below, the resulting IEF banding patterns for the samples of recombinant EPO are each similar to the pattern for Amgen's recombinant EPO product Epogen® and are each different from the banding pattern of naturally-occurring EPO found in the urine of a normal known negative individual and from the International Standard for purified urinary EPO obtained from the World

Health Organization.

III. TESTING FOR EPO DOPING USING ISOELECTRIC FOCUSING

22. In 1998, there arose great concern that cyclists competing in the Tour de France were doping with recombinant human erythropoietin ("rHuEPO"). The discovery of rHuEPO and other drugs in the trunk of automobile just before the race led to a widespread investigation that eventually resulted in recovery of many drug products, confessions from several athletes, and evidence of widespread involvement of cycling teams and coaches.[4] In response to these revelations, the World Anti-Doping Agency was formed. Thus, in 1998, there was great impetus to develop a technique that could accurately and reliably detect the presence of recombinant EPO in the human body separate and apart from the presence of any naturally occurring EPO.

A. THE DOPING CONTROL TEST PROCEDURE

23. The first successful attempt to develop a direct test for rHuEPO in urine came in 1995 with the description of a method for detecting rHuEPO in urine by isoelectiric focusing gel electrophoresis.[5] Although this test had practical limitations, it demonstrated conclusively that the isoform[6] pattern and content of native human EPO present in urine and that of rHuEPO

---

[4] Swift EM, "Drug pedaling," *Sports Illustrated* 7-5: 60-65 (1999); Anonymous, "A sport in shame," *Sports Illustrated* 7-27: 28-33 (1998); Jarvis CA (1999) "Tour de Farce," *Br. J. Sport Med.* 33: 142-143

[5] Wide, L., Bengtsson, C., Berglund, B., Ekblom, B., "Detection in blood and urine of recombinant erythropoietin administered to healthy men," *Med Sci Sports Exerc* 27: 1569-1576 (1995).

[6] "Isoforms" are different versions of the same protein which differ in some measurable characteristic. In this report, "isoforms" are versions of EPO which differ in their isoelectric focusing point. "Glycoforms" are versions of the same protein which differ specifically in the structure of their attached sugar structures.

differ.

24. In 2000, the direct detection of rHuEPO in urine was further improved when Lasne and de Ceaurriz combined isoelectric focusing with a double immuno-blotting procedure ("double blotting").[7] In my testimony, I intend to explain this method.

25. During my testimony, I intend to explain what isoelectric focusing is and how it is performed. See Exhibit K, attached. For instance, I intend to explain that isoelectric focusing is a well-established and scientifically validated laboratory procedure for detecting whether or not the electrical charge of proteins that are being compared differs. It is performed by applying protein samples in lanes on a gel-like substance that has been prepared such that it has a pH[8] gradient across the length of each lane. In our laboratory the pH range of the gel is 2 - 6. Thus, one end of the gel lanes is relatively more acidic,[9] and the other end is relatively more basic.[10] When an appropriate electric field is applied to the gel, a sample protein will migrate (travel) across the gel until it reaches its own state of balanced charge with the pH at such location; the corresponding pH number is said to be the "isoelectric point" or pI for that

---

[7] Lasne, F., "Double blotting: A solution to the problem of non-specific binding of secondary antibodies in immunoblotting procedures," *J. Immunol. Methods* 253: 125-131 (2001); Lasne, F., Martin, L., Crep, N., de Ceaurriz, J., "Detection of isoelectric profiles of erythropoietin in urine: Differentiation of natural and administered recombinant hormones," *Anal. Biochem.* 311:119-126 (2002). Streptavidin-horseradish peroxidase is a protein that will bind to the second antibody, after binding, it can act as an enzyme which performs a chemical reaction on the substrate molecule to generate light ("chemiluminescence") which can be captured as an image of where the erythropoietin protein molecules were on the gel.

[8] "pH" is a measure of the acidity or alkalinity of a solution. Solutions with a pH less than seven are considered acidic, while those with a pH greater than seven are considered basic (alkaline). pH 7 is considered neutral because it is the pH of pure water at 25° C. pH measurements generally range between 0 and 14.

[9] An "acid" is any chemical compound that, when dissolved in water, gives a solution with a pH less than 7.0. Acids are compounds that can donate a hydrogen ion (H^+, also known as a "proton") to another compound (called a "base").

particular sample.

26. Every protein molecule has a characteristic pI. pI is a reflection of all the charged groups[11] attached to the protein molecule. These charged groups can include certain amino acids.[12] These charged groups can also include post-translational modifications that carry a charge, such as some sugar groups like sialic acid. Often, particular proteins like erythropoietin are made up of a mixture of molecules which differ in their composition of these charged groups. Individual members of such a molecular mixture are known as isoforms. Such proteins and their isoforms can be distinguished by differences in their pI.

27. The entire analysis consists of four steps: sample preparation; isoelectric focusing, *immuno-blotting*, and visualization. The following description is intended to be a summary for the non-specialist.

1. Sample preparation

28. The task is to prepare the urine sample so that it is suitable for IEF analysis. This involves two steps. The first involves inactivating enzymes that could destroy the erythropoietic proteins before the sample gets to the IEF phase of the analysis. The second is to remove materials in the urine that are detrimental to the analysis. This step involves concentrating the proteins (rHuEPO is a protein). First, we add protease inhibitors (chemicals) to the urine to deactivate enzymes which might otherwise destroy the erythropoietic proteins thereby foiling the analysis. Next, we use specialized filters that remove molecules with low

---

[10] A "base" is a substance that can accept protons from acids.

[11] Certain chemicals, known as "ions" can gain or lose atoms or electrons in such a way to attain either a positive or negative electrical charge. A protein molecule can have numerous different charged groups, some of which may be negative, and others positive.

[12] Of the twenty amino acids which make up proteins, aspartic acid and glutamic acid are acidic.

molecular weight. Erythropoietins are high molecular weight proteins. We do this by placing the urine in a cup that has the specialized filter at the bottom of the cup. The cup is placed in a centrifuge which spins around like a merry-go-round. As the spinning takes place, the low molecular weight material passes through the filter and the EPO (in this case, EPO and certain other substances is retained on the filter. This material is called the retentate.

29. This step is repeated one more time. If rHuEPO is present in the urine, it will be in the final retentate along with any endogenous naturally occurring EPO. The final retentate is a liquid. A small portion of it is used to estimate the concentration of EPO by an immunoassay. Another portion is spotted on the gel after adjustment of the concentration. From the immunoassay we obtain an estimate of the amount of rHuEPO in the urine sample. We use this number to adjust the concentration of rHuEPO in the retentate to a uniform value. We do this so as to 'spot' a relatively uniform amount of material on the gel. If we did not spot a relatively uniform amount of material on the gel the lanes in the electropherograms would have widely variable amounts of material and they would be difficult to interpret.

2. Isoelectric focusing

30. Before we can perform the electrophoresis we must prepare a gel. The gel is about 25 by 12 cm (length and width) and about 1 mm thick. The gel is a jelly-like material that serves as the 'platform' for the electrophoresis. The gel is prepared by mixing various chemicals in a flask and pouring the mixture into a cast. Just before the pour, a reagent is added that causes the materials to "gel." After the gel "hardens" to the consistency of a flexible jelly-like material, the cast is removed and the gel is placed flat on the surface of the electrophoresis instrument. The electrophoresis unit is designed to accommodate the gel on its flat surface. Electrodes are

---

Histidine, lysine, and arginine are basic.

attached to the gel and connected to the electrophoresis unit such that a current of electricity can be applied to the gel.

31. The samples and standards that are to be electrophoresed are "spotted" onto the gel by adding a small volume (20 micro liters) of each sample to a piece of filter paper (10 by 5 mm) that has been placed on the gel. The pieces are placed 1 cm apart close to one edge of the gel. The gel can accommodate about 13 such pieces. In this way, the surface of the gel is divided into 13 imaginary lanes. One of the electrodes attached to the gel is the anode and the other is the cathode. The electrophoresis unit is turned on. This sets up an electric circuit between the cathode (negative or minus pole), and the anode (positive or plus pole). Electrical current flows and the glycoproteins that have been spotted on the gel begin to move.

32. Glycoproteins such as rHuEPO are molecules that carry positive or negative charges. The net charge of the glycoprotein is the algebraic sum of all the positive and negative charges. Each molecule has one net charge at a given pH. When the current is applied, a pH gradient is established between the anode and the cathode. For the electropherograms that our laboratory produces, the anode or plus pole is generally oriented along the upper or top margin on the gel, and the cathode or minus pole is at the opposite side of the gel, that is along the bottom or lower edge of the gel. This is a convention and other laboratories may use the reverse orientation. It does not matter.

33. One of the characteristics of molecules is the pH of their water solutions. It is a unit of measurement that extends from 1 to 14, with 1 being acidic and 14 being basic. If the water solution of a molecule has a low pH we say it is an acid or acidic. If the pH is high we call it a base or basic. For example, orange juice is acidic and has a low pH, while milk is more basic and has a higher pH. The pH refers to the relative amount of acid (or base) in a material.

34. As I mentioned above, the charged molecules migrate (move) when the current is applied. Under the influence of the electrical field, charged molecules migrate in the direction of the electrode bearing the opposite charge. Thus, negatively charged or more acidic molecules migrate toward the anode — the electrode with the positive charge — and positively charged basic molecules move in the other direction. The molecules move slowly along the vertical axis of the gel to their isoelectric point. The isoelectric point (pI) is a fundamental characteristic of proteins. The pI of the molecule is the pH value at which the molecule is electrically neutral. In our work, the isoelectric point is the specific location on the gel where the number of positive charges on the molecules of EPO are each exactly balanced by the number of negative charges. The current is applied to the gel for three hours. During the first half hour, before the samples are spotted onto the gel, the pH gradient is being established. Then the samples are spotted and the process continues. The total number of hours is selected such that all the molecules have sufficient time to migrate or move to their isoelectric point. Once they reach the isoelectric point they remain stationary.

35. Each sample or standard is spotted in one lane. The number of samples and standards that are processed on one gel is determined by the intent of the study. Typically we use one or two different standards, one or more control samples (urines with known content), and several unknowns (content not known). Typically the standard is pure rHuEPO, or pure darbepoetin, or a mixture of the two. Typical control urines are a urine obtained from a subject to whom we have administered darbepoetin or rHuEPO and a urine from a subject whom we are certain has not received any erythropoietic agent. With this background, one can now interpret an electropherogram that shows rHuEPO. Not all the molecules of rHuEPO have exactly the same chemical structure and therefore the same charge. All the molecules in one band will have

the same isoelectric point, which means they will have the same net charge. That is not to say that all molecules in one band have an identical molecular structure — different structures can have the same isoelectric point. Naturally occurring endogenous EPO found in the urine has many different isoforms that are spread from the anode (positive) end of the gel to the center. In contrast, rHuEPO migrates toward the cathode (negative) end because it has fewer negative charges. This explains how rHuEPO and endogenous EPO can be discriminated in the gel.

3. Immuno-blotting

36. At this stage the bands are separated on the gel. The task now is to remove them from the gel and to mark them in some fashion so that their location can be observed with the naked eye. This is accomplished by 'blotting' and many more steps. Blotting is a procedure for transferring proteins from one surface to another. Two blotting procedures are used (blot 1 and blot 2). The first blot transfers the proteins from the gel to a membrane (membrane #1) and the second transfers the proteins from membrane #1 to membrane #2 (second membrane). The gel is removed from the electrophoresis plate, washed with a buffer, and placed between two stacks of paper that have been soaked is a special blotting buffer. This stack (or sandwich) is placed into an instrument which is designed to apply an electrical charge across two plates. The stack is placed between the two plates and the current is turned on for 30 minutes. The instrument is referred to as the 'Blotting unit.'

37. During this stage, the proteins 'travel' from the gel to membrane #1. Membrane #1 is a mirror image of the material that was on the gel. Next, membrane #1 is bathed in a solution of antibodies.[13] These antibodies are very special and critical to the analysis

---

[13] Antibodies are proteins found in the blood of animals. Many antibodies bind tightly to a single type of molecule, such as a specific protein. Anti-EPO antibodies bind tightly to EPO, but essentially no other molecule. As such, anti-EPO antibodies can be used to detect EPO from

because they specifically bind to human EPO. The antibodies are produced in mice who were immunized with a piece of the EPO protein that causes them to produce antibodies. The specific EPO antibodies are then harvested from the mice and used in various assays for EPO. Because we also use another antibody later in the assay, we sometimes refer to this antibody as the 'first' or primary antibody.

38. In the next stage, referred to as the second blot, the primary antibody is removed from the first membrane and transferred to a second membrane (membrane #2). The molecules of EPO remain on the first membrane, but the antibodies against them are transferred to the second membrane. The test detects these second antibodies, which mark the location of the rHuEPO. The second blot is accomplished like the first blot. Then the second membrane is incubated in a solution containing a second antibody that binds specifically to the first antibody. The first membrane is saved in case the second blotting step needs to be repeated.

4. Visualization

39. The location of the second antibody on membrane #2 matches the location of EPO on membrane #1. We need a way to visualize the second antibody. To this end, a marker protein called strepavidin-horseradish peroxidase which binds to the second antibody is used. Next a substance (or "substrate") is added that emits light when it comes in contact with the marker protein. The emitted light is then captured with a special digital camera. The final image is used to evaluate the results.

40. This method of isoelectric focusing allows us to verify with the naked eye that naturally occurring human EPO as it circulates in normal human bodies and is excreted into urine is composed of a number of different isoforms, which distribute themselves on the

---

amongst a complicated mixture of biological molecules.

isoelectric focusing gel much like a unique bar code or fingerprint. Although recombinant human EPO also is composed of a number of isoforms, the pattern of those isoforms is different from that of naturally occurring human EPO — in other words, this collection of isoforms distribute themselves on the gel as a distinct bar code or fingerprint. Thus, the method also allows us to verify with the naked eye that recombinant human EPO (whether from a vial as sold or as present in the urine of individuals who have been injected with it) has an isoform pattern that is strikingly different from that of naturally-occurring human EPO. This method shows that naturally-occurring human EPO has isoforms not present in recombinant human EPO. Additionally, after sufficient doses of recombinant human EPO have been given to a normal individual, this method of isoelectric focusing shows that the pattern of isoforms corresponding to the individual's naturally occurring EPO no longer appears in the samples of urine as taken from the individual over time. Therefore, this method of isoelectric focusing allows us to determine if EPO is naturally occurring or recombinantly produced.

41. Because there are differences between naturally occurring and recombinant EPO and because this method can reliably and accurately detect them, it can be used to reliably and accurately detect which individuals are blood doping with recombinant EPO and which are not — without being lead astray by the presence of naturally occurring EPO. Without naturally occurring and recombinant EPO being different from one another, the WADA IEF doping test would not work because there would be no means to reliably and accurately detect which individuals are doping with recombinant EPO and which have only naturally occurring EPO present in their bodies.

42. Thus, the IEF test for EPO works by the same general principle as most doping tests. An analytical technique that is capable of discriminating between the chemical

properties of a foreign compound (the drug) and all endogenous compounds normally found in the body is developed. Analytical techniques such as the IEF test are only useful if there is a difference between the chemical structure or makeup of the foreign compound and the endogenous compounds of the body. If there are no differences between the drug and chemicals in the body, then other types of techniques (such as looking for the symptoms of taking the drug) must be used to detect its abuse.

43. To perform the EPO doping IEF test, WADA laboratory regulations require that analyses include references or standards (i.e., a urine sample from a human subject known to have received EPO — the "positive standard" — and a urine sample from a human subject known not to have received EPO — the "negative standard"). The details of this method and the main finding have been described.[14]

B. THE DOPING CONTROL TEST IN PRACTICE

44. In my testimony, I intend to explain how the EPO doping IEF test has been used in practice. For instance, I intend to testify that during the 2002 Games of Salt Lake City, our laboratory analyzed 598 urine samples for the drugs on the IOC List of Prohibited Substances. In addition, 1,222 blood samples were obtained from athletes competing in the endurance sports of cross-country skiing, biathlon, Nordic combined, long-track speed skating, and short-track speed skating. These blood samples were tested at the venues by international sport federations before competition for indicators of EPO doping. If these preliminary tests revealed suspicious results, when the athlete finished competing additional blood and urine

---

[14] Lasne, F., Martin, L., Crep, N., de Ceaurriz, J., "Detection of isoelectric profiles of erythropoietin in urine: Differentiation of natural and administered recombinant hormones," *Anal Biochem.* 311: 119-126 (2002); Catlin, D.H., Briedbach, A., Elliott, S., Glaspy, J., "Comparison of isoelectric focusing patterns of darbepoetin alfa, recombinant human erythropoietin and

samples were obtained for EPO analysis at my laboratory.

45. In total, my laboratory received 77 combined blood and urine samples. Of these, samples from three athletes met our criteria for illicit use of darbepoietin alfa (a new erythropoiesis stimulating protein). All three athletes were cross-country skiers who had won a total of eight medals.

46. According to the procedure in effect at these Olympics, before announcing test results to the public, a committee reviewed the laboratory findings and held two hearings. The hearings were attended by representatives of the athletes and delved into the details of the testing. In each case, the testing results were upheld. The IOC Executive Board reviewed the cases and made the final decision to announce the findings to the public. In addition, the athlete had a right to appeal the IOC's decision to the CAS. The CAS affirmed the decisions of the IOC and each of three cross-country skiers were stripped of their medals.

C. THE VALIDITY, ACCURACY, AND RELIABILITY OF THE EPO DOPING TEST

47. I intend to testify as to the validity, accuracy, and reliability of the EPO doping IEF test. For example, I intend to testify that in my opinion, based on my knowledge, learning, and experience, the test is valid, accurate, and reliable. The method has undergone an extensive scientific validation process and has been used successfully for many years by accredited anti-doping laboratories around the world. It is a well-established procedure widely accepted by the scientific community, as demonstrated by publication in a number of international scientific peer-reviewed journals. Further, in all its decisions relating to EPO, the CAS has confirmed the validity of the EPO detection method. And, at a meeting in September

---

endogenous erythropoietin from human urine," *Clin. Chem.* 48: 2057-2059 (2002).

2005, the WADA Laboratory Committee reiterated its support of the method when properly applied.[15]

48. I intend to testify to the subject of false positives and negatives. For example, I intend to testify that any analytical test may be subject to errors. Such errors may be false positives (e.g., where an athlete appears to have used rHuEPO even though he or she has not) or false negatives (e.g., where a doping athlete has used rHuEPO but had not been detected). In my opinion, however, the risk of false positives in the tests we use is vanishingly low. To date, the EPO doping IEF test described above has been used to test samples from over 30,000 individuals. Out of all of these tests done by multiple laboratories in multiple locations, over many different occasions, there has been only one false positive. An investigation of this case led to discovery and definition of an "active" urine. This led to a change in the criteria for reporting a positive case. WADA discussed this one potential source of false positives, and determined that under the proper procedures, "there is no risk of a false positive":

> In certain rare circumstances, normal endogenous EPO shifts into the recombinant EPO area. This phenomenon is clearly identified by accredited laboratories so that these rare profiles are labeled properly and are not reported as adverse results due to EPO doping. WADA was informed of this phenomenon by accredited laboratories in the spring of 2005. As a result, WADA instructed all accredited laboratories to integrate this information into their interpretation of results. In addition, laboratories are required to seek a second independent opinion before reporting any adverse result. There is no risk of a false positive reading; all accredited laboratories are in a position to distinguish between this rare profile and exogenous EPO.[16]

49. Conversely, the IEF test does produce some false negative results. This

---

[15] http://www.wada-ama.org/en/dynamic.ch2?pageCategory.id=527.

[16] http://www.wada-ama.org/en/dynamic.ch2?pageCategory.id=527; *See also* WADA 2005 Adverse Analytical Findings Reported by Accredited Laboratories.

logically follows from the way in which WADA has written the criteria for a positive. WADA's idea is to set the criteria for a positive very, very high, so that no one is falsely accused, even at the expense of being unable to punish some cheaters.[17]

50. In the last five years, the UCLA Olympic Laboratory has performed more than 7000 urine analyses for EPO by this method. These analyses were performed on samples collected by the United States Anti-doping Agency (USADA) from Olympic athletes mainly in cycling and track and field events. About ten percent were collected from athletes competing in National Collegiate Athletic Association (NCAA) events. Out of these 7000 samples, we identified 9 athletes who had improperly used recombinant EPO. Of these, three have publicly confessed to using rHuEPO, three have accepted penalties and not contested the laboratory result, the physician for a seventh has been indicted for distributing rHuEPO, the eighth maintains his innocence and has appealed to CAS, and no information is available on the ninth case.

IV. ORIGIN OF SAMPLES

51. In my testimony, I intend to describe the samples that I tested in connection with this litigation. Exhibit C is the chain of custody documentation for the samples I received from Amgen. Exhibits D through F are declarations from Amgen's employees who sent the samples, and explain what the samples are, how they were obtained, and how they were shipped to me. Exhibit G are the package inserts found in the recombinant EPO packages. Exhibit J is the package insert for the urinary EPO ("2nd IRP).

---

[17] WADA Technical Document of EPO detection. http://www.wada-

52. On April 6, 2007, I received from Amgen its pharmaceutical Epogen®, and a sample of the cell culture medium ("CCM") from the EPO-producing recombinant cells used to make Epogen® — obviously the CCM material was not purified in any way.

53. On April 10, 2007, I received from Amgen seven EPO pharmaceutical products obtained from India, China, Mexico, Argentina, and Korea and not made by Amgen or any company affiliated with it:

Origin Country	Name	Manufacturer
China	China-Epiao	SS-Pharm
China	SEPO	China-SPG
Korea	Espogen	LG
India	Shanpoietin-1	Shantha Biotech
India	Wepox	Wockhardt Limited
Argentina	Zyrop	Bio Sidus S.A
Mexico	Epomax	Cryopharma Lab

54. Each of the samples I received was in good condition, still on ice, with all packaging and seals intact.

55. On February 7, 2007, I purchased and obtained a sample of urinary EPO (uEPO) standard reference material distributed by the World Health Organization ("WHO") from the United Kingdom National Institute for Biological Standards and Control (NIBSC). I understand this uEPO sample to be the 2nd International Reference Preparation as the catalog listing for it reads: "Erythropoietin, human, urinary. Lyophilized. 10 IU / ampoule. 2nd International Reference Preparation, 1970 No. 463, 23rd Report Urinary hormone NIBSC Code:

---

ama.org/rtecontent/document/td2004epo_en.pdf.

67/343 70.1015."[18] The package insert for this sample is attached as Exhibit J. This material was originally concentrated from the urine of patients suffering from hookworm anemia in 1968.[19]

56. On February 10, 2007, we also obtained urine samples from normal healthy males and from a male treated with Epogen®. This is material that we generated in a study in which we administered Epogen® to normal human volunteer subjects, for use as a positive control in our EPO doping test to make sure that we could detect recombinant human EPO.

V. METHODS

57. We tested the samples described here using the same procedure we have developed to test athletes' urine samples for EPO doping. We used the same care to process the samples and document the results as we do when we test to determine whether athletes are cheating.

58. The details of the methods used to generate the data herein are fully described in the peer-reviewed literature. The IEF method for EPO was first described by Lasne in a letter to Nature in 2000.[20] My colleagues and I have published peer-reviewed scientific articles concerned with the detection of darbepoetin alfa and recombinant human EPO in human urine,[21]

---

[18] http://www.who.int/biologicals/reference_preparations/catalogue_de/en/print.html.

[19] Annable, L., Cotes, P.M., and Mussett, M.V., "The second international reference preparation of erythropoietin, human, urinary, for bioassay," *Bull. Wld. Hlth. Org.* 47:99-112 (1972).

[20] Lasne,F., de Ceaurriz, J., "Recombinant erythropoietin in urine," *Nature* 405: 635 (2000).

[21] Catlin, D.H., Breidbach, A., Elliott, S., Glaspy, J., "Comparison of the isoelectric focusing patterns of darbepoetin alfa, recombinant human erythropoietin, and endogenous erythropoietin from human urine. *Clin. Chem* 48:2057-2059 (2002); Breidbach, A., Catlin, D.H., Green, G.A., Tregub, I., Truong, H., Gorzek, J., "Detection of rHuEPO I urine by isoelectric focusing," *Clin. Chem.* 49:901-907 (2003).

and an extensive review of the history, practice, and detection of doping with EPOs.[22] Exhibits H and I describe the methods we used in further detail.

59. The samples of EPO pharmaceutical products obtained from India, China, Mexico, Argentina, and Korea, as well as the samples of Epogen® and the uEPO standard were prepared for spotting according to the protocol described in Exhibit H.

60. The urine samples were prepared for IEF by a series of steps that included centrifugation, filtration, and concentration. The urine samples were included to assess whether both urine samples and pharmaceuticals can be simultaneously analyzed, and whether pharmaceuticals can be detected in urine samples.

61. Approximately 12 mIU of each sample was spotted on the gel and isoelectric focusing gel electrophoresis was performed. Exhibit L shows the image obtained from the gel. Each sample was spotted in one lane. There are 13 lanes numbered consecutively 1-13 from the left. The sample of Normal (known negative) Human Urine (NHU) was spotted in lanes 1 (leftmost lane) and 12 (rightmost lane). The sample of the uEPO International Standard is in lane 13. The sample of Epogen® is spotted in lane 2. The sample of urine collected from a male treated with Epogen® is in lane 3. The sample of cell culture medium (CCM) collected from recombinant EPO-producing cells is in lane 11. The samples of the EPO pharmaceutical products obtained from India, China, Mexico, Argentina, and Korea are in lanes 4-10. The Figure is labeled with the identity of each sample.

---

[22] Catlin, D.H., Hatton, C.K., Lasne, F., "Abuse of Recombinant Erythropoietins by Athletes," In: Molineux G, Foote MA, Elliott S, eds. Erythropoietins and erythropoiesis: Molecular, Cellular, Preclinical, and Clinical Biology. Birkhäuser Verlag, 2003:205-227.

VI. RESULTS

62. I intend to testify as to the results obtained from this experiment and in particular to explain what Figure 1 shows. For example, I intend to explain how Figure 1 shows the relative migration patterns of the samples of the products obtained from India, China, Mexico, Argentina, and Korea as compared on the same gel to those of Epogen®, the unpurified CCM from Amgen's recombinant EPO-producing cells, the purified urinary EPO, and the urine from normal healthy males and from a male treated with Epogen®. Specifically, I intend to explain how the migration patterns of all of the recombinant EPO samples compare with that of all of the samples of urine normal healthy males. I intend to explain how the migration patterns of all of the recombinant EPO samples compare with that of the sample of purified urinary EPO. I intend to explain how the migration patterns of all of the recombinant EPO samples compare with that of the sample of urine from a male being treated with Epogen®. I intend to explain how the migration patterns of all of the recombinant EPO samples compare with that of the sample of the unpurified CCM from Amgen's recombinant EPO-producing cells. I intend to explain how the migration pattern of the sample of the unpurified CCM from Amgen's recombinant EPO-producing cells compares with those of all of the urine samples and of the purified urinary EPO. I intend to explain how the migration patterns of all of the urine samples compare with that of the urinary EPO reference standard.

63. In particular, I intend to explain how and where Epogen® has three distinct bands and two faint bands on Figure 1. In the WADA system,[23] these bands are numbered 1-5, with 5 being the most basic. The two products from China in lanes 4 and 5 were the most similar ones to Epogen®, although both had at least one extra faint band in the more basic region of the

gel. All other products clearly had more bands in the basic region of the electropherogram. I intend to testify that none of the EPO pharmaceutical products obtained from India, China, Mexico, Argentina, and Korea had bands of higher acidity than those of Epogen® and will show where and how on Figure 1 this is proven. I also will testify that the negative control — the normal human urine (NHU) sample — had at least 11 bands, the vast majority of which were in the more acidic region of gel and at a location different from where the bands for the various recombinant EPO products can be found, and will show where and how on Figure 1 this is proven. This result is typical of the many thousands of normal urine samples we analyzed in our laboratory. As expected, the concentrated uEPO reference standard material (lane 12) appears quite similar to NHU. I also will testify that the positive control — the urine from the Epogen®-treated subject — had normal bands like the NHU control samples but that, like the Epogen® sample, it also had three very dense bands in the basic region, and will show where and how on Figure 1 this is proven. I will testify that in my opinion the data in this lane — i.e., the positive control lane — clearly fulfills the World Anti-Doping Agency (WADA) criteria for reporting the sample as containing rHuEPO. In other words, if the data in this lane had been obtained from an athlete for testing purposes, I would be duty bound to report them to WADA as consistent with that which one would expect for an individual who was in fact doping with recombinant EPO.

VII. INTREPRETATION

64. This experiment demonstrates that with the appropriate equipment and technique a gel can be generated that reliably and accurately displays the pattern of EPO isoforms either derived from human urine or from pharmaceutical preparations of EPO.

---

[23] WADA Technical Document of EPO detection. http://www.wada-ama.org/en/

65. As expected, the concentrated human urine EPO reference standard gives a band pattern similar to a normal human urine sample.

66. The sample of urine from an Epogen® treated individual was clearly positive under the WADA testing criteria. If a person is treated with Epogen®, the pattern clearly shows two sets of bands: the normal bands and the bands due to Epogen®. The density and location of the bands that overlap with the Epogen® bands, however, reveal that the sample came from an individual who was using recombinant human EPO. This is the basis for the WADA urine test for the presence of rHuEPO in human urine.

67. The unpurified CCM from Amgen's recombinant EPO-producing cells shows that Amgen's unpurified EPO starting material as it is produced by mammalian cells grown in culture does not possess any EPO glycoforms that are more acidic than the isoforms found in the purified Epogen® pharmaceutical product.

68. My experiment also reveals that the other recombinant EPOs are somewhat different from Epogen®. The Chinese EPOs are most similar to Epogen®. On close inspection there is a faint band 6. The products from India, Korea, Argentina, and Mexico have more prominent bands 6 and 7. The Mexican product (Epomax) in lane 10 is missing or has fainter bands 1-4. When compared to Epogen®, none of the other recombinant EPO pharmaceutical products have any additional acidic isoforms that are lacking from Epogen®.

69. Based on these data, my learning, and experience, I make the following conclusions:

(i) The EPO isoforms observed in purified urinary EPO standard obtained from the NIBSC are almost indistinguishable from the EPO isoforms observed in the whole urine of a normal individual.

(ii) All recombinant EPOs tested could clearly be distinguished from both EPO in normal urine and the international standard for urinary EPO. The difference in each case is the presence of several isoforms in urinary EPO which are lacking for each recombinant EPO.

(iii) Amgen's unpurified recombinant EPO contains all of the same glycoform bands as Epogen®, except that it has a lower proportion of the most acidic isoforms and it appears to have 3 additional basic isoforms. Unpurified recombinant EPO could also readily be distinguished from both EPO in normal urine and the international standard for purified urinary EPO.

Executed this 11th day of May, 2007 at Los Angeles, California.

DON H. CATLIN M.D.