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UCLA OLYMPIC ANALYTICAL LABORATORY
DEPARTMENT OF PHARMACOLOGY
UCLA SCHOOL OF MEDICINE
2122 GRANVILLE AVENUE
LOS ANGELES, CALIFORNIA 90025
PHONE (310) 825-2635
FAX (310) 206-9077

CONFIDENTIAL

May 27, 2004

Terry Madden
The United States Anti-Doping Agency
2550 Tenderfoot Hill St., Suite 200
Colorado Springs, CO 80906-7346

RE: Specimen number [redacted]

Dear Mr. Madden

Please find enclosed the documentation package for the screen, the A confirmation, and the B confirmation on the case identified above.

Enclosed are computer generated images and graphs supporting our conclusion and the drug testing report.

Please feel free to call if you have any questions

Sincerely

Don H. Catlin, M.D.
Director

06/01/04

CONFIDENTIAL
DOCUMENTATION

SAMPLE IDENTIFICATION:
Organization requesting test: USADA
Date of sample collection: Mar 16, 2004
Site ID: OOC
[redacted]
Substance identified: **recombinant human Erythropoietin (rHuEPO)**

TABLE OF CONTENTS

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BATCH CHAIN OF CUSTODY

SHIPMENT RELEASED BY	TO	DATE/TIME	PURPOSE
SIGNATURE - PRINT - COURIER	FILL OUT SECTION RIGHT BELOW		
[signature] Montgomery	Fereshteh Oeshad	3/18/04 10:10AM	TRANSFER

Said shipment consists of:

Airbill # or Package Tracking # or description

[ ] Bags
N=____
(Color)_____

[ ] Boxes
N=____

[X] Envelopes
N=____

12 1A1 UV4 22 1008 0795 (A)
12 1A1 UV4 22 1008 3069 (A)
12 1A1 UV4 22 1013 2041 (A)
12 1A1 UV4 22 1013 3223 (A)

[other] explain:_____

INITIAL NEXT TO THE AIRBILL # TO INDICATE THE BATCH.

Integrity of the shipment is (check one):

[X] undamaged [ ] damaged (describe:_____)

see reverse side for continuation.

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After inspection, custody of the shipment is as follows (document transfers of the shipment to Receiving Chemist, Room 118, Coldroom, etc.):

RELEASED BY	TO	DATE	PURPOSE
Fereshteh Delshad	REFRIGERATOR # 17	MAR 1 8 2004	STORAGE
REFRIGERATOR # 17		MAR 1 8 2004	Accessioning

The shipment is unpacked in the Accessioning Room and assigned a UCLA folder label:

Client: USADA Folder #: [redacted] UCLA CODE: [redacted]

The number of **A samples** is: [redacted] number of **B samples** is: [redacted]

The number of **Athlete Signature Forms/** Official Records is: 84

The number of **Specimen Custody Documents (2624)** is: ____

After unpacking and batching the shipment, **BOTTLE** custody is as follows:

RELEASED BY	TO	DATE	PURPOSE
			Aliquot As in Rm 118
	COLDROOM	MAR 1 8 2004	STORAGE
			4 3/23/04

G:\SOP\LC\SAMPM\CUSBATCH.2003

Form approved AR 7/7/03

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USADA

UNITED STATES ANTI-DOPING AGENCY

Sample Manifest

USADA 6 of 12

Sample Information

1. DATE(S) OF COLLECTION: 03-16-09 (MM/DD ALL DATES 2 DIGIT/SINGLE DATES)
2. NAME OF EVENT (If OOC write "OOC"): OOC
3. SITE ID Number: (If OOC write "OOC"): OOC
4. CITY & STATE where samples shipped from: Albuquerque (City), N.M. (State)
5. TEST KIT NUMBERS SHIPPED:

[redacted]

Lab Codes: A=problems w/A bottle; B=problems w/B bottle; AB=problems with both bottles

6. COURIER: ☐ World Courier ☒ UPS ☐ Other: _____
7. WAYBILL/ Tracking NUMBER: 1Z 1A1 7V4 22 1013 327 3
8. BAG SEAL NUMBER (If OOC write "OOC"): OOC
9. DATE SEALED: 03 (Month) 17 (Day) 09 (Year)
10. TIME SEALED: 9:00 ☒ AM ☐ PM
11. Replacement Seal Number (If needed): _____
12. Reason for Replacement: _____
13. Date of Replacement: _____ (Month) _____ (Day) _____ (Year)
14. Time of Replacement: _____ ☐ AM ☐ PM
15. LEAD DCO: Micky Williams (Signature) Micky Williams (Printed Name)

For Laboratory Use Only

LABORATORY CONFIRMATION OF RECEIPT OF SAMPLES: PLEASE COMPLETE & FAX TO USADA AT (719) 785-2001

1. DATE RECEIVED: 3 (Month) 18 (Day) 09 (Year)
2. BAG SEAL NUMBER: (If OOC write "OOC"): OOC
3. COMMENTS: _____
4. LABORATORY REPRESENTATIVE: _____ (Signature) Fereshteh Delshad (Printed Name)

Rev. 2/03 White=USADA Yellow=To Laboratory With Samples ©USADA

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SAMPLE CUSTODY - DOCUMENTATION OF SEAL INTEGRITY

FOLDER: USADA [redacted]

UCLA CODE	BOTTLE NUMBER	* A	* B	If not intact, describe below
[redacted]	C	O	O	
	B	O	O	
		X	X	
		X	X	
		X	X	
		X	X	
		X	X	
	D	O	O	
	E	O	O	
	O	O	O	

* Instructions: Verify by "X" that the custody of each specimen is intact. If not intact, record "NO" and describe in the space provided. Note: Entries for internal QC are listed as "O".

The above samples were received in the condition stated above.

Signature [signature] Date 3/18/04

Print Fereshteh Dalahad Time 12:50PM

Verified (initials and date) ASJ 3/18/04

4p 3/22/04

SJ. 4/5/04

4/5/04

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CHAIN OF CUSTODY DOCUMENTATION

Circle: BOTTLE OR (ALIQUOT(S)) (A OR B

Aliquot (SCREEN (BATCH OR INDIVIDUAL) OR INDIVIDUAL CONFIRMATION

Organization: USADA UCLA folder no. USADA [redacted]

If batch, UCLA code numbers: NA

If individual, bottle no. [redacted] = UCLA code no [redacted]

If multiple replicate aliquots indicate here N = ___

Circle assay: 1 E CF FF DB TE ONTRAK DIU OTHER LPO
AC-BASE

IT IS REQUIRED TO PRINT EACH NAME AT LEAST ONCE ON THIS PAGE

Released by:	To	Date	Purpose
Fereshteh Delshad	REFRIGERATOR #22	MAR 1 8 2004	STORAGE
REFRIGERATOR #22	Annie Ramsey	MAR 1 9 2004	Begin Assay
A Ramsey	FREEZER #24	MAR 1 9 2004	STORAGE
FREEZER #24	A Ramsey	MAR 2 4 2004	Continue Assay
A Ramsey	Electrophoresis unit #2	MAR 2 4 2004	IEF
Electrophoresis unit #2	A Ramsey	MAR 2 4 2004	Continue Assay
A Ramsey	Henry Truong	MAR 2 4 2004	Continue Assay
Henry Truong	REFRIGERATOR #22	MAR 2 4 2004	Incubation
REFRIGERATOR #22	A Ramsey	MAR 2 5 2004	Continue Assay
A Ramsey	FLUOCHEM CAMERA	MAR 2 5 2004	DATA AQUISITION 3.4.15.04

C:\SOP\LOGS&FRM\CUSTODY

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IT IS REQUIRED TO PRINT EACH NAME AT LEAST ONCE ON THIS PAGE

Released by:	To:	Date	Purpose
FLUOCHEM CAMERA	A Ramseyer Annie Ramseyer	MAR 2 5 2004	Transfer
A Ramseyer	long Term STORAGE	MAR 2 5 2004	End Assay SS. 4/5/04
			KB 4/2/04

U:\SOP\LOGS&FRM\CUSTODY

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[initials] 11/15/04

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CHAIN OF CUSTODY DOCUMENTATION

Circle: BOTTLE OR ALIQUOT(S) (A) OR B

Aliquot: SCREEN (BATCH OR INDIVIDUAL) OR INDIVIDUAL CONFIRMATION

Organization: USADA UCLA folder no. USADA [redacted]

If batch, UCLA code numbers: A

If individual, bottle no. [redacted] = UCLA code no. [redacted]

If multiple replicate aliquots indicate here N = ___

Circle assay: I II CF FF βB TE ONTRAK DIU OTHER___
AC+BASE

IT IS REQUIRED TO PRINT EACH NAME AT LEAST ONCE ON THIS PAGE

Released by:	To:	Date	Purpose
COLDROOM	Fereshteh Delahad	MAR 2 5 2004	Aliquot in Room 108
	Positive Freezer	MAR 2 5 2004	STORAGE

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Circle: BOTTLE OR ALIQUOT(S) A OR B

Aliquot: SCREEN (BATCH OR INDIVIDUAL) OR INDIVIDUAL CONFIRMATION

Organization: USADA UCLA folder no: USADA [redacted]

If batch, UCLA code numbers: NA

If individual, bottle no: [redacted] = UCLA code no: [redacted]

If multiple replicate aliquots indicate here N = _____.

Circle assay: T II CF FF βB TE ONTRAK DIU OTHER EPO
AS+BARR

IT IS REQUIRED TO PRINT EACH NAME AT LEAST ONCE ON THIS PAGE

Released by:	To:	Date	Purpose
Fereshteh Dolatkhah	REFRIGERATOR # 22	MAR 2 5 2004	STORAGE
REFRIGERATOR #22	Andreas Breidbach	MAR 2 5 2004	Transfer
	FREEZER #24	MAR 2 5 2004	STORAGE
FREEZER #24	Henry Truong	MAR 2 9 2004	Begin Assay
	REFRIGERATOR 22	MAR 2 9 2004	STORAGE
REFRIGERATOR #22	Annie Ramseyer	MAR 3 0 2004	Continue Assay
	Electrophoresis unit #2	MAR 3 0 2004	I.E.F.
Electrophoresis unit #2		MAR 3 0 2004	Continue Assay
	REFRIGERATOR #22	MAR 3 0 2004	Incubation
REFRIGERATOR #22		MAR 3 1 2004	Continue Assay 53-X1504

AR 4/2/04

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Released by:	To:	Date	Purpose
Annie Ramseyer	FLUOCHEM CAMERA	MAR 3 1 2004	DATA ACQUISITION
FLUOCHEM CAMERA		MAR 3 1 2004	Transfer
	long Term STORAGE	MAR 3 1 2004	End Assay 5). 4/5/04
			4/2/04

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Circle: BOTTLE OR ALIQUOT(S) A OR B

Aliquot: SCREEN (BATCH OR INDIVIDUAL) OR INDIVIDUAL CONFIRMATION

Organization: USADA UCLA folder no USADA [redacted]

If batch, UCLA code numbers: NA

If individual, bottle no. [redacted] = UCLA code no. [redacted]

If multiple replicate aliquots indicate here N = NA

Circle assay: I JI CF FF βB TE ONTRAK DIU OTHER EPO Activity
(C) BASE

IT IS REQUIRED TO PRINT EACH NAME AT LEAST ONCE ON THIS PAGE

Released by:	To:	Date	Purpose
FREEZER 2-4	Henry Truong	MAR 2 9 2004	Aliquoting
Henry Truong	Water bath Room #125	MAR 2 9 2004	Incubation
WATER BATH ROOM #125	A Ramsey Annie Rameyer	MAR 3 0 2004	Continue Assay
A Ramsey	unit #2 Electrophoresis	MAR 3 0 2004	IEF
Electrophoresis unit #2	A Ramsey	MAR 3 0 2004	Continue Assay
A Ramsey	REFRIGERATOR #2	MAR 3 0 2004	Incubation
REFRIGERATOR #2	A Ramsey	MAR 3 1 2004	Continue Assay
A Ramsey	FLUOCHEM CAMERA	MAR 3 1 2004	DATA AQUISITION
FLUOCHEM CAMERA	A Ramsey	MAR 3 1 2004	Transfer
A Ramsey	long term STORAGE	MAR 3 1 2004	End Assay

S: 4/5/04

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AS 4/2/04

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Circle: BOTTLE OR ALIQUOT(S) A OR B

Aliquot: SCREEN (BATCH OR INDIVIDUAL) OR INDIVIDUAL CONFIRMATION

Organization: USADA UCLA folder no. USADA [redacted]

If batch, UCLA code numbers: LA

If individual, bottle no. [redacted] = UCLA code no. [redacted]

If multiple replicate aliquots indicate here N = ____

Circle assay: I II CF FF βB TE ONTRAK DEU OTHER____
AC-BANK

IT IS REQUIRED TO PRINT EACH NAME AT LEAST ONCE ON THIS PAGE

Released by:	To:	Date	Purpose
COLDROOM	Fereshteh Dolshad	MAR 25 2004	Transfer
[signature]	Positive Freezer #2	MAR 25 2004	STORAGE
Positive Freezer #2	[signature]	MAY 2 4 2004	Aliquot in Room 108
[signature]	Positive Freezer #2	MAY 2 4 2004	STORAGE 05/24/04

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Circle BOTTLE OR ALIQUOT(S) A OR B

Aliquot SCREEN (BATCH OR INDIVIDUAL) OR INDIVIDUAL CONFIRMATION

Organization USADA UCLA folder no. USADA [redacted]

If batch, UCLA code numbers [redacted]

If individual, bottle no. [redacted] = UCLA code no. [redacted]

If multiple replicate aliquots indicate here N = [redacted]

Circle assay: I II CF FF DB TE ONTRAK DIU (OTHER) LABCORP

AD-BASE

IT IS REQUIRED TO PRINT EACH NAME AT LEAST ONCE ON THIS PAGE

Released by	To	Date	Purpose
Fereshteh Delshad	Andreas Breitbach	MAY 2 4 2004	Begin Assay
[signature]	FREEZER #2	MAY 2 4 2004	STORAGE
FREEZER # 2	[signature]	MAY 2 5 2004	Continue Assay
[signature]	REFRIGERATOR 22	MAY 2 5 2004	Incubation
REFRIGERATOR 22	[signature]	MAY 2 6 2004	Continue Assay
[signature]	Lou Tom STORAGE	MAY 2 6 2004 ~~MAY 2 6 2004~~	SS 5/26/04 End Assay 05/26/04

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Circle: BOTTLE OR ALIQUOT(S) A OR B

Aliquot: SCREEN (BATCH OR INDIVIDUAL) OR INDIVIDUAL CONFIRMATION

Organization: USADA UCLA folder no: USADA [redacted]

If batch, UCLA code numbers:

If individual, bottle no. [redacted] = UCLA code no: [redacted]

If multiple replicate aliquots indicate here N = _____

Circle assay: I II CF FF 2B TB ONTRAK DRI OTHER [illegible]

IT IS REQUIRED TO PRINT EACH NAME AT LEAST ONCE ON THIS PAGE

Released by:	To:	Date	Purpose
Fereshteh Dehshad	Andreas Waldbach	MAY 2 4 2004	Begin Assay
	Water bath R125	MAY 2 4 2004	Incubation
Water bath R125		MAY 2 5 2004	Continue Assay
	REFRIGERATOR 22	MAY 2 5 2004	Incubation
REFRIGERATOR 22		MAY 2 6 2004	Continue Assay
	Long term STORAGE	MAY 2 6 2004	End Assay 05/26/04

[illegible] SCHEDULE/FORM CUSTODY Form approved [illegible]

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DESCRIPTION OF METHODS

Detection of Recombinant Erythropoietic Proteins

SCREENING PROCEDURE

Urine samples that are tested for erythropoietic proteins are screened first. If the screening results suggests that a prohibited substance is present the samples are subjected to an A-confirmation procedure. In the UCLA laboratory the screen and the A-confirmation are performed by the same method. The main difference between the screen and the A-confirmation is that in the former several samples are electrophoresed on one gel next to each other, whereas in the latter the samples are separated by extra space.

The procedure described below detects recombinant human erythropoietin (rHuEPO) and darbepoetin. The analysis consists of four steps: sample preparation; isoelectric focusing (IEF), immuno-blotting, and visualization. The following description is intended to be a summary for the non-specialist.

Sample preparation

Preparing samples for IEF analysis involves two steps: 1) enzyme deactivation, 2) filtration, and concentration. The first step is to deactivate enzymes that could destroy the rHuEPO thereby foiling the analysis. It is done by adding protease inhibitors (chemicals) to the urine. The second step is to remove materials from the urine that are irrelevant and to concentrate the proteins. This is accomplished with specialized filters that retain molecules with high molecular weight, such as rHuEPO. The urine is placed in a cup that has the specialized filter. The cup is placed in a centrifuge which spins at high speed. This forces the low molecular weight material to pass through the filter. The rHuEPO and other proteins with similar molecular weights are retained (the retentate) on the filter. This step is repeated one more time. If rHuEPO is present in the urine, it will be in the final retentate. The final retentate is a liquid. A

small portion of it is used to estimate the concentration of rHuEPO by an immunoassay. Another portion is spotted on a gel (described below). The immunoassay provides an estimate of the amount of rHuEPO in the urine sample. This number is used to adjust the concentration of rHuEPO in the retentate to an optimal value. No adjustment is needed if the estimated concentration is low. If the estimate is high the retentate is diluted. Twenty microliters of the retentate are applied to the gel.

Isoelectric focusing

Glycoproteins such as darbepoetin and rHuEPO are molecules that carry positive and 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 which is positive, negative, or neutral depending on the pH. The isoelectric point (pI) is a fundamental characteristic of proteins. It is the pH value at which the molecule is electrically neutral because the number of positive charges on the molecules are exactly balanced by the number of negative charges.

The gel is a jelly-like material that serves as the 'platform' for the electrophoresis. The gel is about 25 by 12 cm (length and width) and about 1 mm thick. 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.

Before the samples are put on the gel, a pH gradient must be set up. Electrodes are attached to the gel and connected to the electrophoresis unit. One of the electrodes attached to the gel is the anode and the other is the cathode. When the electrophoresis unit is turned on it sets up an electric circuit between the cathode (negative pole), and the anode (positive pole). When the current is applied the molecules used to set up the pH gradient migrate. The 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 vice versa. A current is applied to the gel for half an hour to establish the pH gradient.

The current is turned off. The samples and standards that are to be electrophoresed are 'spotted' onto the gel by adding a small volume (20 microliters) 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 24 such pieces. In this way the surface of the gel is divided into 24 imaginary lanes.

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 analysis. Typically we use one or two different standards, one or more control samples (content known), and several unknowns (content not known). Typically the standard is pure rHuEPO, or pure darbepoetin, or a mixture of the two. A typical control urine is a urine obtained from a subject to whom we have administered rHuEPO.

The current is turned back on which causes the glycoproteins that have been spotted on the gel to begin to move or migrate. 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.

With this background, one can now interpret an electropherogram that shows rHuEPO (see figure 1). rHuEPO has 5 bands which are referred to as isoforms. An isoform is a subset of the rHuEPO molecules that has a defined pI. Not all the molecules of rHuEPO have exactly the same chemical structure and therefore the same pI. All the molecules in one band will have the same pI, that is they will become neutral at one and the same pH. The molecules of darbepoetin focus near the anode side of the gel because it has a low pI. In contrast, rHuEPO focuses closer to the cathode because it has a higher pI. This explains why rHuEPO and darbepoetin are separated on the gel by several centimeters of physical space.

Immuno-blotting

At this stage the bands are separated on the gel. The task now is to transfer them off the gel. This is accomplished by 'blotting'. 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 rHuEPO from the gel to membrane #1. Membrane #1 is incubated with antibodies against EPO proteins. The second blot transfers the antibodies from membrane #1 to membrane #2.

The gel is removed from the electrophoresis plate, washed with a buffer, and placed between two stacks of paper that have been soaked in 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.' During blot #1 the rHuEPO 'travels' 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 against EPO. These antibodies are very special because they specifically bind to endogenous human EPO, rHuEPO, and closely related proteins such as darbepoetin. The specific EPO/darbepoetin antibodies are obtained from mice that have been immunized and therefore make antibodies that react with darbepoetin and rHuEPO. Because we also use another antibody later in the assay, we sometimes refer to this antibody the 'first' or primary antibody.

In the next stage, referred to as the second blot, the primary antibody is transferred from the first membrane to a second membrane (membrane #2). The molecules of EPO and darbepoetin remain on the first membrane, but the antibodies against them (primary antibody) are transferred to the second membrane. The test detects the primary antibodies that mark the location of the darbepoetin and 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 primary antibody.

Visualization

The location of the second antibody on membrane #2 matches the location of EPO or darbepoetin on membrane #1. We need a way to visualize the second antibody. To this end, a marker protein which binds to the second antibody is used. Next a special substance 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 (electropherogram) is used to evaluate the results.

CONFIRMATION PROCEDURE

The confirmation procedure is very similar to the screening procedure. The sample preparation, isoelectric focusing (IEF), immuno-blotting, and visualization are identical to the screening procedure. The only difference is the types and number of samples spotted on the confirmation gel. For the A-confirmation four lanes contain a standard (darbepoetin and rHuEPO), and there is one lane each for the Positive Quality Control, the Negative Quality Control, and the sample that is being confirmed.

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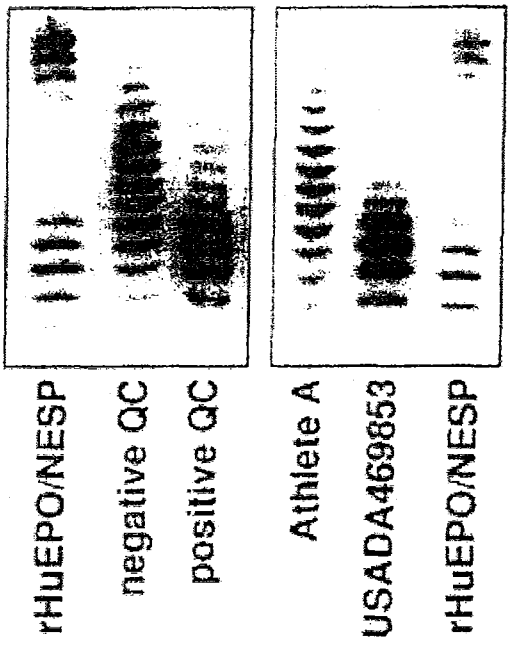

Figure 1: Screen of the A sample

Electropherogram corresponding to screening a batch of urine samples for erythropoietic proteins. Each sample is contained in one lane. There are six lanes. The bands or isoforms corresponding to rHuEPO, darbepoetin (NESP), or endogenous EPO have the appearance of rungs of a ladder. There are two lanes (lanes 1 and 6, numbering from left to right) containing a standard consisting of a mixture of rHuEPO and darbepoetin, lane 2 contains a negative quality control urine sample, and lane 3 a positive quality control urine sample. Lane 4 is a sample from another athlete and lane 5 is sample #[redacted]

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SAMPLE A SCREEN CONCLUSION

The two-band ratio is greater than 1.19 and the percent basic isoforms is greater than 80%, therefore an A-confirmation is carried out. (For the origin of the number 1.19 see publication: Breidbach A, Catlin DH, Green GA, Tregub I, Truong H, Gorzek J. Detection of rHuEPO in Urine by isoelectric focusing. Clin Chem 2003; 49:901-907)

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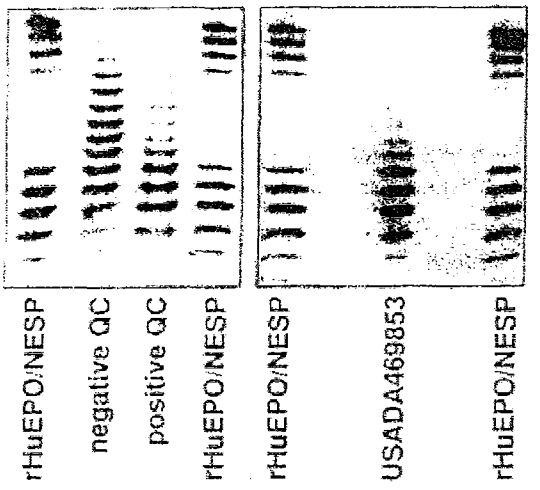

Figure 2: Electropherogram corresponding to the A confirmation of sample #

Each sample is contained in one lane. There are 7 lanes in the figure. The bands or isoforms corresponding to rHuEPO, darbepoetin (NESP), or endogenous EPO have the appearance of rungs of a ladder. There are four lanes containing a standard consisting of a mixture of rHuEPO and darbepoetin. These are lanes 1, 4, 5, and 7 with the numbering starting from the left-most lane. The negative QC sample is in lane 2 and the positive QC sample is in lane 3. The lane containing urine # is lane 6.

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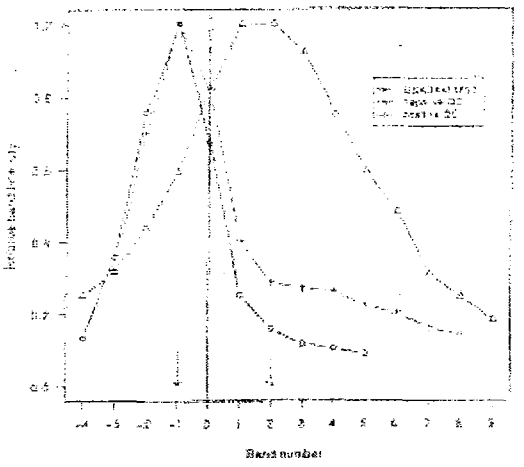

Figure 3: Graphic representation of the band pattern - A confirmation

Densitometry has been used to express the darkness (density) of the bands in Figure 2 as numerical values. Plotting these values as a function of the band number results in graphs showing the relative peak height of the bands on the Y-axis and band number on the X-axis. The band labeled 0, which is marked by a vertical line, is defined as the band with the same isoelectric point (pI) as the most acidic band in the rHuEPO standard. Starting from band 0, the successive bands in the direction of the anode are considered 'acidic' and are labeled 1, 2, 3, 4,... Similarly the bands in the direction of the cathode are considered 'basic' and are labeled -1, -2, -3, and -4.

The shape of the graph for the negative QC is characteristic of a negative sample. The peak apex occurs at band 2 and the area under the curve is predominantly to the right of the vertical line. The shape of the graph for the positive QC is characteristic of a positive sample. The peak apex occurs at band -1 and the area under the curve is predominantly to the left of the vertical line.
For sample #[redacted]e peak apex is on the left of the vertical line like that of the positive QC and the area under the curve is predominantly on the left of the vertical line.

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SAMPLE A CONFIRMATION CONCLUSIONS

1) The two-band ratio:

The A confirmation data indicate the presence of rHuEPO according to criteria published in a peer-reviewed journal by the UCLA Olympic Laboratory (Breidbach A, Catlin DH, Green GA, Tregub I, Truong H, Gorzek J. Detection of rHuEPO in Urine by isoelectric focusing. Clin Chem 2003; 49:901-907.) In summary these criteria are:

1) the isoform bands that focus in the same area as the rHuEPO standard are darker than other isoform banos of the sample.

2) these bands have the same pI as the corresponding bands of the rHuEPO standard;

3) the sample contains the three bands that correspond to the pI of bands 0, -1, and -2 of the rHuEPO standard (see description for figure 3);

4) the 'Two-Band Ratio' is greater than 7. The upper 99% confidence interval for the two-band ratio is 1.19. (Note: the ratio is not a linear function (see Breidbach et.al.Clin Chem 2003; 49:901-907)

2) Percent basic isoforms:

In addition, the sample is positive for rHuEPO according to the 'percent basic isoforms' criteria. The percent basic isoforms is 90%. This method has been the subject of discussion in various expert committees hosted by the IOC and it has been described in an extended abstract by Pascual et. al. (Schaenzer et.al.(eds.) Recent Advances in Doping Analysis/ Proceedings of the Manfred Donike Workshop. 2002:135-144). In addition the method is discussed in a Court of Arbitration for Sport decision (CAS 2001/A/345 Roland Meier v/ Swiss Cycling) wherein a value greater than 80% was deemed to be positive

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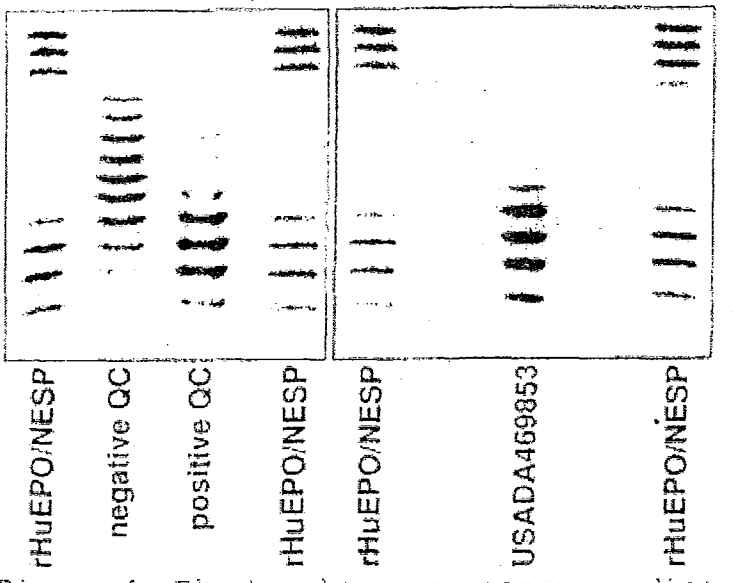

Figure 4: Electropherogram corresponding to the B confirmation of sample #[redacted]

Each sample is contained in one lane. There are 7 lanes in the figure. The bands or isoforms corresponding to rHuEPO, darbepoetin (NESP), or endogenous EPO have the appearance of rungs of a ladder. There are four lanes containing a standard consisting of a mixture of rHuEPO and darbepoetin. These are lanes 1, 4, 5, and 7 with the numbering starting from the left-most lane. The negative QC sample is in lane 2 and the positive QC sample is in lane 3. The lane containing urine #[redacted] is lane 6.

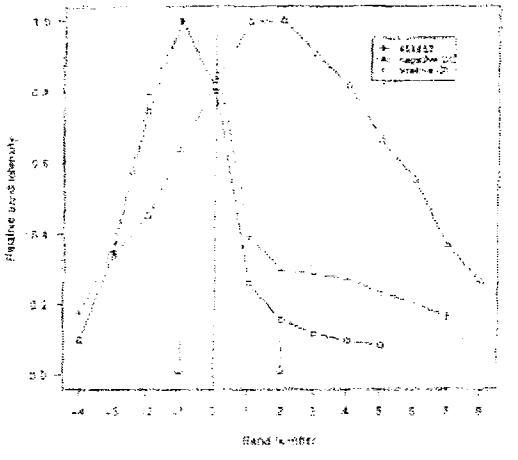

Figure 5 : Graphic representation of the band pattern - B confirmation

Densitometry has been used to express the darkness (density) of the bands in Figure 2 as numerical values. Plotting these values as a function of the band number results in graphs showing the relative peak height of the bands on the Y-axis and band number on the X-axis. The band labeled 0, which is marked by a vertical line, is defined as the band with the same isoelectric point (pI) as the most acidic band in the rHuEPO standard. Starting from band 0, the successive bands in the direction of the anode are considered 'acidic' and are labeled 1, 2, 3, 4,... Similarly the bands in the direction of the cathode are considered 'basic' and are labeled -1, -2, -3, and -4.

The shape of the graph for the negative QC is characteristic of a negative sample. The peak apex occurs at band 2 and the area under the curve is predominantly to the right of the vertical line. The shape of the graph for the positive QC is characteristic of a positive sample. The peak apex occurs at band -1 and the area under the curve is predominantly to the left of the vertical line.
For sample #[redacted] the peak apex is on the left of the vertical line like that of the positive QC and the area under the curve is predominantly on the left of the vertical line.

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SAMPLE B CONFIRMATION CONCLUSIONS

1) The two-band ratio:

The A confirmation data indicate the presence of rHuEPO according to criteria published in a peer-reviewed journal by the UCLA Olympic Laboratory (Breidbach A. Catlin DH, Green GA, Tregub I, Truong H, Gorzek J. Detection of rHuEPO in Urine by isoelectric focusing. Clin Chem 2003; 49:901-907.) In summary these criteria are:

1) the isoform bands that focus in the same area as the rHuEPO standard are darker than other isoform bands of the sample.

2) these bands have the same pI as the corresponding bands of the rHuEPO standard;

3) the sample contains the three bands that correspond to the pI of bands 0, -1, and -2 of the rHuEPO standard (see description for figure 3);

4) the 'Two-Band Ratio' is greater than 6. The upper 99% confidence interval for the two-band ratio is 1.19. (Note: the ratio is not a linear function (see Breidbach et.al.Clin Chem 2003; 49:901-907)

2) Percent basic isoforms:

In addition, sample #[redacted] is positive for rHuEPO according to the 'percent basic isoforms' criteria. The percent basic isoforms is 89%. This method has been the subject of discussion in various expert committees hosted by the IOC and it has been described in an extended abstract by Pascual et. al. (Schaenzer et.al.(eds.) Recent Advances in Doping Analysis/ Proceedings of the Manfred Donike Workshop. 2002:135-144). In addition the method is discussed in a Court of Arbitration for Sport decision (CAS 2001/A/345 Roland Meier v/ Swiss Cycling) wherein a value greater than 80% was deemed to be positive

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To verify that the isoforms are stable in the sample, we adjusted the pH to 5 with 0.1 mol/L HCl. We added Pepstatin (15μmol/L to 500μL) and Complete stock solution (1% of a 1 tablet/2mL solution) to 0.5 mL of sample. (Pepstatin and Complete are protease inhibitors.). After 15 min at room temperature 300mU rHuEPO were added and the

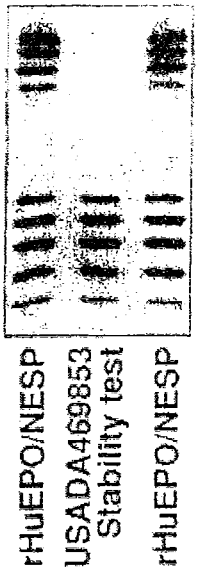

Figure 6: Stability test - A confirmation

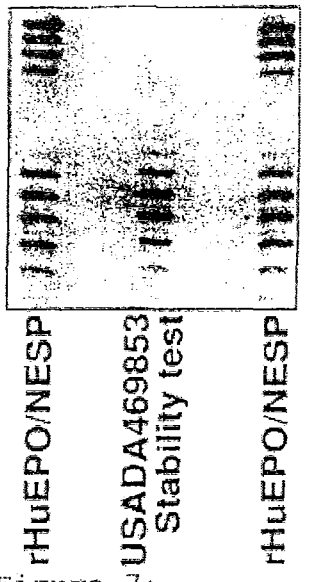

Figure 7: Stability test - B confirmation

urine was incubated at 37°C overnight. 20μL of the treated urine sample were subjected to isoelectric focusing and immunoblotting. This study was carried out with sample #[redacted] The rationale is if there is anything that causes a shift of the isoform pattern of the urine sample the well-known pattern of the added rHuEPO would change.

Figures 6 and 7 show the electropherograms of the above experiments. It can be seen that the isoform pattern of the added rHuEPO did not change.

Figure 8 shows an overlay of the isoform patterns of the screen, the A-, and the B-confirmation of sample # [redacted]. This further verifies that there is no change in the location of the isoforms between the three analyses.

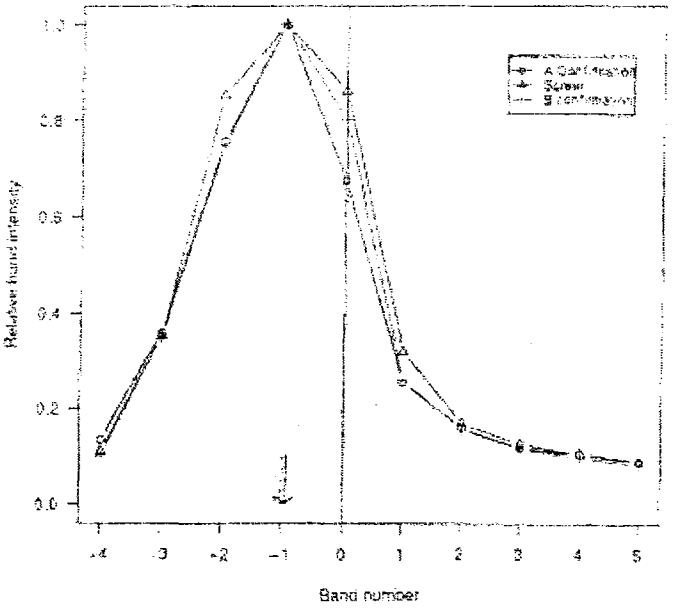

Figure 8: Overlay of the graphic representations of the band pattern of screen, A-, and B-confirmation of sample # [redacted]

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CONFIDENTIAL

June 21, 2004

Terry Madden
The United States Anti-Doping Agency
2550 Tenderfoot Hill St., Suite 200
Colorado Springs, CO 80906-7346

RE: Specimen number [redacted]

Dear Mr. Madden

Please find enclosed an addendum to the documentation package for the case identified above.

Please feel free to call if you have any questions.

Sincerely,

Don H. Catlin, M.D.
Director

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DOCUMENTATION

SAMPLE IDENTIFICATION:
Organization requesting test: USADA
Date of sample collection: Mar 16, 2004
Site ID: OOC
[redacted]
Substance identified: **recombinant human Erythropoietin (rHuEPO)**

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Band	Peak area
-4	37357
-3	232807
-2	463736
-1	553275
0	382344
1	99292
2	39495
3	24331
4	14888
5	10689

Table 1: Peak areas of the A confirmation of this sample

Densitometry has been used to convert the band densities (darkness) into areas (see documentation package page 27). From these areas the 'Two-band ratio' and the 'Percent basic isoforms' have been calculated. For the 'Two-band ratio' the sum of the areas of bands '-1' and '-2' is divided by the sum of the areas of bands '1' and '2'.

The value for the TBR is 7.3 ((553275+463736)/(99292+39495)).

For the 'Percent basic isoforms' the sum of the areas of the basic bands ('0', '-1', '-2', etc.) is divided by the sum of the areas of all bands and then multiplied by 100.

The value for the 'Percent basic isoforms' is 89.8 %.

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Band	Peak area
-4	22160
-3	204283
-2	510906
-1	665432
0	522979
1	125182
2	47359
3	29572
4	21253
5	12197

Table 2: Peak areas of the B confirmation of this sample

Densitometry has been used to convert the band densities (darkness) into areas (see documentation package page 27). From these areas the 'Two-band ratio' and the 'Percent basic isoforms' have been calculated. For the 'Two-band ratio' the sum of the areas of bands '-1' and '-2' is divided by the sum of the areas of bands '1' and '2'.

The value for the TBR is 6.8 ((665432+510906)/(125182+47359)).

For the 'Percent basic isoforms' the sum of the areas of the basic bands ('0', '-1', '-2', etc.) is divided by the sum of the areas of all bands and then multiplied by 100.

The value for the 'Percent basic isoforms' is 89.1 %.

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CONFIDENTIAL

November 12, 2004

Terry Madden
United States Anti-Doping Agency
2550 Tenderfoot Hill St., Suite 200
Colorado Springs, CO 80906

RE: Specimen number [redacted]

Dear Mr. Madden,

In response to your fax of today, please find enclosed an addendum to the documentation package for the case identified above.

In our publication (Breidbach A, Catlin DH, Green GA, Tregub I, Truong H, Gorzek J. Detection of rHuEPO in Urine by isoelectric focusing. Clin Chem 2003; 49:901-907) we state that a TBR value greater than 1.19 is associated with a risk of 1 in 100. Since that publication we have increased our rHuEPO-free control population to 685 samples, and based on that data, a TBR value of greater than 1.8 is associated with a risk of 1 in 100,000. That is, if 100,000 samples were analyzed we would expect to find one sample with a TBR greater than 1.8.

Please feel free to call if you have any questions

Sincerely,

Don H. Catlin, M.D.
Director

CONFIDENTIAL
DOCUMENTATION

SAMPLE IDENTIFICATION:
Organization requesting test: USADA
Date of sample collection: Mar 16, 2004
Site ID: OOC
[redacted]
Substance identified: recombinant human Erythropoietin (rHuEPO)

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CRITERION FOR POSITIVITY

As stated in the documentation package for this sample, dated Apr 15, 2004, our primary criterion for positivity is the "two-band ratio" (TBR). The concept of this ratio has undergone peer review and was published in 2003 (Breidbach A, Catlin DH, Green GA, Tregub I, Truong H, Gorzek J. Detection of rHuEPO in Urine by isoelectric focusing. Clin Chem 2003; 49:901-907).

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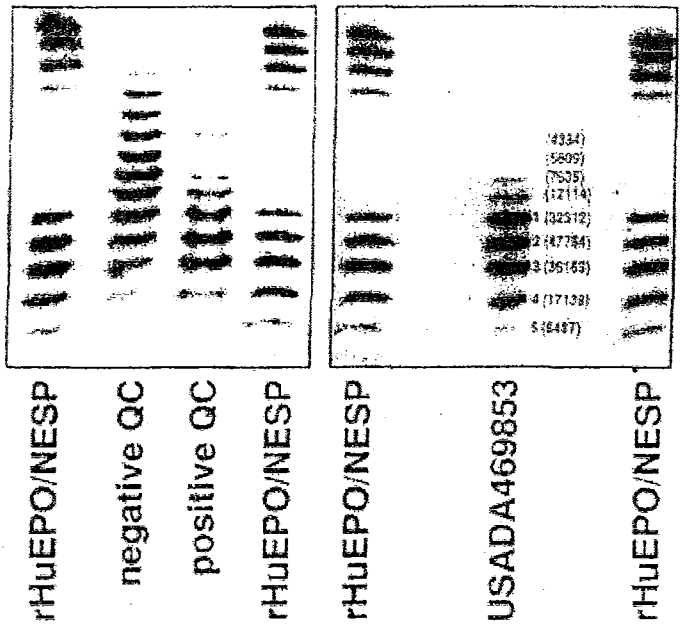

Figure 1: Electropherogram corresponding to the A confirmation of this sample.

Densitometry has been used to convert the band intensities (darkness) into numerical values (see documentation package page 27). Those numerical values (peak heights) are displayed next to the individual bands in parentheses. Also shown are the band IDs (1, 2, 3, 4,...) according to the World Anti-Doping Agency's (WADA) technical document TD2004EPO draft of Oct 13, 2004.

It can be seen that band 2 is the most intense band in the entire lane, that the second most intense band is band 3, and that there are three consecutive, acceptable bands (bands 1, 2, and 3) in the "basic" area. Therefore the identification criteria of WADA TD2004EPO draft of Oct 13, 2004 are satisfied.

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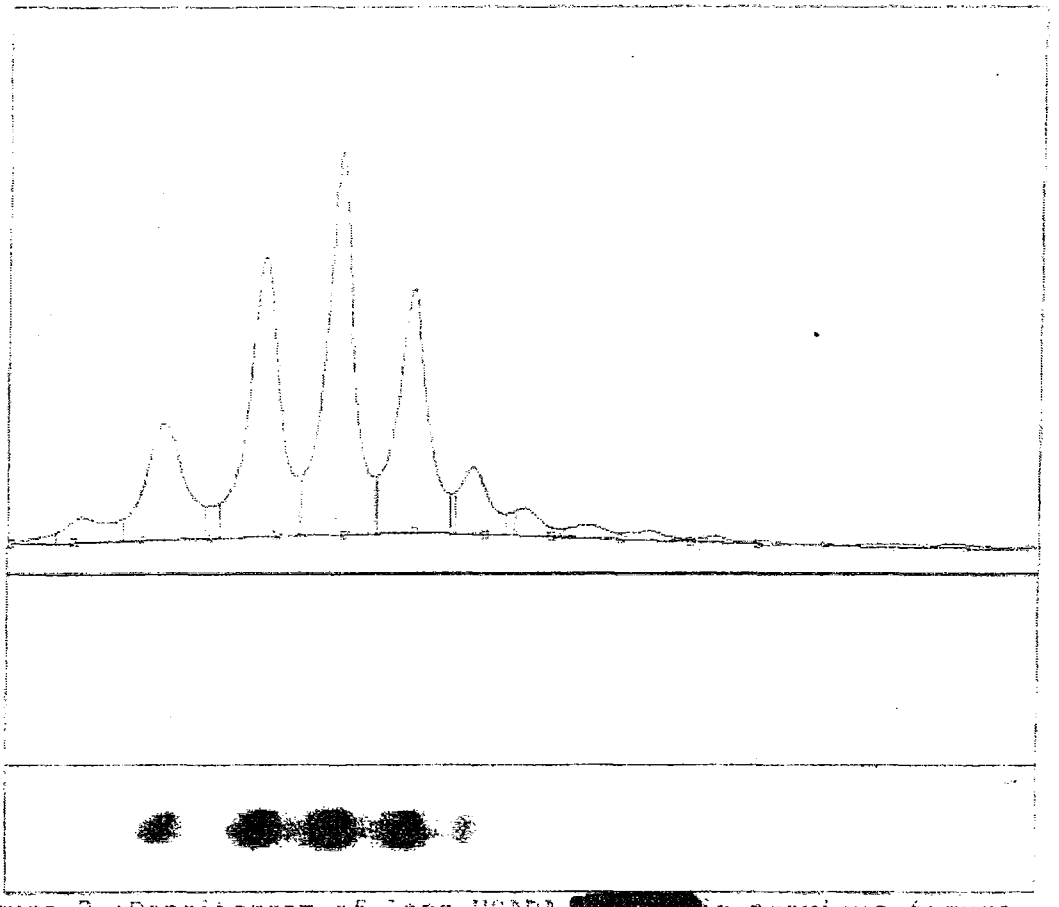

Figure 2 :Densitogram of lane USADA [redacted] in previous figure

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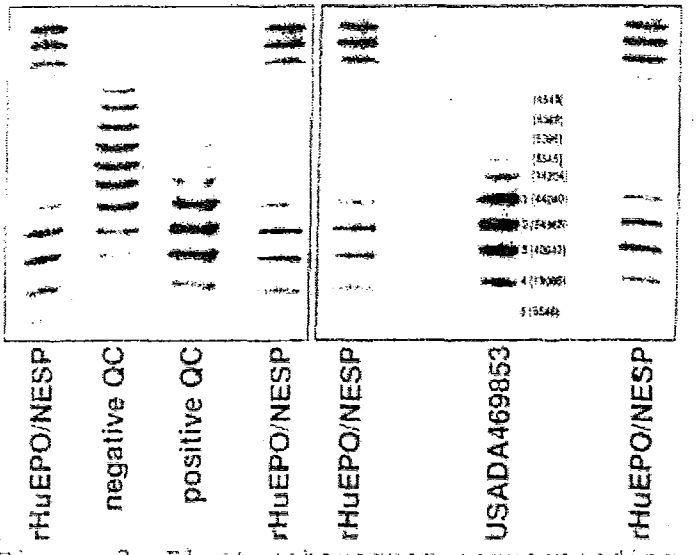

Figure 3: Electropherogram corresponding to the B confirmation of this sample

Densitometry has been used to convert the band intensities (darkness) into numerical values (see documentation package page 27). Those numerical values (peak heights) are displayed next to the individual bands in parentheses. Also shown are the band IDs (1, 2, 3, 4....) according to the World Anti-Doping Agency's (WADA) technical document TD2004EPO draft of Oct 13, 2004.

It can be seen that band 2 is the most intense band in the entire lane, that the second most intense band is band 1, and that there are three consecutive, acceptable bands (bands 1, 2, and 3) in the "basic" area. Therefore the identification criteria of WADA TD2004EPO draft of Oct 13, 2004 are satisfied.

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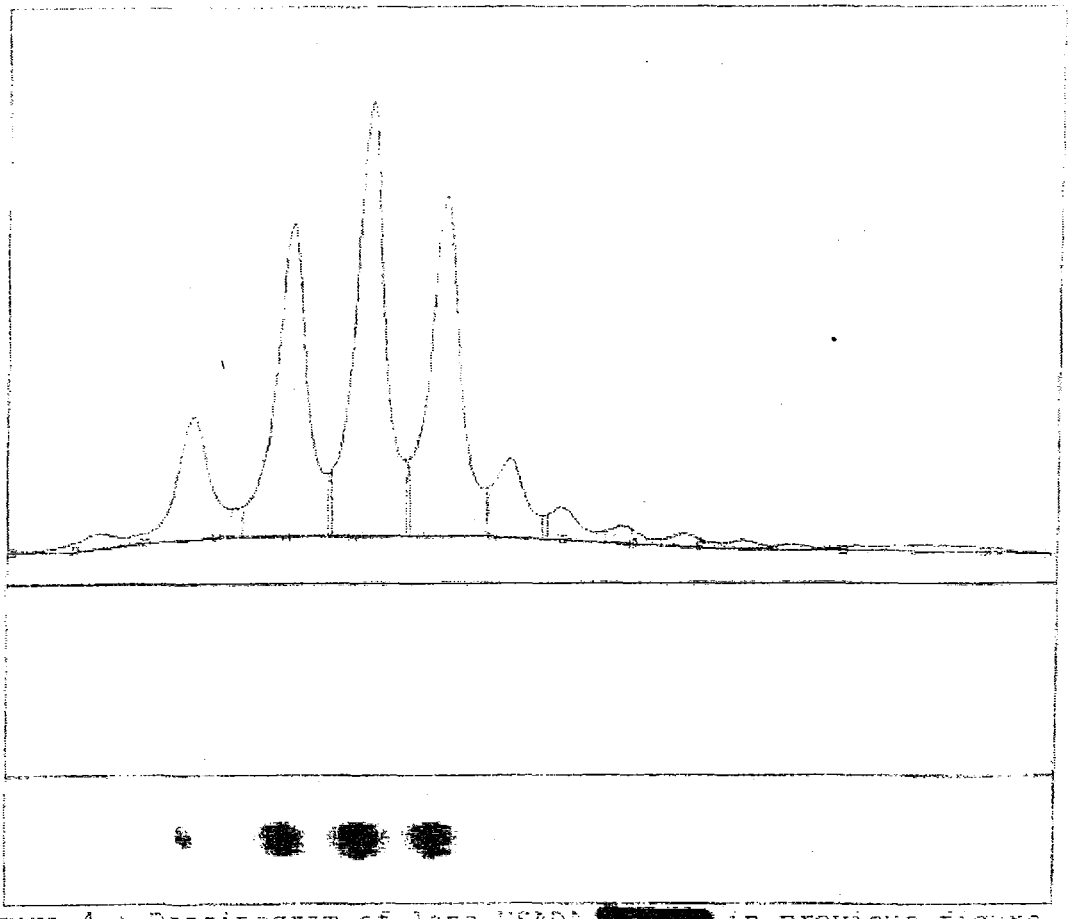

Figure 4 : Densitogram of Jane USADA [redacted] in previous figure