SUPREME COURT OF THE STATE OF CALIFORNIA COPY

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| THE PEOPLE OF THE STATE OF CALIFORNIA,<br>Plaintiff-Respondent,<br>vs.<br>KEVIN COOPER,<br>Defendant-Appellant. | CR 72787<br>Supreme Court No. Crim24552 |

APPEAL FROM THE SUPERIOR COURT OF SAN DIEGO COUNTY

HONORABLE RICHARD C. GARNER, JUDGE PRESIDING

REPORTERS' TRANSCRIPT ON APPEAL

APPEARANCES:

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24

VOLUME ~~1~~ of __ volumes.
Pages 1039 to 1168, incl.

JILL D. MC KIMMEY, C.S.R., C-2314
and
BRIAN V. RATEKIN, C.S.R., C-3715
Official Reporters

SUPERIOR COURT OF THE STATE OF CALIFORNIA

FOR THE COUNTY OF SAN BERNARDINO

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| THE PEOPLE OF THE STATE OF CALIFORNIA, Plaintiff, vs. KEVIN COOPER, Defendant. | NO. OCR-9319<br>VOLUME 14<br>Pgs. 1039 thru 1168, incl. |

REPORTERS' DAILY TRANSCRIPT

BEFORE HONORABLE RICHARD C. GARNER, JUDGE

DEPARTMENT 3 - ONTARIO, CALIFORNIA

Monday, April 23, 1984

APPEARANCES:

For the People: DENNIS KOTTMEIER
District Attorney

DENNIS KOTTMEIER
District Attorney
By: JOHN P. KOCHIS
Deputy District Attorney

For the Defendant: DAVID McKENNA
Public Defender
By: DAVID NEGUS
Deputy Public Defender

Reported by: JILL D. McKIMMEY
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C.S.R. No. 3715

# I N D E X



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ONTARIO, CALIFORNIA; MONDAY, APRIL 23, 1984; 9:40 A.M.

DEPARTMENT NO. 3 HON. RICHARD C. GARNER, JUDGE

APPEARANCES:

The Defendant with his Counsel, DAVID NEGUS, Deputy Public Defender of San Bernardino County; DENNIS KOTTMEIER, District Attorney of San Bernardino County, JOHN P. KOCHIS, Deputy District Attorney of San Bernardino County, representing the People of the State of California.

(Jill D. McKimmey, C.S.R., Official Reporter, C-2314, Brian Ratekin, C.S.R., Official Reporter, C-3715)

(Whereupon, the following proceedings were had in chambers:)

THE COURT: All right. With reference to People versus Kevin Cooper, Mr. Cooper's here with all three counsel.

Last week we discussed a stipulation to be presented to the Court. I would imagine that would be the first order of business this morning.

Has that been worked on?

MR. NEGUS: I submitted it in the form that I was submitting it, and I believe that Mr. Kochis agreed to it.

THE COURT: I am not even sure where it's at.

All right. I have it here. I made some notes for and aft on it, but --

MR. NEGUS: I can get you another copy.

THE COURT: -- I don't know if that's agreeable with you. This is entitled "Defendant's Proposed Stipulation Regarding Transfer of Case and Hearing In Limine Motions in San Bernardino County."

Mr. Kochis, have you examined that? I will cross off my handwriting at some point, if that's agreeable. I kind of thought --

MR. NEGUS: I have another copy, if you'd rather sign that.

THE COURT: I thought you might have a more formal document. A cleaner copy might now be appropriate.

MR. NEGUS: And it is my understanding as well that from the previous stipulations, that jeopardy is likewise attaching at this point in time.

MR. KOCHIS: This stipulation is acceptable, and I am going to sign it and indicate our acceptance.

THE COURT: All right. I will formally approve it and sign it myself and date it.

I am marking on the front "Original" and crossing off "Defendant's Proposed" to where now it just reads "Stipulation", and I will write "Approved and Accepted April 23, 1984."

Before I sign it, I'd like Mr. Cooper to personally

acknowledge and agree to it.

MR. NEGUS: Is the stipulation that I told you about where we're agreeing that in exchange for the prosecution not being able to relitigate the change of venue motion and preserving my right to make a change of venue motion out of San Diego County, we are agreeing to do the trial motions in this county?

THE DEFENDANT: Yes, I agree.

THE COURT: All right.

MR. KOCHIS: Your Honor, the one thing I would like to clarify for the record, Mr. Negus' reference to our agreement not to relitigate the change of venue motion, in effect, was limited to our rights pursuant to Penal Code 1036.5, our right in the future to relitigate our ability to hear the case in this county.

MR. NEGUS: True.

MR. KOCHIS: And I don't think we are limited if something would result in the course of this trial that would result in what we call a hung jury and the case were later retried some years down the road.

MR. NEGUS: I don't know about that, but certainly all we are intending to do is whatever effect Penal Code Section 1036.5 has, that's what we're --

THE COURT: I'm not quite sure I follow that. Could you spell it out for me? I don't have that code section before me.

MR. KOCHIS: Yes, Your Honor. Under that section of the Penal Code, the prosecution has a right any time prior to the transfer order being filed to request another evidentiary hearing and to relitigate the Court's decision to transfer the case out of San Bernardino County, and to say, Judge, for example, four or five months have passed since you have made that decision, the feelings in the community have subsided, we now feel Mr. Cooper can get a fair trial in San Bernardino County, Penal Code Section 1036 says we have to have that hearing before the case is transferred out of the county.

THE COURT: I understand. I understand now. All right.

The document signed and dated by myself is given to the clerk for filing.

I am also giving to you the two confidential statements filed previously by counsel back middle of April which were ordered sealed, and the clerk can seal those documents.

MR. NEGUS: Your Honor, I am going to be presenting to you later today a proposed order releasing those documents to me to take to the Court of Appeal, so if that makes a difference as to how you handle it --

THE COURT: You don't have a copy of those?

MR. NEGUS: Well, I'm requesting that all the exhibits and transcripts be released to me so I can lodge

them with the Court of Appeal in connection with my writ.

THE COURT: That would leave this Court without the documents.

MR. NEGUS: That's normally what happens with writs, and they give it back as soon as they get through deciding whether they want to hear it or not.

THE COURT: Well, I guess they will be encompassed within your motion in the other exhibits and orders then.

All right. Next order of business.

MR. NEGUS: The next thing I'd like to bring up just in chambers is that the prosecutor has filed with this Court a notice of intention to introduce prior acts pursuant to Evidence Code Section 1101(b), and they have also introduced a statement which is required by Penal Code Section 190.3 of acts that they intend to introduce at the penalty phase, if we ever get to a penalty phase. There are some 24 acts that the prosecution has listed. At some point in time we are going to have an in limine motion as to the admissibility of those acts. Prior to that in limine motion, I feel it is necessary for me to investigate those acts because I believe that some of them involve people other than my client, some of them didn't happen, and a variety of other different reasons.

Judge Kayashima has requested that before he approves the large sum of money that would be required to send my investigator hither and yon to investigate these,

that I request that this Court require the prosecution to make a preliminary offer of proof as to how those acts would be relevant and the Court then rule on that preliminary offer of proof, so that if there were any that could be excluded, we could save the money that would be involved in investigating them; therefore, I would request that the Court ask the prosecutor to make a preliminary offer of proof as soon as the prosecutor can.

THE COURT: Well, that's fine, as far as I'm concerned. I'm not sure that this precise moment is the time for doing that.

MR. NEGUS: Well, the reason is that Mr. -- that we're talking about going back to Pennsylvania, and I need to send Mr. Forbush back there as soon as possible. I don't -- I don't expect that Mr. Kochis is going to be able to rattle off his reasons off the top of his head, but I wanted to set the Court on notice that I am going to be -- that I need to have that as soon as possible.

THE COURT: That's fine.

Any problem with that, Mr. Kochis? When would we want to handle it?

MR. NEGUS: Perhaps the end of next week.

MR. KOCHIS: I will attempt to be ready by the end of next week to articulate my reasons on the record.

THE COURT: End of next week?

MR. KOCHIS: Before the Court leaves on vacation,

that would be either next Wednesday or Thursday, which would be May the 2nd of May the 3rd.

THE COURT: That's fine.

MR. NEGUS: Thank you.

That's all I had to do in chambers, I think.

THE COURT: Anything further, Mr. Kochis?

MR. KOCHIS: Not at this time.

THE COURT: We are going to proceed this morning on what, now, precisely?

MR. KOCHIS: The reliability and validity and acceptance within the scientific community of --

THE COURT: Is this Kelly-Frye then?

MR. KOCHIS: Yes, Your Honor.

MR. NEGUS: I am prepared to make a formal motion on that in court, if you want me to. I didn't think that needs to be back here.

THE COURT: I'm ready to proceed outside. You can make your statements outside, if you wish, sir.

MR. NEGUS: Fine.

THE COURT: One other thing. As I indicated to you, I believe, Friday, I am going to be calling about 11:00 o'clock or at the morning recess the judge in San Diego, and as best I could figure at this time as far as when to tell him, I would expect us to tentatively set the 9th of July.

MR. NEGUS: I hope so.

THE COURT: Does that sound okay?

MR. KOCHIS: Yes.

THE COURT: All right. Thank you.

Let's go back outside.

(No omissions.)

(Whereupon the following proceedings were held in open court.)

THE COURT: All right, Counsel. Now, in open court, Mr. Negus, did you have something?

MR. NEGUS: Yes, Your Honor. Basically what, at this point, I'd like to do is -- is have a -- the foundational hearing which is required by People versus Kelly and People versus Frye and People versus Shirley for the following scientific tests that may come up in the evidence of this particular case.

First of all, with respect to a technique called electrophoresis, what is referred to as Group I testing for the enzymes PGM, EsD and GLO; Group II testing for ACP, ADA and AK; Group III testing for transferrin and group specific component; and Group IV testing for CA II and PEP A. In addition, the testing procedure technique known as isoelectric focusing with respect to PGM subtypes; a gradient gel technique for the typing of haptoglobin; the technique used by Mr. Gregonis to do Lewis typing of blood. The test used by Mr. Gregonis to determine secretor or non-secretor status of semen; the test used by Mr. Gregonis to determine the secretor or non-secretor status of saliva; the test used by Mr. Gregonis to determine whether or not the chemical p30 was present -- was present in certain stains; and the testing procedure with respect to determining amylase in saliva stains.

THE COURT: Mr. Kochis, we both anticipated commencement

of the Kelly-Frye issue today. We didn't talk about the specific batting order, so to speak. Are you prepared to proceed as indicated?

MR. KOCHIS: Your Honor, with some deviations. I have some concerns about two of the enzymes, at least, some of the tests Mr. Negus wants me to raise a foundation for.

In the Group I, of the GLO, it's not my understanding my laboratory performed those tests on any of the stains. And on the PGM subtyping, I believe at that time my laboratory did not engage in PGM subtyping and we did not type any of the known bloods for their PGM subtypes nor any of the stains for their PGM subtypes.

THE COURT: What group is that?

MR. KOCHIS: That's the separate group, Your Honor, that Mr. Negus touched on when he finished enumerating the enzymes in Group IV. And it was not my intention to lay a foundation for enzymes he did not type for, obviously.

THE COURT: Okay.

MR. KOCHIS: And with the other items, the amylase, I had not intended to go into that, but I can. And the Lewis blood typing, I had not intended, but, again, I -- I can and do intend to go into the ABO system and the method Mr. Gregonis used to determine those.

But, at this point, I'm not stipulating by laying a foundation that this is covered by the Kelly-Frye-Shirley line of cases in that those cases involve voice print

identification and hypnosis. And it's going to be my position, and I do not intend to waive my position by laying a foundation, that serology is a field of science apart from those particular suits and that it has been accepted in courts in this state at the trial level in many cases.

THE COURT: Well, I think, for purposes of proceeding, you may as well assume that it is a proper subject for -- for objection to the foundation and that it is not all universally accepted in courts. And, to some extent, it is different techniques. So I'm going to have to listen to it, reserving your right to argue the point, however, at a later time.

How long had you expected this to last?

MR. NEGUS: Until the middle of next week or the end of next week.

MR. KOCHIS: That's correct, Your Honor.

THE COURT: I think in some manner from the reporters, then, I would like a copy of the transcript on this as well.

Yes, sir?

MR. NEGUS: Mr. Kochis is correct that Mr. Gregonis did not do any GLO typing or PGM subtyping. However, I put those in there because I may be eliciting testimony from Mr. Kochis' experts on those subjects. And I believe that they will come up in the course of the trial. And rather than try and distinguish foundations later, I thought it would be more convenient to do them all now.

THE COURT: Call your first witness.

MR. KOCHIS: People would call Dan Gregonis.

D A N I E L J. G R E G O N I S, called as a witness by and on behalf of the People, was sworn and testified as follows:

THE CLERK: You do solemnly swear the testimony you are about to give in the action now pending before this Court shall be the truth, the whole truth and nothing but the truth.

THE WITNESS: I do.

THE CLERK: Please be seated.

State your name, please, for the record and spell your last name.

THE WITNESS: Daniel J. Gregonis, G-r-e-g-o-n-i-s.

THE COURT: Counsel,before you start, he's testified before, and I'm not at all sure that we can save any at all, but have you thought about that? Is there any way we can expedite?

MR. NEGUS: No. Basically, Mr. Gregonis' testimony before was -- I doubt that duplicates hardly any of what we will do now. And I think it will be confusing to me to try and keep them straight. Basically, what he's -- his subject of his testimony before was not the subject of the testimony we're going to have now.

MR. KOCHIS: Although I can't agree with it, you need two people to stipulate. If Mr. Negus is not going to, it's not

necessary for me to consider it.

THE COURT: All right. Go ahead.

MR. KOCHIS: May I proceed.

THE COURT: Yes.

DIRECT EXAMINATION

BY MR. KOCHIS:

Q. Mr. Gregonis, what is your business or profession?

A. I'm employed as a criminalist with the San Bernardino County Sheriff's Crime Laboratory.

Q. What educational background do you have in terms of a college education which qualifies you for that position?

A. I have a Bachelor of Science in Criminalistics from Metropolitan State College in Denver, Colorado.

Q. How long have you been employed by this county as a criminalist?

A. Approximately four and a half years.

Q. Are you familiar with the concept of serology?

A. Yes, I am.

Q. And would you define for this Court the term serology.

A. Serology, or in particular, forensic serology, is the identification and subsequent grouping of body fluids, mostly in their dried state.

Q. What education did you receive at the undergraduate level specifically that dealt with the issue or issues involved in serology?

A. Okay. Among my graduate level courses that I took include basic chemistry, also biochemistry, genetics. Along with this, I've had seminars dealing with criminalistics in general. And also I have spent an approximately nine month internship with a crime laboratory, the Colorado Bureau of Investigation in Denver, Colorado, working the majority of time with two forensic serologists there.

Q. What additional training in serology did you receive at that particular laboratory in addition to the training you would have received at the college level?

A. Basically the -- the hands-on technique, along with several papers and articles which were available to me there which were not available to me at the college itself. Plus the experience of the examiners that I was working with.

Q. Since you left college, have you attended any seminars which dealt in the field of serology?

A. Yes, I have.

Q. When was the first one?

A. I believe it was in April of 1980. And this was a subject called Basic Forensic Serology, put on by the FBI Academy in Quantico, Virginia. It was a two-week seminar.

The next one entitled -- well, it was advanced serology, essentially, also by the FBI. And it was also a two-week seminar. Believe that was in July of 1982.

Q. Do you belong to any professional societies or organizations?

A. Yes, I do.
Q. What do those consist of?
A. I belong to the California Association of Criminalists.
Q. And do you belong to any subgroup within that association which deals with this issue of serology?
A. Yes, I do.
Q. What's the name of that organization?
A. Okay. Basically it includes a serology study group which meets approximately four to six weeks -- every -- every four to six weeks.
Q. How many individuals are in that particular study group?
A. In the southern section, there's approximately, I would say, 30 individuals that do serology.
Q. And for what purpose does the group meet?
A. Basically for the discussion of various theories, techniques, any problems that we may have or any new fields which come up in the field of forensic serology.
Q. In the past, have you held any position within that organization?
A. I have been chairman of that group.
Q. Do you regularly subscribe to any scientific literature which deals with the field of serology?
A. Along with the membership in the California Association of Criminalists, I get the Journal of Forensic Science Society. Along with that, the laboratory that I work for gets a number of journals concerning forensic

serology and criminalistics in general.

(No omissions.)

3-1

Q. Are you familiar with the term "biochemical markers of individuality"?
A. Yes, I am.
Q. To what does that term apply?
A. That applies to the various markers, including the ABO or common blood type system which occur in the body which may be different for different individuals.
Q. Is it possible to distinguish between individuals further than their ABO blood types?
A. Yes, it is.
Q. And do individuals have various enzymes in their blood as well as a specific ABO type?
A. Yes, they do.
Q. And were you in court when Mr. Negus listed a number of specific enzyme types?
A. Yes, I was.
Q. Are those examples of the various enzymes that may exist, for example, in human blood?
A. Yes, they are.
Q. And do all people, for example, have the same enzyme type, whether it's PGM or EsD?
A. The great majority of -- you mean the specific type or just the existence of the enzyme itself?
Q. Well, first let's start with the enzyme itself. Do all individuals have, for example, certain enzyme types in their body?

A. Okay. The great majority of people do. There are some people who are genetically lacking some of the enzymes.

Q. Among the people that do have the enzymes, do they all have the same enzyme type? For example, is everybody a PGM Type 1?

A. No, sir.

Q. Now, in the past, as a criminalist, have you examined samples of whole blood to determine their ABO blood type?

A. Yes, I have.

Q. Using what procedure and method?

A. Basically, something called the slide agglutination technique.

THE COURT: I'm sorry. Say that again.

THE WITNESS: Slide agglutination technique.

Q. BY MR. KOCHIS: And briefly, Mr. Gregonis, how is that technique employed?

3a

A. Okay. Briefly, what is done is you take a -- the blood itself. You centrifuge it. You separate the serum from the red blood cells. You then react the serum against known ABO blood and look for agglutination or clumping of the red blood cells in one of those, or two of those. Then you take the red blood cells and you react that against known anti A, anti B, anti H and what's called anti A "comma", B antisera, and then again look for clumping or agglutination.

Q. When you see clumping or agglutination, what will that tell you?

A. Okay. When you're using it on the red blood cells from the unknown blood, you're looking for what's called the antigen. If you get, for instance, a reaction or clumping in the well where you put the anti A antisera, that indicates that there's an antigen present, and so on and so forth for the anti B, anti H and anti A "comma", B. As far as the reverse or the one where you're using the serum from the individual's blood, you're looking for the antibodies, and that will -- you're looking -- say, if a person has A blood, he will have the B antibody in his own blood, and that will react against the B cells that you add in.

Q. Mr. Gregonis, you have used two terms, antigen and antibody. Could you define for the Court what each one of those consists of and how they in fact differ from each other in terms of function?

A. Okay. The basic definition of antigen and antibody can kind of go together. An antigen is a substance which, when introduced into a body, elicits either a protein or something like that. It will elicit what's called the immune response. The immune response is simply the production of antibodies.

Q. Is a person's ABO blood type determined genetically?

3b

A. Yes, it is.
Q. And does a person's blood type change during the course of their life?
A. No, sir, it does not.
Q. Approximately how many times have you performed the tests you've just described to the Court on samples of whole blood to determine the person's ABO blood type?
A. It has to be a thousand, maybe two thousand times.
Q. Have you likewise testified in a court of law in this state as an expert in ABO blood typing in the past?
A. Yes, sir, I have.
Q. Approximately how many times?
A. Approximately 90 times.
Q. Turning your attention to the enzymes, starting first with the enzymes which are often placed in what has been referred to as the Group I category, specifically which two enzymes are we talking about?
A. As far as the way I run the test, we are talking about one enzyme called EsD and the other one called PGM.
Q. Is there a particular test you use to determine what a person's EsD and PGM enzyme type are?
A. That would be the electrophoresis method which has been called the Group I.
Q. Would you tell this Court what electrophoresis consists of.

A. Okay. Simply stated, electrophoresis, it's carried out in a gel type material, Jello type material, if you will. It's clear, and what you do is you simply put the sample or samples into multiple slots with their standards on the plate itself. You put electricity across the plate, and what happens is the various enzymes will separate due to the charge on them, either being attracted to the positive or negative side of the electrical poles, and will separate out into various patterns.

Q. What particular type of gel do you use on the Group I system?

A. This is a gel that consists of a one percent agarose and one percent starch.

Q. And essentially is the gel something you make in the laboratory?

A. Yes, it is.

Q. Do you start with a beaker and some type of water?

A. Okay. We start with a -- if you will, a beaker and what's called a buffer along with the dried starch and the dried agarose.

Q. And then do you follow a schedule adding certain things to the buffer?

A. Okay. Basically, the only thing that's added to the buffer at this point is starch and agarose.

Q. And then do you heat the mixture?

A. We heat the mixture up to -- the agarose and starch dissolve at approximately 95 to a hundred degrees Celsius, which would be about 212 degrees Fahrenheit.

Q. And then at some time after that, you pour it on a glass plate?

A. Yes, we do.

Q. And then after you pour the gel on a glass plate, do you allow it to sit so that it solidifies and forms this gel-like substance?

A. Yes, we do.

Q. Then do you take some type of sharp instrument, for example, a razor and cut various slots in the glass itself?

A. It's a slot maker that's made for the technique itself, yes.

Q. And then in the slots do you place what are known as standards?

A. In some of the slots, yes.

Q. And could you tell this Court what a standard consists of. For example, which one of the enzyme tests do you as a matter of habit run first?

A. As far as the Group I?

Q. Yes.

A. Okay. As far as that is concerned, the one I develop first is the EsD. After that is developed and read, I will develop the PGM.

Q. Do you use standards when you do your EsD run?
A. Yes, I do.
Q. And do you place those standards on certain slots on the glass plate themselves?
A. They are put on known slots that we keep a log of the run itself where each sample is.
Q. And would you tell the Court what a standard is.
A. The standard in electrophoresis consists of known enzyme types. In this case I use a standard which is a known EsD and a known PGM type. This is simply to see where the various bands appear, that they are all there, and they are all separated properly.
Q. Where do you get your standards for your EsD type?
A. The standard I get either from people in the laboratory which I've typed before or I get them from SERI, Serological Research Institute, in Emeryville, California.
Q. Who is the director of that particular institute?
A. The person in charge of that laboratory is a person named Brian Wraxall.
Q. And is it his system that you employ in your laboratory?
A. Yes, it is.
Q. Once you have the standards on certain slots on the glass plate, do you then take various unknown blood-stains that you have in your possession but whose genetic content you're not aware and place those on

other slides?

A. Yes, I do.

Q. If your sample is a whole blood, what procedure is involved in taking whole blood of which you do not know the genetic composition and placing it on the appropriate slot in the tray?

A. There's one of two things that you can do. First of all, you can dry down the whole blood onto a piece of white cotton and make a stain out of that and run it just like any -- any other stain. If you -- you can also take the liquid blood after it has been washed, the red blood cells have been washed. You slice or break open the cells and then put that extract onto a piece of thread and put the thread into the slot itself.

Q. After you had the standards on various slots on the tray and your unknown blood on various slots in the tray, what do you do with the tray next?

A. The next thing that I would do is I would put it onto what's called a cooling platen. It's simply aluminum -- a piece of aluminum with a circulating bath going underneath it to keep the plate itself cool during the analysis.

I will then put electricity across the plate itself, in this case for about three hours at 300 volts, take it off and then develop it.

Q. After it is developed, how do you go about actually

reading what is on the plate?

A. Okay. Basically, it depends on the enzyme. The first one, EsD, would be developed by putting a filter paper overlap containing chemical called MUA on it over the plate in a certain area, letting it develop at room temperature for approximately 10 minutes, and then taking it into a darkened area and shining ultraviolet light onto the plate itself, whereupon you can see bands which fluoresce under the ultraviolet light.

Q. Is this essentially the same type of procedure you use when you run a stain to determine its PGM enzyme type, with the exception of using a different reagent, for example?

A. With the exception of using a different reagent, and also that the PGM is developed so that you can see it in regular daylight.

(No omissions.)

Q. Now, is this method, this use of electrophoresis, something you yourself developed?
A. No, sir, it is not.
Q. Are you aware of approximately when, through your reading in the literature, it was first developed?
A. At least in the 1950's. It may be before that that it was developed for use.
Q. Are you familiar, at least by name, of a person named Bryan Culliford?
A. Yes, I am.
Q. And do you know where in the '70's, through your reading, he was employed?
A. He was employed at the London Metropolitan Laboratory.
Q. And at that time did he involve in implementing this procedure into use in his laboratory?
A. Yes, he did.
Q. Are you aware of when the technique began to be employed in this country?
A. Basically through probably 1965 on it started being employed in -- regularly in forensic work.
Q. Approximately how many times in the past have you engaged in analyzing whole blood to determine, for example, its EsD and PGM enzyme type?
A. That's, again, going to be in thousand.
Q. Have you likewise testified before as an expert in courts in this state on PGM and EsD type analysis?

A. Yes, I have.
Q. Approximately how many times?
A. I'd say around 80 to 90 times.
Q. Mr. Gregonis, turning your attention for a minute to bloodstains, could you describe for the Court what the difference is between a bloodstain and a whole sample of blood.
Q. Okay. Basically, bloodstain would be something which is deposited and has dried at some point. Whole blood is taken from -- directly from the vein, put into a vial. In this county we use what's called an EDTA blood vial for a standard.
Q. For example, if you took an EDTA blood vial with an eye dropper, took blood out of it, placed it on a cloth, on a desk and on the wall and allowed it to dry, would those three situations be an example of bloodstains?
A. Yes, they would.
Q. Do you likewise engage in the analysis of bloodstains to determine the PGM, EsD enzyme type of the actual stain itself?
A. Yes, I do.
Q. Do you likewise use electrophoresis to make that determination?
A. Yes, I do.
Q. And in attempting to determine the EsD and PGM type of a bloodstain,would you likewise use the starch/agarose gel

medium?

A. Yes, I would.

Q. And in your reading, have you determined that you are the only person who uses electrophoresis to determine the EsD enzyme and PGM enzyme types of bloodstains?

A. No, sir. There are many -- many more people that use it.

Q. Returning to your serology study group, approximately how many people are members of that particular group?

A. As I stated before, approximately 30 people in Southern California.

Q. And are those individuals people who are employed by a forensic laboratory somewhere in Southern California?

A. Yes, they are.

Q. Do you talk to all those individuals?

A. The majority of them, yes.

Q. And, to your knowledge, do all of those individuals use electrophoresis in determining the EsD and PGM enzyme types, for example, of whole blood?

A. All except for one person that I know, which they do not do electrophoresis, period. But the remainder do.

Q. Likewise, with the bloodstains, with the exception of the one person who does not engage in electrophoresis, does -- does every member of the serology study group use electrophoresis to determine the EsD and PGM enzyme types of dry blood?

A. Yes, they do.

Q. Mr. Gregonis, is it fair to say that the members of your serology study group are people who are employed by various law enforcement agencies throughout Southern California?

A. Some are law enforcement; some are private laboratories.

Q. Are you aware as to whether or not the use of electrophoresis to determine enzyme type is limited to use by forensic scientists, specifically, people who work for law enforcement?

A. It is not limited to strictly forensic scientists, no.

Q. Is it -- is it employed by people in the medical field?

A. Yes, it is.

Q. Could you give me an example of such a person in another field.

A. One person I know of is Dr. Robert Sparks at UCLA Medical Center. Another person I know of, she's a med tech specialist, is a girl named Barbara Bryan at University of California, Irvine.

Q. Are you familiar with the term "proficiency testing"?

A. Yes, I am.

Q. And could you tell the Court what proficiency testing consists of.

A. Basically what a proficiency test consists of is someone -- a sample of blood or body fluid, unknown, basically, which is sent out to various laboratories, and the laboratory will analyze that blood in a blind or a -- not knowing

what it is, obtain the results that they get from their analysis, and then give the results back to the person who originally sent the bloods or blood or body fluid out to the laboratory.

Q. Does your laboratory engage in proficiency testing, for example, for ABO blood typing?

A. Yes, we do.

Q. And do you receive communication back from the person who submitted the sample to you as to how you performed on the test?

A. Yes, we do.

Q. Approximately how many times have you personally engaged in proficiency testing in your laboratory here in San Bernardino in terms of ABO blood analysis?

A. Okay. As far as individual proficiency tests that were sent out, it would be about 12 times.

Q. And did you receive communication back as to how you performed on each of those 12 proficiency tests as to ABO blood typing?

A. Yes, I did.

Q. How did you perform?

A. I got all of them right, none of them wrong.

Q. And to clarify for the Court, essentially proficiency testing involves someone sending you a sample which has a particular ABO blood type on it; is that correct?

A. Yes, sir.

Q. And that has previously been determined by the laboratory or sender?
A. Yes, it has, yes.
Q. And they do not communicate to you in any fashion what the ABO blood type is?
A. No, they do not.
Q. And then you type it to determine, I assume, whether or not it is blood?
A. Whether it is blood, whether it is human blood, and then to go on with the ABO and the enzyme types, yes.
Q. And then you return all that information, the results of your tests to the person -- to the laboratory that sent you the sample in the first place?
A. That is correct, yes.
Q. And then they compare your results of the unknown sample with their known results?
A. Yes, sir.
Q. And they communicate that to you whether or not you're making correct analysis, essentially; isn't that true?
A. Essentially, yes.
Q. The procedure you employed to determine ABO blood type on the 12 proficiency tests, is that the same procedure that you informed the Court of earlier this morning?
A. On the 12 proficiency tests, no, it is not.
Q. What method did you use in those particular proficiency tests?

A. As far as the bloodstains were concerned, they are analyzed differently from the whole blood. And it's two methods. One is called the absorption-elution; the other one is called Lattes.

Q. Could you explain to the Court, first of all, the absorption-elution method.

A. Okay. The absorption-elution method, again, is looking for the antigen.

Q. If I could stop you for a minute, Mr. Gregonis, by that, can we assume that when you engage in proficiency testing with ABO, it's always been a bloodstain and never whole blood; would that be fair to say?

A. That is correct, yes.

Q. If you could return now to your explanation of the absorption-elution method.

A. Okay. Again, you're looking, as far as the absorption-elution, you're looking for the antigen in the bloodstain. What is done, a sample of this bloodstain is taken. You add, again, known anti-sera, anti-A, anti-B and anti-H to the blood stain itself. You then let that sit in a refrigerator for approximately four to six hours. If there is an antigen present, that will react with the antibody.

For instance, if you have an A blood stain, it will react the anti-A anti-sera. And -- what happens there is that the anti-A will be bound up by the A antigen on

the bloodstain.

You then take and you wash away any excess unbound anti-sera, leaving simply any reacred anti-sera, in the bloodstain, and that's about it.

You will then take this, add some saline to it, put it in an oven at approximately 58 degrees Celsius. What this does -- and that's over a time period of 15 to 20 minutes. What that does is it frees the reacted antibody into solution.

And you take that, you add known A, B and O cells. If you have the freed antibody in there, for instance, in this case, the -- the A antibody which had reacted with the antigen, it will then be free to react with any known cells or red blood cells that you add to it.

So you add A, B and O red blood cells that will react with the A red blood cells and make for clumping or agglutination.

Q Returning, for a moment, to the technique you use to analyze whole blood to determine its ABO blood type, is that a method that you developed?

A No, sir, it is not.

Q Is that a method that is regularly employed by every member of your study group, serology study group?

A In some form or another, yes.

Q Is that a method that is limited in its application to people in the forensic science field?

A. No, sir, it is not.
Q. Is it also used by hospitals?
A. Yes, it is.
Q. Blood banks?
A. Yes, it is.
Q. By people who do paternity testing?
A. Yes, it is.
Q. And have you read the literature that pertains to the reliability and validity of this technique that you use for typing whole blood to determine its ABO blood type?
A. Some of it, yes.
Q. In which particular journals or books or handbooks have you done that reading?
A. Okay. As far as whole blood typing, this is concerning -- if I can think of the titles -- most of them deal with blood bank books. One is by Race & Sanger. Those are the authors. I do not recall the name of the book itself. There are also a number of blood bank books which give examples and techniques for doing ABO typing.
Q. What is the definition in scientific terms of the term "reliability"?
A. Reliability in scientific terms would be simply that you can reproduce, using the same methods, you can reproduce the results.

(No omissions.)

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Q What does the word "validity" mean in scientific terms?
A Validity would mean that you can -- it's justifiable that you can apply the test that you're applying to the sample.
Q Based on the blood typing analysis that you have done with whole blood to determine the ABO blood type, based on your education and your experience and your conversations with members of your serological group and the reading that you've done, do you have an opinion as to whether or not the technique you employed is reliable and valid --

MR. NEGUS: Objection.

Q BY MR. KOCHIS: -- to determine the ABO blood type of whole blood?

MR. NEGUS: Objection. His opinion is irrelevant. I have not heard any foundation which would establish Mr. Gregonis as the sort of person who is qualified to give an opinion on scientific reliability.

People versus Kelly specifically goes to the kind of persons who can give such testimony. The example that they had in People versus Kelly was a man who was, similar to Mr. Gregonis, employed by a crime laboratory. He apparently had done more voice print comparisons than anybody in the known world, or at least he was certainly the person who had done the most of any -- of anybody. He had extensive training of the type that Mr. Gregonis has

in how to perform the techniques, but the court in Kelly, nonetheless, found that that individual was not qualified to testify on the Kelly-Frye issue because his qualifications were those of a technician and law enforcement officer, not of a scientist.

Mr. Gregonis has his -- has a bachelor's degree in criminalistics, which is basically a field which technicians are involved in. He has testified to his -- his technical experience in performing the experiments, but his only scientific background that I could recall was a course in basic chemistry, biochemistry and genetics at the undergraduate level, and that hardly qualifies him as a scientist.

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MR. KOCHIS: Well, Your Honor, our position is Mr. Gregonis has a college degree, which I believe sets him apart from the expert in Kelly. He's also testified as an expert many times in this area. He does reading in the field. He is a member of a professional society that is involved in the field. He's been a chairman of that particular portion of the society in the past. He's done extensive research in the -- reading in the area in the past. He's done an extensive work in the area in the past in terms of the volumes of the samples that he's engaged in. He's engaged in proficiency testing as to dried blood, and he's never apparently made a mistake on that, and it is our position that we can call a number of

people to give their opinion. We don't intend, as they did in Kelly, to rest our position on the opinion of one particular expert, and we feel that Mr. -- the foundation has been established that Mr. Gregonis is a serologist. He's qualified as an expert in the past. He's certainly qualified to give an opinion as to the reliability and validity of a technique that he has read about, that he has employed himself over a thousand times, and that he has testified to as an expert over 90 times.

We're dealing with a field that is quite a bit different than voice print identification in that Mr. Gregonis has testified that literature indicates that it's used -- the technique is used in other fields -- blood banks, genetics, and the medical field.

MR. NEGUS: Your Honor, Mr. Nash, the expert in Kelly, had done over a hundred and eighty thousand tests, I believe, if I recall correctly. He had taken courses in -- from all the leading experts in the field, Dr. Tosi and Mr. Kersta, in fact, including people that were pioneers in the field in terms of the actual technique. His education was -- was certainly broader than Mr. Gregonis' in terms of the -- of the underlying theories behind his technique; nonetheless, the court in Kelly said that on the issue of testimony on Kelly-Frye, it is an area where -- and I'm quoting from page 139 -- in which only another scientist in regular communication with other colleagues in

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the field is competent to express such an opinion. They distinguish Mr. Nash as a technician from the scientists in the field.

Mr. Kochis may well bring in those -- bring in some of those scientists, but that doesn't bootstrap Mr. Gregonis into being -- into being one of them. His qualification -- the fact that he has testified in court before merely says that he is qualified as a technician, not that he is qualified as a scientist, which is the difference between the two -- between the foundation necessary to testify in Kelly-Frye and the foundation necessary to testify as to his results.

THE COURT: Anything further?

MR. KOCHIS: Your Honor, I may add that Kelly-Frye had to do with a new field, and I believe in our points and authorities we both indicated that ABO blood typing is something that has been accepted by the courts in this state as being valid and reliable. At least the Huntington case and the other cases Mr. Negus and I cited indicated that, and as far as ABO blood typing is concerned, this is not a case of first impression in front of any court.

MR. NEGUS: That doesn't mean that Mr. Gregonis is qualified to give an opinion.

THE COURT: Counsel, I am going to admit it subject to striking the whole thing later on.

Contrary to the Kelly case, I think there will be

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further evidence on the point, and I don't know what weight his testimony or opinion will have on the issue at the moment, but let's proceed on it. Overruled.

MR. NEGUS: Just so we don't have to go through this same dialogue every time that it comes up, can I have a continuing objection to Mr. Gregonis' qualifications to testify as a scientist on reliability of all these different techniques?

THE COURT: Yes. Proceed.

Q BY MR. KOCHIS: Mr. Gregonis, what is your impression -- excuse me. I'll strike that. What is your opinion as to the reliability and validity of the technique to determine the ABO type of whole blood that you employed in this particular case?

A That it's reliable and valid.

Q Likewise, based on your experience, your education, your seminars, the work that you've done in the laboratory and the literature that you've read, do you have an opinion as to the reliability and validity of the two techniques that you employed in this particular case to determine the ABO type of a bloodstain?

A Yes, sir, I do. They are both reliable and valid.

Q Before we move to the enzyme typing, could you explain briefly to the Court what the Lattes procedure consisted of.

A Okay. The Lattes procedure is looking for the

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antibody, and simply what you do is you take a bloodstain and add dilute suspension of known cells, ABO. If the bloodstain contains the A or the A antibody, it will react and clump the A cells that you add to it, and so on and so forth for the B or the anti B. If a bloodstain contains the anti -- or the B antibody, it will clump the B cells.

Q. Likewise, Mr. Gregonis, do you have an opinion as to the acceptance in your community, the community of your friends that are serologists, as to the reliability and the validity of the technique you employed in typing whole blood to determine its ABO blood type?

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A. It's accepted both as reliable and valid for typing bloodstains as far as the ABO blood type.

Q. In the forensic community?

A. In the forensic community, yes.

Q. And through your reading and your conversations, do you have any knowledge as to whether or not people in the medical field, for example, people who work with blood banks and paternity testing, accept the method that you employed to determine ABO blood type of whole blood as valid and reliable?

A. Yes, they do.

Q. Do they in fact use the same technique?

A. Or variations of, yes, same principles involved.

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Q. To your knowledge, do people that work in hospitals or in a medical setting analyze bloodstains?

A. Very rarely will they do such thing, not normally.

Q. Turning your attention again to the -- to the enzyme typing and the use of the agarose/starch gel electrophoresis, do you have an opinion as to whether the people in your community, the forensic serologists, consider that technique, the agarose/starch gel electrophoresis technique, a valid and reliable means of determining the enzyme content of whole blood as it relates to EsD and PGM?

A. Yes, they do consider it valid and reliable for whole blood.

Q. And do you likewise have an opinion as to whether or not this technique is accepted as a reliable and valid means of determining the EsD enzyme type and PGM enzyme type of a bloodstain?

A. Again, they generally consider it reliable and valid as to the determination of bloodstains for EsD and PGM.

Q. Returning for a moment to the medical community, for example, Dr. Sparks, are you aware of whether his laboratory employs electrophoresis to determine enzyme types?

A. Yes, they do.

(No omissions.)

Q. And are you aware of whether or not in his community, the medical community, medical doctors in their laboratories use electrophoresis to determine the enzyme type, for example, P -- PGM and EsD of whole blood?

A. Yes, they do.

Q. And do you have an opinion as to whether or not the literature indicates that, within that community, that community accepts electrophoresis as a valid and reliable means of determining the EsD and PGM enzyme type of whole blood?

A. Yes, they do.

Q. What is the difference, if any, that's involved in testing a particular bloodstain to determine its EsD or PGM enzyme type, that difference being difference from the steps you must take to determine the EsD and PGM enzyme content of whole blood?

A. There's not really a big difference. The -- the biggest difference, I would say, is for bloodstains you must get them in the solution first, whereas the blood or whole blood itself is already into a solution, liquid form.

Q. How do you get a bloodstain into a solution so you can place it on the plate?

A. Basically what I'll do is I'll put the bloodstain into a what is called a reducing reagent in the EsD and PGM. It's something called the Cleland's reagent. And that essentially brings the enzymes back to their original

state, along with putting them into solution.

Q. Are the steps that you take before you put the unknown blood samples on the plate the same whether you're going to run a plate for whole blood or dry blood? By that I mean, for example, for the Group I enzymes, and let's take $PGM_1$, do you use the same gel for a whole blood sample as you would a bloodstain?

A. Yes, we do. They are generally run side by side.

Q. Use the same type of plate?

A. Yes, sir.

Q. Use the same electrical charge?

A. Yes, sir.

Q. Use the same -- what's the stain called? I forget. What for the PGM? Not the stain but the overlay.

A. Reaction.

Q. Use the same reaction?

A. Yes, I do.

Q. And, likewise, for the PGM, other than getting the bloodstain into a liquid form to put on the plate, are the steps the same in analyzing whole blood from dry blood?

A. Yes, they are.

Q. And this all involves the principal of electrophoresis?

A. Yes, it does.

Q. Now, essentially with all the group systems, the Group I, the Group II, the Group III and the Group IV, do you use electrophoresis to attempt to determine the enzyme type of

those various blood groups?

A. Yes, I do.

Q. And is the difference in the testing procedure, for example, between Group I and Group II, the mixture of the gel that you use?

A. Yes, it is. It uses a different buffer or liquid, and it uses a 10 percent starch rather than a one percent agarose-one percent starch solution.

Q. And what type of gel did you use for Group II?

A. It's a starch gel.

Q. Once the gel itself is mixed, are the steps that you take to do your reading off the Group II system essentially the same as the steps you take to do the Group I system?

A. Essentially, yes.

Q. Do you use different reagents to get a reading in the Group II system than you would, for example, the Group I system?

A. Yes, we do.

Q. But you use the same type of plate?

A. Same -- well, as opposed to the Group I and Group II?

Q. Yes.

A. They are -- well, first of, the -- the starch, as opposed to the starch/agarose.

Q. I'm not talking about the gel; I'm talking about the glass plate itself.

A. Yes, we do, exactly the same.

Q. And the machine that you use is the same for both groups?
A. Yes.
Q. Likewise, with the Group III and the Group IV systems, is the technique essentially the same?
A. Essentially the same, yes.
Q. How does the technique for the Group III system differ, if it does differ, from the technique that you have described for the Group I system?
A. The development is slightly different. But, other than that, the -- there is one thing in the group -- or, in the Group III which is called haptoglobin which is done in a different manner. It's done with a what's called a gradient acrylamide gel, which is -- most all the Group I, Group II, Group III and Group IV are done on a horizontal surface, whereas what -- the haptoglobins will be done on a vertical surface.
Q. Mr. Gregonis, at the time that you were doing the work in this case, were you running the haptoglobin separately as almost a fifth system or were you running them together with the Group III system?
A. Essentially I run separately.
Q. And when you did the work in this system -- in this case, excuse me, you got to the Group III system, did you use one gel to do the transferrin and the GC?
A. Yes, I did.
Q. And did you make those readings essentially off the same

plate?

A. Yes, I did.

Q. Now, in -- is there any particular term that applies to getting more than one reading off the same plate? Is that referred to as a multi-system?

A. A multi-system approach, yes.

Q. Are you the only person in the field of forensic serology that uses a multi-system?

A. No, sir.

Q. To your knowledge, is a multi-system used by the other serologists in your study group that engage in electrophoresis?

A. Yes, it is.

Q. And to your knowledge, are you the only 40 people in this country that uses a multi-system in electrophoresis?

A. No, sir, we're not. There are others that use it.

Q. Other people in other law enforcement agencies in other states that use a multi-system?

A. Yes, sir.

Q. Likewise, in the -- in the medical field, for example, with Dr. Sparks, does his laboratory use a system which allows them to run more than one enzyme type of a particular plate?

A. Yes, they do.

Q. Do they use the exact same multi-system that you use?

A. No, sir, they do not.

Q And are you familiar as to whether or not the use of a multi-system is accepted as a -- a valid and reliable means of enzyme typing in the medical field by people, for example,such as Dr. Sparks?

A Yes, it is accepted.

Q At the time that you did the work on this particular case, the Cooper case, was your laboratory set up to do PGM subtyping?

A No, sir, it was not.

Q Did you analyze any of the whole blood samples that you had in this particular case to determine their PGM subtypes?

A No, sir.

Q Likewise, the Group I enzyme type, GLO, were you doing that type of enzyme analysis in your laboratory at the time this case arrived in your laboratory?

A No, sir, we were -- were not.

Q Did you analyze any of the whole blood samples in this case to determine the GLO enzyme type?

A No, sir.

Q Likewise, any of the stains?

A No.

Q Did you do any PGM subtyping on any of the stains?

A No, sir.

MR. KOCHIS: Your Honor, I was wondering if we could take a recess at this point.

THE COURT: Sure. About 15 minutes.

(Recess.)

THE COURT: Please continue.

Q. (BY MR. KOCHIS:) Mr. Gregonis, in this case, did you conduct a serological analysis of at least six known blood types, whole bloods?

A. Yes, I did.

Q. For example, did you conduct an analysis of the blood of each of the victims, that being Christopher Hughes, Joshua Ryen, Jessica Ryen and both Mr. and Mrs. Ryen?

A. Yes, I did.

Q. Did you likewise conduct an analysis of the whole blood sample from the defendant in this case, Mr. Cooper?

A. Yes, I did.

Q. And did you analyze each of those six samples to determine their ABO blood types and in the procedure that you have previously described?

A. Yes, I did.

Q. And referring to the enzymes, did you examine each of the six whole blood samples to determine their Group I enzyme type, the EsD and the PGM?

A. Yes, I did.

Q. And did you analyze each one of the six whole blood samples to determine their type of Group II enzymes, the EAP, the AK and the ADA?

A. Yes, I did.

Q. And, likewise, did you analyze each of the six whole blood samples to determine the particular serum protein type, the transferrin and the GC, the Group III system?
A. No, sir. I analyzed only Mr. Cooper's blood for those.
Q. Did you analyze any of those six samples to determine the enzyme types that fall in the Group IV category, the peptidase A and the CA II?
A. Yes, I did.
Q. Which ones?
A. Both. All -- I analyzed all six for those.
Q. And then at that time you were running the haptoglobin as a separate system?
A. Yes, I was.
Q. And did you run all six samples to determine the haptoglobin type or just Mr. Cooper's?
A. Just Mr. Cooper's.
Q. Now, does your laboratory engage in proficiency testing as to the enzyme types themselves?
A. Yes, we are.
Q. For example, have you engaged yourself in proficiency testing on the Group I enzymes?
A. Yes, I have.
Q. Approximately how many times?
A. Again, probably -- about 12 times.
Q. And what were the results of your examinations under the proficiency tests?

A. I got them all right.
Q. Likewise, do you personally engage in your laboratory in proficiency testing for the enzymes in the Group II system?
A. Yes, sir.
Q. About how many times?
A. I believe that would be -- I don't believe I did those on the first sample, so it would be 11 times.
Q. And what were the results?
A. I got them all right.
Q. The Group III system, do you engage in proficiency testing on the enzyme -- serum proteins within that group?
A. Yes, we do.
Q. About how many times have you participated in proficiency tests in that group?
A. Believe it's two times.
Q. And what were the results?
A. I got them all right.
Q. Do you engage in proficiency testing in the enzymes found in the Group IV system, the PEP A and the CA II?
A. Yes, sir.
Q. How many times have you personally engaged in proficiency testing for those enzymes?
A. Approximately four times.
Q. And what were the results of the proficiency tests?
A. I got them all right.

Q. Have you ever done any proficiency testing in the laboratory for haptoglobin?
A. Yes, sir.
Q. How many times?
A. Again, approximately two times.
Q. And what were the results?
A. Again, all correct.
Q. Now, are you familiar with the term that Mr. Negus used with the Court this morning, the Lewis system?
A. Yes, I am.
Q. And could you tell the Court what that consists of.
A. Okay. The Lewis system, or, the Lewis antigen is the antigen which is found on the red blood cell. And basically what interest it is to forensic science is that you can tell from the blood whether a person is a secretor or a non-secretor.
Q. And is the Lewis system, is that the name of the test that you employ to determine whether someone is a secretor or not or does the test have another name?
A. That's the name of the -- of the antigen that I'm looking for. The test that I employ to determine the Lewis is called the Ficin microcapillary tube test.
Q. And is that a test that can only be used on whole blood or can it be used on stains as well?
A. There is some research for stains. However, I have only seen it done on whole blood -- on whole blood and also

have only seen it done in case work on whole blood.

Q. And is that particular procedure accepted within the community of forensic serologists as a valid and reliable method of determining from a person's blood, red blood cells, whether or not they're secretors?

A. Yes, it is.

Q. Can you explain to the Court if a person is a secretor what type of information that may give you as to the person's blood type being manifest in other body fluids.

A. Okay. If a person is a secretor -- first of all, "secretor," "non-secretor" applies only to the ABO blood group system, not to the enzymes, not to the serum proteins. If a person is -- is a secretor, he will secrete his blood group antigens, his ABO blood group in the body fluids other than blood. That includes semen, saliva, perspiration and even gastric juices.

As far as -- as an example, I myself, what I'm called is an A secretor, which means I'm an ABO Type A in my blood; I am also a secretor. So I will have in other body fluids, my semen, saliva, what are called the A and H antigens.

Q. Then in theory it's possible to get an indication as to whether or not you have left saliva at a particular location by testing that; is that true?

A. Part of the test, yes.

Q. Now, in this particular case, did you perform the test

that you have described on the six whole blood samples, the five victims and the defendant, to determine whether or not they were secretors?

A. Yes, I did.

Q. And, likewise, did you test numerous bloodstains that were submitted to your laboratory in this case?

A. Yes, I did.

Q. And, specifically, did you test stains that were removed from a hatchet?

A. Yes.

Q. From a button?

A. Yes, I did.

Q. From a nylon rope?

A. Yes, I did.

Q. And a stain that was found on the wall of the Ryen home?

A. Yes, I did.

Q. And did you test all those stains to determine, for example, their ABO blood type?

A. Yes, I did.

Q. And did you test those stains to determine what their enzyme types were using the Group I through Group IV systems and testing for the haptoglobin where possible?

A. On all the bloodstains that you mentioned, if there was quantity enough, I tested for all the enzymes. As far as the serum proteins including the haptoglobin, the item marked A-41, the bloodstain from the hallway in the

Ryen home was the only bloodstain that I tested for.

(No omissions.)

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Q. In this particular case, did you perform an examination on a stain from a green blanket that was removed from the Lease house?

A. Yes, I did.

Q. And did you conduct a test on the semen stain to determine whether or not the person who deposited that stain was a secretor or not?

A. Yes, I did.

Q. What type of test did you perform?

A. This is a test called the absorption-inhibition test.

Q. And what does that test consist of?

A. Okay. Basically, you're detecting again the ABO antigens, and you're looking for either secreted antigen or a person who is a non-secretor.

Do you want the procedure also?

Q. If you could briefly explain the procedure.

A. Okay. My procedure, as I do it in the laboratory, first take the stain and the approximate size of the stain, and put it into 15 drops of saline. This essentially elutes any of the stain into the saline itself and gives all the blood substances and everything into that saline. I then centrifuge the tubes to get rid of any cellular material. I will then take extracts or a drop of each extract and simply mix that with known anti A, anti B and what's called anti H antiserum in this particular test. If the

antigen is present, for instance, if I tested myself -- I have the A and the H antigen -- it will react with the A and the H -- or the anti A and the anti H antibodies in test tubes essentially. What I will then do after a period of time in the refrigerator that it's allowed to react, I will mix in known A, B and O cells into these test tubes. If the stain has reacted with the antisera, none of the antisera, or a reduced quantity of the antisera, will be available to react with the A -- or with the known red blood cells.

For instance, if I put my own saliva in there, it will bind up the A -- or the A antibody, and once I put in the A cells, that A antibody will not be available to react with the A cells that I put in, and, therefore, when I mix it, it will not clump. If I were to not be a secretor, in that same instance, and put that in there, I would not have the A antigen present; therefore, the A antibody would be free to react with the known red blood cells that I put in, and it would clump.

Q. Mr. Gregonis, the technique that you employed to determine whether or not the stain, semen stain on the blanket, was left by a person who is a secretor or not, was that a technique that is accepted in your community, the community of forensic serologists, as a valid and reliable means of testing a semen stain to determine if

the person who deposited it was a secretor or not?

A. Yes, it is.

Q. Likewise, did you conduct a test on a cigarette butt or cigarette butts that were submitted to your laboratory to determine whether or not there was any saliva on that particular butt?

A. Yes, I did.

Q. And what technique did you use?

A. It is a test that determines the presence of amylase or not, and it is a test that is called phaedebas test. What happens in this technique is that amylase itself, which is an enzyme found in saliva in a large quantity, is designed to break up starch into smaller molecules. What this test does is it takes the starch molecule bound with a blue dye, and you mix in an extract of your stain. If there's amylase present, it will react, break apart the starch and free the blue dye into solution, and you essentially get a blue colored solution if there's amylase present.

Q. Within your community of forensic serologists, is that accepted as a valid and reliable means of determining whether or not there is any saliva on a particular sample, for example, a cigarette butt?

A. Yes, it is.

Q. And for us laymen, what is the significance of finding amylase on an object such as that?

A. Okay. Not necessarily just finding amylase, but finding amylase in a high quantity is a good indication that you have saliva present.

Q. What is the technique that you employed on the cigarette butt to determine whether or not the individual was a secretor and, if so, what his ABO blood type was?

A. Okay. Again, the -- that would be the absorption-inhibition test.

Q. The same test that you performed on the blanket that had what appeared to you to be the semen stain?

A. Yes, sir.

Q. And is that absorption-inhibition test accepted within your community of forensic serologists as a reliable means for testing for saliva stains as well as semen stains?

A. Yes, it is.

Q. In this particular case, did you likewise attempt to determine what the enzyme types were that were found in the semen on the blanket?

A. Yes, I did.

Q. And is it possible to make that determination from a body fluid such as semen?

A. Yes, it is.

Q. And what types of enzymes did you look for in this particular case on the blanket?

A. Okay. First of all, the two enzymes that I analyzed for that are present in high enough quantity in semen to analyze are the PGM and the peptidase A, and those are the ones that I looked for in the semen.

Q. Were those the only enzymes you looked for in the semen stain?

A. Those are two that I was definitely looking for. I also tested for the CA II and the EsD, although I did not at the time expect to find those, because they are not present in very high quantity.

7b Q. And to determine what type of enzymes exist in a semen stain, do you use electrophoresis as you do in bloodstains?

A. Yes, I do.

Q. And once you get the sample on the plate, by that, I mean the semen sample, is the procedure essentially the same as the procedure you follow with a bloodstain?

A. Yes, it is.

Q. And is that the procedure that you employed in this case?

A. Yes, it is.

Q. Within your community, the community of forensic serologists, is the use of electrophoresis accepted as a reliable and valid means of determining what the enzyme types are in semen stains?

A. Yes, it is.

Q. Mr. Gregonis, based on the reading that you've done, the conversations that you have had, your education and your experience, do you have an opinion as to whether electrophoresis, starch gel electrophoresis, is a valid and reliable means of determining the enzyme types that existed in bloodstains?

MR. NEGUS: Objection, irrelevant for essentially the reasons stated before.

THE COURT: Overruled.

THE WITNESS: Yes, I do.

Q. BY MR. KOCHIS: And what is your opinion?

A. That would be that it's very reliable and very valid for the examination of bloodstains and semen stains for the presence of enzymes and serum proteins.

Q. Mr. Gregonis, are you familiar with a term "population genetics"?

A. Yes, I am.

Q. And to what does that apply?

A. That applies to the distribution of various factors or traits in the general population.

Q. Is a person's enzyme type, for example, EsD enzyme type or PGM enzyme type, related in any fashion to their ABO blood type?

A. No, sir, it is not.

Q. Likewise, is a person's PGM enzyme type related in any fashion to their EsD enzyme type?

A. No, sir, it is not.
Q. Are there any of the enzymes that you've testified to in this case that you tested for in this case that are related to each other. By that, I mean that have any particular -- for example, CA II enzyme type would cause you automatically to have a particular EsD enzyme type?
A. No, sir, there is not.
Q. Now, have studies been conducted by individuals in the field of serology as to the frequency with which particular genetic markers appear?
A. Yes, there has.
Q. For example, do geneticists and people that work in paternity keep statistics on the frequency of occurrence of a particular ABO blood type?
A. Yes, they do.
Q. And do people in those fields keep track of the frequency with which a particular EsD enzyme type appears in a given population?
A. Yes, they do.
Q. Have you relied -- or did you rely on particular persons and their studies when you gave your opinion at the preliminary hearing in this case?
A. Yes, I did.
Q. And what studies specifically did you rely upon?
A. The biggest study that I relied upon was one by

Dr. Grunbaum where he analyzed 22 various factors in the blood.

Q. And by factors, are you talking about not only ABO blood type, but enzyme type?

A. Yes, sir.

Q. And did he conduct a study to determine the frequency in certain populations by which those factors appear?

A. Yes, he did.

Q. Are you familiar with the term "power of discrimination"?

A. Yes, I am.

Q. And to what does that term apply?

A. Discrimination power would apply to if you take a system, what is the probability that you can take two random individuals and discriminate between them using that system.

Q. Returning again to the -- to the topic of population genetics, is there a procedure, a mathematical procedure, by which one can determine or estimate the frequency of occurrence of a particular genetic makeup within a given population? And by that, I mean is there a procedure that you employ to determine, for example, how many times within a given population of a thousand people a particular genetic makeup will occur, for example, the same ABO type, EsD type, PGM type, all the way through the four groups?

A. Yes, there is.

(No omissions.)

Q. And how is that particular procedure employed?
A. Basically just to take the population that you have, say, eight thousand people, and analyze for any number of systems that you're looking for and find the percent or numbers that you have -- that have a particular type.
Q. For example, the tables that you used that were published by Dr. Grunbaum, did they indicate the frequency within given populations of, for example, how a particular EsD enzyme type will occur?
A. Yes, he did.
Q. And a particular CA II enzyme type?
A. I don't believe that he has included CA II. I believe I got that one from another publication.
Q. Are there any of the enzyme types that are related to race?
A. Yes, there are.
Q. Which particular enzymes?
A. As far as enzymes go, that would include the peptidase A, the CA II, and also the G6PD.

MR. KOCHIS: Your Honor, I have no further questions at this point.

THE COURT: Mr. Negus.

CROSS EXAMINATION

BY MR. NEGUS:

Q. When you gave your opinion that the different tests that

you used were reliable, did you mean to imply that -- that the same factors go into determining reliability as to all the different tests? Or, let me rephrase that.

Is your -- is your opinion that the tests are reliable a -- a group opinion or do you have an opinion as to each of the tests individually?

A. That would be as to each individual test.

Q. In -- in coming to an opinion as to whether a particular technique is reliable for a particular enzyme in a particular -- particular body fluid, there's a bunch of factors that -- that have to be considered; is that right? I mean, there's more than just --

A. Yes, there is.

Q. And those factors will vary with, for example, whether your sample is gathered at a crime scene or whether it's gathered in a laboratory?

A. As to whether it's a -- a bloodstain that's case material or whether it's prepared stains, yes, that's part of it.

Q. So you -- so you, if you're looking at -- at -- if you're analyzing blood, whole blood that you scoop up at a crime scene, there may be different factors as -- as to reliability as opposed to whole blood which is taken from a person's arm and put into a test tube in a laboratory?

A. Given those two circumstances, yes.

Q. One of the factors that differen -- differentiates

between crime scene blood and laboratory blood is, on the one hand, you have unknown contamination, on the other hand, steps are taken to make sure that the sample is pure; is that true?

A. Depends on the studies that are done. Basically the -- the studies in the laboratory may include various things where you have contaminants. Obviously if you're dealing with a whole blood out of a sterile vial, that is different and not as contaminated as, say, a blood stain on a wall someplace.

Q. Well, let's just take the difference between, say, a crime scene sample, and you mentioned medical laboratories In a medical laboratory, there would be an attempt made to make sure that the -- the blood sample was pure; is that correct?

A. That is true, yes.

Q. In a crime scene blood sample, there would also be -- you would have the difference that there was -- you would know the different physical conditions -- excuse me, you would not know the different physical conditions under which the blood had undergone, like, heat, temperature, duration, various things like that, whereas, in a medical laboratory, they would know precisely when it was drawn and under what conditions it was stored; is that correct?

A. For the most part, yes.

Q. Similarly, in a crime scene blood stain, you would not

know whether or not the blood had undergone any deterioration, whereas, in the laboratory, medical laboratory, there would be an attempt made to make sure that the blood was well preserved; is that true?

A. As far as before you collect it, yes.

Q. In a crime scene situation, it would be unknown whether the blood was in fact blood or a mixture of fluids or what it was when it was collected; is that correct?

A. At the time of collection, no. But after the analysis, I think that you can make some statements as to whether it is a mixture of blood or mixture of fluids or not, yes.

Q. Well, that depends on the analysis, whereas, in a medical laboratory, if it were blood, they would know it was blood; is that correct?

A. Yes, I would hope so.

Q. And in a crime scene, you would not be aware if there was a mixture of body fluids from different individuals or if it all came from the same individual; is that correct?

A. Are you talking at the initial sampling or at the -- after the analysis?

Q. At the time the sample is -- at the time when -- the time the sample comes into your laboratory, when you have to start analyzing it, you don't know?

A. Okay. Before the analysis, no, you don't know that.

Q. Whereas in a medical laboratory efforts are made to make sure that you don't mix up one person's blood with another?
A. That is correct, yes.
Q. Do all the -- there's also a difference, is there not, in the chemical environment in which a crime scene sample is kept as opposed to the chemical environment which a laboratory sample is kept; is that correct?
A. As opposed to a whole blood sample, yes.
Q. In the whole blood there would be some sort of anti-coagulant added which wouldn't be true in a crime scene sample?
A. Generally, yes.
Q. Do all those factors that I just mentioned with respect to crime scene versus medical laboratory samples affect the reliability of the testing?
A. They can, yes.
Q. With respect to individual markers, that is, the individual enzymes, proteins, antigens that you have described within the human body, there's hundreds of those different markers; is that correct?
A. Yes, there are.
Q. And they all vary in their reliability as far as forensic uses is concerned; is that true?
A. I would say you -- they vary in their reliability to some extent. But, however, they are all reliable.

Q. All 150?
A. Well, no. I'm talking about the ones that I analyze.
Q. Okay. But in -- in -- in picking the ones that you analyzed, you're picking out of a larger -- larger group of polymorphous markers; is that correct?
A. That is correct, yes.
Q. And the -- even amongst the -- the ones that you analyze, the different markers vary in terms of their -- how long they will persist in both room temperature, dry, wet, frozen states; is that correct?
A. Yes, they do.
Q. They also vary in the types of changes that they will undergo both in preserved and non-preserved states; is that correct?
A. Yes, they do.
Q. And they even vary amongst the types of changes that they will undergo in a wet state and vary in the types of changes they will undergo in a dry state; is that correct?
A. Mostly in regards to speed of degradation, yes.
Q. Well, they will also -- I mean some -- some particular -- some particular enzymes will, in a -- a wet state, will -- will change by the protein, the enzyme undergoing a reaction with glutathione and having its charge changed through the formation of mixed disulfides; is that correct?
A. It happens in the dry state also. It just happens in the

wet state faster.
Q. In the wet state some will do that, right?
A. Yes, they will.
Q. Some won't?
A. That is correct, yes.
Q. So the proteins will vary in the types of chemical changes that they'll undergo; is that correct?
A. Yes, sir.
Q. And some proteins will undergo some changes in a wet state that they wouldn't undergo in a dry state; is that correct?
A. I would say again it depends on the -- on the speed and what you're calling dry and what you're calling wet. The relative humidities, what you're looking at, if it's obviously moist, then you're looking at the speed of the degradation. If it's dry but you're talking about a 10 percent humidity as opposed to a 1 percent humidity or something like that, then there are -- there are changes that are similar.
Q. Well, what -- what -- in the literature is there recognized a relative humidity which basically distinguishes wet from dry?
A. Not that I recall, no.
Q. Are you familiar with the work of a man named George Sensabaugh?
A. To some extent, yes.

Q. Are you familiar with articles that he has written on protein degradation in a journal called Isozymes?
A. No, sir, I am not.
Q. The reliability of the various markers will also vary as to the -- the chemical environment in which they are found; is that true? That is, a marker will react differently, for example, to blue denim than to glass?
A. Yes, sir. You're talking about the substrate that the stain or whatever is on, yes.
Q. And that will again vary with each individual marker?
A. I think there's basically some general things that you can look at. There are -- blue denim material, for most of the enzymes, including the ABO, is very difficult or more difficult to get results off rather than, say, a stain that's clean on a piece of glass.

(No omissions.)

Q. Are there any substrates that will affect different enzymes in different manners?

A. As far as different from what I just stated, no, not that I know of.

Q. The reliability of the different enzymes, proteins, what have you, as markers also varies with differences in their chemical structures; is that true?

A. The reliability of the testing? Is that what you're getting at?

Q. Yeah. The reliability of a marker as something that can be used in forensic serology will vary with its chemical structure; is that true?

A. That is true, yes.

Q. You will have different problems of reliability with markers that undergo -- well, back up. The difference between, say, one particular phenotype of an enzyme and another could be a difference of a single amino acid in the molecular structure of the protein; is that right?

A. That is correct, yes.

Q. And the nature of that particular kind of substitution will affect the -- how reliable a particular marker is for forensic work; is that true?

A. I don't think I can agree with the statement in that context. I don't know what -- I don't understand your question.

9-2

Q. Some amino acid substitutions will, for example, result in a clear difference in the protein's total electrical charge; is that right?

A. Yes, sir.

Q. Others the difference may be less clear?

A. That is true, yes.

Q. In other cases, there may be no difference?

A. That is true.

Q. If you're using electrophoresis, won't that fact change the usefulness and reliability of the particular enzyme as the marker?

A. It may change the different patterns that you see due to the difference in the substitution of the amino acids.

Q. Does the rate of creation of these different enzymes vary genetically?

A. It can, yes.

Q. Can that affect their reliability as markers?

A. It can affect the quantity that you find in the blood in the first place.

Q. Does that affect their reliability as markers?

A. No, sir, it does not. It affects how much you're going to find and whether you're going to find it or not.

Q. Will different phenotypes of a single enzyme vary in their rate of synthesis?

9-3

A. They can, yes.
Q. Will that fact affect the enzyme's reliability as a marker?
A. I would say in that context, no. Again, you are still looking at the presence or absence of that phenotype.
Q. Are there hidden variations in phenotypes, that is to say, variations which the electrophoretic means that you use don't detect?
A. Yes, there are.
Q. Which of the enzyme systems that you tested have these hidden variations?
A. The two that I can think of right now -- well, actually three. One is haptoglobin. Another one is the peptidase A, and the other one would be the PGM.
Q. Can those -- the existence of those hidden variations affect the accuracy and reliability of your typing calls of those enzymes?
A. No, sir.
Q. You mentioned that you don't necessarily use the same technique exactly for each enzyme; is that correct?
A. That is correct, yes.
Q. Can the reliability of electrophoretic testing vary with the type of gel that you use?
A. Yes, it can.

Q. Can it vary with the identity of the buffer that you use?

A. You mean the particular makeup of the buffer?

Q. Well, the particular chemical that you use, the particular chemicals that you use in the buffer.

A. Yes, it can.

Q. Can it also vary with the pH of the buffer?

A. Yes, it can.

9a

Q. Can it also vary with the staining technique that you use?

A. Yes, it can.

Q. Can it vary with the temperature that you carry out the reaction?

A. Yes, it can.

Q. Different genetic markers also vary in their reliability according to the body fluid that you're looking at; is that true?

A. They vary in the quantity that is there, yes.

Q. What about the reliability?

A. Not really, no.

Q. Mention was made in the -- that in addition to electrophoresis which tries to -- well, when you're using the word "electrophoresis", you're using that to mean that the different phenotypes of the enzyme in question are separated on the basis of differentials in their electrical charge?

A. Along with if you look at the haptoglobin and the size of the molecule.

Q. Well, the haptoglobin test -- the haptoglobin testing where you're looking at the size of the molecule is unique; is that correct?

A. In the tests that I'm using, yes.

Q. In addition, there is also another type of test that can be done called isoelectric focusing; is that correct?

A. Yes, there is.

Q. And in that particular test procedure, the different phenotypes are being separated on the basis of differences in the pH at which they reach a certain equilibrium?

A. Simply stated, yes.

Q. Do the different enzymes vary in their reliability depending upon whether or not you're separating them by electrical charge, molecular weight or pH?

A. They can, yes. As far as molecular weight and electrical charge, if you will, I would say those are about the same; however, with the pH, there are some that appear to degrade faster than others.

Q. For example, esterase D is not considered to be reliable using the isoelectric focusing approach; is that correct?

A. That is not correct. As far as an initial technique,

yes, I would not use it as an initial technique, but as far as a further differentiation of esterase D, yes, it is reliable.

THE COURT: Can we break it now, Counsel?

MR. NEGUS: Fine.

THE COURT: 1:30, please.

(Whereupon, at 12:00 o'clock noon a recess was taken.)

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ONTARIO, CALIFORNIA; MONDAY, APRIL 23, 1984; 1:45 P.M.

DEPARTMENT NO. 3 HON. RICHARD C. GARNER, JUDGE

(Appearances as heretofore noted.)

THE COURT: Everybody's present.

Go ahead, Mr. Negus.

CROSS EXAMINATION (Resumed)

BY MR. NEGUS:

Q. Did you read the journal of your association for last year, 1983?

A. Yes, I did.

Q. In the journal for last year, there was an article about isoelectric focusing and esterase D; is that correct?

A. I can't remember specifically, no.

Q. Do you remember an article -- do you remember reading an article by a man named Horscroff and a man named Sutton which indicated that for bloodstains over 48 hours old isoelectric focusing did not produce reliable results in analyzing esterase D?

A. No, sir, I do not.

Q. In assessing the reliability of different techniques and different markers, there are also variations in reliability with respect to the interpretation of the measurements produced by the various techniques; is that true?

A. I don't know what you mean by "the measurements."
Q. Well, electrophoresis, using the starch gel technique that you use for Group II, measures how far different proteins will go, varying according to their electrical charge; is that right?
A. It shows how far they'll go, yes.
Q. So that is a measurement?
A. In the crude sense, yes.
Q. The measurement in itself doesn't tell you what phenotype, if any, of acid phosphatase, for example, you have; is that correct?
A. Simply the distance that something will go or where they'll be found on the plate? It is part of the analysis but it's not specific, no.
Q. All right. In order to get to the -- in order to come up with the sort of conclusions that are significant as far as forensic serology is concerned, you have to interpret the results; is that correct?
A. That is true, yes.
Q. And as far as the interpretation of the results is concerned, is there a factor of subjectivity involved that affects the reliability?
A. Yes, there is.
Q. Is there a factor of the education and background of the interpreter which affects reliability?
A. There can be, yes.

MR. NEGUS: Could I have one of these pieces of paper up here marked as whatever? Oh, we haven't decided what -- what marking, exhibit marking numbers we're going to use for this hearing.

THE COURT: Let's figure out some classification that will be used just for these particular -- for this period.

MR. KOCHIS: How about the initials K. F. For Kelly-Frye hearing, Exhibits 1 through --

THE COURT: Kelly-Frye,

THE CLERK: Your Honor, can we just use the "K"?

MR. NEGUS: The "K" is fine with me, or just -- or -- I don't -- do we need or, as far as all the trial motions are concerned, all the in limine motions, do we need to have a different set of numbers for each motion or can we just use one set of numbers for the full schmear?

MR. KOCHIS: I have no preference. But, in terms of later locating them, a letter which may indicate which hearing this came from might be simpler.

MR. NEGUS: Okay. "K-1" is fine. "K-1" through whatever is fine with me, then.

THE COURT: You may use that number again, then.

All right. We'll start off, till we hear otherwise, all exhibits, and that will be "K-1" then.

Q (BY MR. NEGUS:) Can you go to the board to Exhibit K-1, which is the piece of paper, I guess, behind you, the piece of paper I have marked as K-1.

THE COURT: I think maybe we ought -- I'm not used to this courtroom. Can he stand there conveniently or should we move the paper, Mr. Negus?

MR. NEGUS: I don't know. I'm -- whichever way --

THE COURT: Bailiff step over, help him move the paper, if you will.

Q. (BY MR. NEGUS:) Down the left hand side, could you write "Group I, Group II, Group III, Group IV and haptoglobin or HP."

A. (Witness complies.)

Q. Then along the top could you write "gel" on one column; another column for buffer; another column for PH; and a final column for staining.

A. (Witness complies.)

Q. Now, in the Group I that you do, which is just for esterase D and PGM, you use a 1 percent agarose-1 percent starch gel; is that correct?

A. That is correct, yes.

Q. Could you put "agarose/starch" there in the Group I.

A. (Witness complies.)

Q. And for the Group II, you use a starch that is 10 percent starch; is that correct?

A. That is correct.

Q. And put a "starch" there.

A. (Witness complies.)

Q. What do you use for Group III?

A. Agarose.
Q. Would you put "agarose."
A. (Witness complies.)
Q. What about Group IV?
A. Same thing with that. It's agarose. (The witness marked the diagram.)
Q. And then for the haptoglobin.
A. It's acrylamide.
Q. Put that --
A. See if I can get the spelling.
Q. A-c-r-y-l-a, I believe, m-i-d --
A. (Witness complies.)
Q. What buffer -- what substance do you use to buffer your Group I?
A. It's a substance called TRIS and maleic acid.
Q. Would you write that in,then, there.
A. (Witness complies.)
Q. And what pH is the -- is that buffer in the gel?
A. Seven point four.
Q. Put that in.
A. (Witness complies.)
Q. What buffer do you use on the Group II?
A. That is a citrate/phosphate.
Q. Would you write that citrate/phosphate in.
A. (Witness complies.)
Q. And what pH do you keep that phosphate in the gel?

A. I believe it's five five. If I could look at my manual --
Q. Sure.
A. (The witness referred to the document.) Yes, it is.
Q. And what buffer do you use for the agarose in the -- in the gel?
A. For the --
Q. Excuse me, Group III.
A. Group III, that is a glycine. It's -- again, it's a glycine/TRIS buffer.
Q. Okay. Put that.
A. (Witness complies.)
Q. And what pH do you -- do you keep that at?
A. I keep it at eight point three.
Q. Put that down.
A. (Witness complies.)
Q. What buffer do you use for the Group IV?
A. That's again a -- a TRIS/phosphate buffer, seven point four. (The witness marks the diagram.)
Q. And you have written that on K-1?
A. Yes, I have.
Q. What buffer do you use for the haptoglobin?
A. That is also a TRIS/glycine buffer.
Q. And what pH?
A. That is eight point four.
Q. Could you write that on the --
A. (Witness complies.)

Q. Now, using the multi-system, you use the same gel buffer and the same pH for the electrophoresis runs for all of the different -- different proteins that you're -- you're measuring in any particular multi-group system; is that correct?

A. That is correct, yes.

Q. But when you get to the -- when you get to the staining procedures, you use different stains to bring out the different enzymes?

A. That is correct, yes.

Q. For Group I, what -- could you again return up there and then indicate for PGM what stain you use for -- for Group -- to bring out the PGM.

A. You want all the ingredients, all the ingredients in the stain?

A. Well, are there various different stainings -- standard staining combinations of -- of that that one uses? For example, there's -- in developing, for example, the PGM, there's about four or five different chemicals that are -- that are added; is that correct?

A. That is true, yes.

Q. And are those -- is that particular procedure used for other -- for other -- for other of the enzymes?

A. No, sir, it is not.

(No omissions.)

11-1

Q. One of the things that you used to bring out the PGM is a substance called G6PD; is that correct?
A. Yes, it is.
Q. Is that the -- is that one of the more critical of the substances?
A. Yes, it is.
Q. And what is the substance that you use to -- to develop the final stain?
A. All of the things that are included in there are necessary for the development of the stain. They include glucose 1 phosphate, a substance called NADP, a substance called MTT, another one called Phenazine Methosulfate, and along with what you mentioned, the G6PD.
Q. Is it the MTT that brings out -- is the last stage?
A. That's the final result. That's what you see, yes, but you would not see that without the rest of the things in there.
Q. Is that final result obtained with any other chemicals -- with any of the other enzymes?
A. Yes, it is.
Q. Which other ones is it?
A. That would include ADA, AK, along with peptidase A.
Q. Are the -- were those -- with the ADA, AK and peptidase A, do you likewise also use the NAD and the NADH?
A. On the peptidase A, no. You don't need it. The AK --

11-2

or the ADA, no. You don't need it. The AK, I believe you do need it on that.

Q Is there any convenient way that you distinguish between the different staining techniques that you use, any particular names, or you just called them by all the chemicals in them?

A Basically, I have never -- except for calling them by what they develop, I haven't really named what they are.

Q Okay.

A Except fluorescent as opposed to a visible dye.

Q Starting with the PGM, what are the substances that you add to bring out -- to develop the pattern?

A There's a substance called glucose 1 phosphate with approximately 1 percent glucose 1, 6-diphosphate contained within it. Also included in that is the NADP as mentioned before, along with the MTT, the PMS and the G6PD.

11a

Q Could you -- would a fair designation of that be then that G-1-6, G6PD and with a dash to MTT, would that be sufficient to distinguish that from all the other staining techniques?

A I think in any of this, you have to include all of them.

Q Then why don't you do that on the chart, if you would, for PGM.

A. Just for abbreviations?
Q. Yeah, and then for esterase D, what do you add to esterase D to bring it out?
A. I add MUA.
Q. What is MUA?
A. Stands for methylumbelliferyl acetate.
Q. Could you put MBA for --
A. MUA?
Q. Yes.
A. (Witness complies).
Q. And that has to be done in the dark; correct?
A. It has to be read in the dark, yes.
Q. With respect to the acid phosphatase, what do you add to bring that out?
A. That's a substance called MUP.
Q. And what is that?
A. It stands for methylumbelliferyl phosphate.
Q. Could you put MUP after EAP there.
A. (Witness complies).
Q. For the ADA in that system, what do you add to bring that out?
A. ADA consists -- the reaction buffer consists of adenosine plus MTT and PMS.
Q. Could you write those -- could you write adenosine, MTT and PMS after ADA?
A. Along with the enzymes included in that also or just --

Q. That will be sufficient to designate the method that you used to some other scientist; is that not correct?
A. I think we'd better include them all in there.
Q. Okay. Do that, please.
A. (Witness complies).
Q. For the AK, what staining system do you use?
A. That includes glucose, adenosine diphosphate, NADP, PMS, MTT, again the G6PD and the hexokinase.
11b Q. Okay. Could you -- basically there's two main systems for staining AK; is that correct?
A. I don't know.
Q. Okay. Well --
A. I know the one.
Q. Would you put then the chemicals that you used for the one.
A. (Witness complies).
Q. What do you use to develop the transferrin?
A. That would be both the transferrin and the Gc are developed by antisera, and after they're reacted with that, they are stained with a stain called Coosmassie Blue.
Q. So that in order to develop the transferrin and the Gc, you have to have a serum which is specific for the particular enzymes that you're talking about?
A. Essentially, yes. (Inaudible).
THE REPORTER: I'm sorry. You were both speaking

at once.

MR. NEGUS: He said, I believe not the enzymes; serum proteins. He said serum proteins, and I said not the enzyme serum proteins.

Q. Is that correct?

A. It sounds good to me.

Q. Could you then write antiserum next to the Group III in the staining --

A. Yeah. I'll draw a line up there.

Q. And what staining procedure did you use to develop the PEP A?

A. That would be -- include a peptide plus PMS, MTT and L-amino acid oxidase.

Q. Would you write those five, I believe it was, chemicals down after PEP A.

A. (Witness complies).

(No omissions.)

Q. And what do you use to develop CA II?
A. That's a substance called fluorescein diacetate.
Q. And could you write "fluorescein diacetate" on the board, CA II.
A. (Witness complies.)
Q. And, finally, what do you use to develop the stains in haptoglobin?
A. I use either benzidine or ortho-tolidine. In this case I use benzidine.
Q. Could you write "benzidine" down.
A. (Witness complies.)
Q. There's an "n" in there.
A. Yes, there is.
Q. Are there any problems that affect reliability connected with the use of agarose/starch gel with Group I?
A. No, sir.
Q. Are there any mistakes that you can make in the preparation of that gel which will affect the results?
A. There's a possibility that you could make the buffers the wrong pH or put the wrong ingredients in the buffers, yes.
Q. What would that do?
A. That would change the mobility of some of your enzymes, where it would -- where they would be on the plate themselves. This would -- you would detect by the standards that you put on the plate.

Q. Did you do anything to measure the pH in the -- in the buffer?
A. Yes, I do.
Q. What do you do?
A. I standardize the pH against -- using a -- a Beckman-Altex pH meter. And in this case I standardize the pH meter first and arrange from pH 7 to pH 10, and then I'll standardize the buffer using either sodium hydroxide or hydrochloric acid to a pH of seven point four
Q. So you'll either add something to make it more -- more alkaline or more acid in order to get it to the right pH?
A. That is correct, yes.
Q. Do you check it again after you're done?
A. With the run?
Q. Yeah.
A. No.
Q. Is it possible that the -- the pH in the gel buffer can change during the run?
A. In the realm of possibilities, yes, I would say -- say it is possible, yes.
Q. How does that happen?
A. Basically, the concentration of various substances in the gel may change due to the heating and the -- simply putting the electricity across the gel.
Q. Well, aren't -- as the -- the way that the -- way that

this particular process works under group -- under Group I, you have -- you have a glass plate with the gel in a little mold; is that correct?

A. Simply stated, yes.

Q. And at either end you have a sponge of some sort?

A. I use filter paper, but it's a wick into another solution.

Q. And that other solution is likewise a gel with a certain -- excuse me, likewise a buffer with a certain pH to it?

A. Yes, it is.

Q. And you have a positive -- there's two; one's positive and one's negative, right?

A. When you apply the run, yes, that's true.

Q. And as you -- as you apply the electricity, does the pH in the -- at the positive pole tend to change differently than the pH at the negative pole?

A. Yes, it would.

Q. How does that work?

A. Basically because you're at the different poles, you're freeing different types of substances from the buffer itself or the water in the buffer, and you're creating an acid on one end and the base at the other end. So you are going to have switches, essentially, opposite of each other.

Q. Is there any way of making sure that, having these three different pH's, your -- your plate -- your plate buffer and your two tank buffers, that you don't end up having

the plate buffer get changed along with the other two?

A. First of all, as far as the change, it's pretty much designed into the system. You expect it to change somewhat. And so it doesn't really become a factor in the system, since that is expected to happen in the first place.

As far as the change in the gel itself and also the change in the buffers on the other side, one of the definitions of a buffer is to prevent -- or a substance, liquid, which prevents the gross change of pH due to addition or subtraction -- or, the addition of the acid or base.

Now, as far as a change in the gel, it say change to some extent. But, then, again, you're doing this, repeating it each time that you are running the gel.

Q. In the buffer, the -- the way that a buffer works, is it not, is that there is in the buffer in the substance that has that where if you plotted the pH on one axis and the amount of protons, for example, that you're adding on another axis, that there will be a sort of a long flat period before -- before it either falls off or ascends the scale; is that true?

A. Essentially, yes.

Q. So there's only so many, for example, protons that you can add before the pH in the buffer will start to change; is that right?

A. That is true, yes.
Q. How do you guard against getting beyond that particular -- that particular stage in your buffers?
A. Essentially making the buffer properly in the first place.
Q. Any way you can tell by looking at it if you make a mistake?
A. You can tell basically by, since you're the one, or, I'm the one who makes up the buffer, I can tell how much substance I put in there. Also when I'm standardizing the pH of the buffer, I can tell from experience that if I add a certain amount of hydrochloric acid or a certain amount of sodium hydroxide what I would expect the buffer to change at what rate. If it changes more than that, that indicates a weak buffer, and I may throw it out in that case. Probably -- I'm sure I would throw it out if it changes more drastically than I expect it to. If it changes too slow than what I expect it would, then, again, it seems like it's more concentrated and, again, I would throw that buffer out before I used it.
Q. Do you make up a new buffer each time you do a different -- a new run?
A. No, I do not.
Q. How often do you change it?
A. Approximately ever five runs or five days.
Q. Do you keep track of how many -- how long it's in or just

by memory?
A. We keep track.
Q. Do you include that record in the record that you make of each of these different runs?
A. No, I do not.
Q. Is there any way that an outsider coming in and looking at the record that you keep of your experiments can tell whether or not you had the wrong pH in your buffer?
A. Except for possibly the photographs, no.
Q. How would the photographs tell a person?
A. Possibly if you included the origin, in the photographs it may show that the enzyme migrated too far or not far enough in the allotted run time.
Q. In the Group I, is there any problems which you -- can affect reliability with the staining that -- you use to bring out the PGM?
A. Not really, no.
Q. Would -- do you prepare each of the reagents that you use in the staining yourself?
A. They are bought from a chemical company, if that's what you mean. I prepare the total reagents from adding each of those together, the final reagent.
Q. Can any of those things that you use in the staining procedure go bad?
A. They can to some extent, yes.
Q. Which one?

A. In particular, the PMS can go bad. Not that it will show reliability problems, but it may show problems with me not being able to get a result at all.
Q. Any other ones lead to that kind of problem?
A. Again, possibly the G6PD if you don't use it up fast enough. That's basically it.
Q. Do you care, if you're not getting the proper results, not to use the same substance over and over again?
A. Yes, I do.
Q. What about the EsD staining? Anything that can cause reliability problems there?
A. No.
Q. If -- what temperature do you -- do you keep the plate at in your Group I?
A. For the EsD, I keep it at room temperature. For the PGM, I keep it at 37 degrees, or body temperature for incubation.
Q. Excuse me. Back up. When you're doing the run, what temperature do you keep it at?
A. The plate or the cooling platen itself is approximately 4 degrees Celsius.
Q. If the cool platen where some other -- well, if the gel were some other temperature than that, would that cause problems in reliability?
A. Okay. First of all, I -- as far as the gel itself, I didn't -- except for various or relatively sophisticated

pieces of equipment, I don't have a way of measuring the actual temperature on the gel. So as long as my cooling platen is at 4 degrees Celsius, then the temperature of the gel should be constant from run to run.

Q. What sort of contact do you have between the cooling platen and the gel?

A. It's direct physical contact.

Q. There's a layer of air in between?

A. There's -- I guess you could say that, yes. Very thin layer of air. Or a lot of times there's condensation that gets on there. Most of the time there is. So there's a layer of water on there.

(No omissions.)

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Q. You don't add any water, though, yourself?
A. No.
Q. Does the condensation cover the whole plate or is it irregular over the surface of the plate?
A. Just as far as looking at it, it appears to be constant.
Q. You don't take any particular cognizance about that one way or the other?
A. I look at the plates to make sure it's even. If there is condensation underneath, I will look at it to make sure it's even across the top or I'll get rid of it completely. The latter is what I usually do.
Q. You wipe it off?
A. Yes.
Q. Is there anything that will tell you if you have a problem with the heating?
A. Yes, there is.
Q. What is that?
A. The milliamps of the run itself will tell me that. The appearance of the gel will tell me that. Also, the appearance of the isozymes at development time will tell me that.
Q. How will the milliamps tell you that?
A. Basically, because if there's an overheating, I will see an increase in the milliamps because of the concentration of the buffer in the gel is going up

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because of evaporation.

Q. How does the appearance of the gel tell you you have heating problems?

A. As far as that is concerned, there may be a visible warp in the gel due to overheating problems.

Q. If you get a visible warp, do you disregard that run?

A. Yes, I will, as far as that which I do not normally experience, yes.

Q. Do you normally experience a warp?

A. To some extent, yes.

Q. Does that indicate that you're having heating problems with your equipment?

A. No, as far as the normal one, no.

Q. How do you distinguish between a normal one and an abnormal one?

A. Basically, the shape and the constant -- if you look at the warp in the gel, it's -- in a normal run, it will be a straight line across the gel essentially where the gel and the action of the electrophoresis has gotten rid of some of the water.

Q. Could you -- K-2 here.

THE COURT: K-2?

MR. NEGUS: K-2.

Q. Could you draw a -- what you would expect a normal PGM gel without any heating problems would look like

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up on the, say, upper left-hand corner, and we'll write that "normal."

A. Okay. As far as if you're going to look at the gel as it's laying flat on the surface, it would look something like -- the origin is right here. You'd find that this area in here will be not as thick, so all this area in here will be not as thick as the area above it.

Q. What happens when you have heating problems?

A. Heating problems you'll -- first of all, if you'll notice, I drew this essentially a straight line. If you have heating problems --

Q. Could you draw a separate one with heating problems.

A. Yeah. You'll find something in the order that you have a warp, maybe something like that going along it. Rather than a straight line, you'll find a lot of waviness, I guess you would call it.

Q. So that there's an area -- the area between the thinned out gel and the non-thinned out gel has a wave to it rather than just being --

A. Essentially, yes.

Q. Will that particular waviness transmit itself into the appearance of the isozymes?

A. Yes, it can, yes.

Q. And how does that -- could you -- let's see. You've drawn the gel pictures in blue. Could you take another

color there, perhaps red, and use that to show the isozyme bands and how they change.

A. Okay. Do you want me to put PGM or esterase D on here?

Q. Let's use AK.

A. That's the wrong one as far as -- I think it would be easier to illustrate it if I was using PGM or esterase D.

Q. Okay. Use esterase D.

A. In a normal one, you'd find something like this where you have just essentially straight lines across. If I was looking at a warped gel, something like this, I'd find possibly patterns that looked somewhat like this, or they are warped along with the edge of the gel -- or the warp of the gel.

Q. In addition to the overall warping of the gel, do you sometimes ever get spot problems?

A. You can, yes. In particular, when you have a sample that's high in concentration in salt or something like that with a semen sample, that occurs occasionally.

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Q. When you get that -- could you write "heated" over the non-normal one there.

A. (Witness complies).

Q. When you get the heated gel, do you attempt to interpret that?

A. Not normally, no. I'll throw that out.

Q. Are there any other problems that are caused by

heating that you get with the Group I?

A. You'll cause in general a degradation of your sample and possibly a movement into a different area because of the difference in the changes in concentration of the sample itself.

Q. How do you tell with that sample?

A. As far as what, the --

Q. When you get -- when you sit there, you've finished your run, how can you tell whether there's -- whether the enzymes have degraded because of the heating of your --

A. Basically, by looking at the gel itself and looking at the results that you have. If it's warped like this, then it's -- it may have some heating or concentration problems. If it's a particular sample that may be streaked or something like that, it may give you -- may be as a result of that too.

Q. You say you can see streaking in the -- in the -- in the plate that you develop?

A. Occasionally, yes.

Q. How big a curve do you require before you throw it out?

A. I really don't have a standard on that. If it looks bad to me, I'll throw it out. If the things are in such a way that they just don't look right from my experience, it's -- if the standards can't be read or

if they don't line up with any of the other things, then it's time to throw it out.

Q Have you -- is there any literature about the problems of heating with Group I?

A. With Group I specifically, I do not know.

Q. Is there any literature about heating problems in general?

A. Yes, there is.

Q. What literature are you aware of?

A. In particular, that would include Culliford's book on the Examination and Typing of Bloodstains in the Crime Laboratory.

Q. And what cures does he propose for the problem?

A. Essentially, cooling, make sure that your cooling apparatus is working properly or I believe at that time he may have also been using runs in the refrigerator.

(No omissions.)

Q. Any other literature that you're aware of?
A. Not specifically, no.
Q. Is there any literature that you're aware of of the problems of staining with PGM?
A. No, sir.
Q. Is there any literature that you're aware of of the problems concerned with variations in PGM in the gel buffer with Group I?
A. Again, that would -- well, not specifically Group I, no.
Q. What general literature are you aware of with that problem?
A. Again, that would be Culliford's book on The Examining and Typing of Bloodstains in the Crime Laboratory.
Q. How many of the techniques that you used are described in Culliford's book?
A. As far as the general technique of electrophoresis, it's described in Culliford's book. As far as the specific items which go into the gels and such, part of them are taken out of there. Most of them are taken out of Brian Wraxall's Bloodstain Analysis System.
Q. Which ones are taken from Culliford?
A. Okay. Basically, the buffer that I use is taken out of Culliford. I believe it's the identical thing for Group I. Also, I believe it's the identical thing for Group II, although the -- the gel is thinner than what Culliford uses. And, basically, the -- the techniques

are out of his book, the general techniques, whereas the methods themselves are taken out of the Bloodstain Analysis System.

Q. The Bloodstain Analysis System, that is a 1978 publication by Mr. -- Mr. Wraxall published by Beckman and Aerospace?

A. Not sure about the date. I think it may be sooner than that.

Q. I'm not sure. Do you have it there?

A. '79, '78 is what it says. October, 1978.

Q. What problems -- well, are there any problems of persistence, stability in PGM?

A. Other than that it degrades, if that's what you're talking about, it will degrade over time.

Q. What problem, if any, does that cause for reliability?

A. As far as reliability, none that I know of. As far as readability, it's either you get it -- get a good result or you can't read it.

Q. Is there any literature on that that you're aware of?

A. There's several pieces of literature on degradation of the various enzymes. As far as specific references, I can't give you -- give you any of those right now.

Q. Other than just not being able to read the -- read the plate, is there any other visible signs of -- of a blood -- a bloodstain containing PGM being too old?

A. Not really, no.

Q. With PGM, when PGM's in the wet state, being defined as

either in liquid form or when the relative humidity is above a certain point, what sort of changes does it undergo?

A. Basically the biggest change is just that the degradation of the protein of it will be broken down and eaten up, if you will, by other -- by other enzymes in the -- in the stain.

Q. And how does that manifest itself?

A. It will reduce the amount of active PGM on the plate as far as electrophoresis is concerned.

Q. Any other wet state problems that you're aware of?

A. Not really, no.

Q. What changes does PGM undergo in the dry stages?

A. Essentially the same thing except that it's at a slower rate.

Q. Do you -- are you aware of any differences between the wet and the dry changes in PGM?

A. Except for the rate, no.

Q. Do you know any place in the literature where these changes the enzyme undergoes are discussed?

A. Not specifically, no.

Q. Are you aware of anyplace in the literature where it discusses how to avoid any type of problems from those changes?

A. I can't give you any specifics, no.

Q. What would you look for to avoid typing problems as far

as changes in PGM?
A. I don't -- I don't understand your question.
Q. Is there anything that you would look for on the -- on a PGM plate that you have developed to keep in mind as sort of diagnostic indicators that PGM may have undergone some sort of change?
A. I would look for a streaking of the bands, which may indicate the presence of bacteria. Also the possibility that I may have a very faint sample where I would have expected a strong result.
Q. Does PGM undergo mixed disulfide reactions?
A. No, sir, it doesn't.
Q. Does it undergo a deamination, deamidation?
A. It's possible that it could, yes.
Q. How would you tell that if it -- if it were to show up in your plate?
A. If I was looking for that, I would -- if it occurred to a significant degree, I would look for PGM bands that were lower or more towards the negative side.
Q. Do you know anyplace in the literature where readings from deamidation are discussed?
A. Not specifically, no.
Q. Is there anything that you can do to prevent the deamidation, deamination of PGM?
A. Except for the drying and the freezing of the -- when you preserve the sample, not that I know of, no.

Q. Is there anything in the chemical structure of PGM that causes any difficulty in interpreting the results of electrophoresis?
A. No, sir.
Q. In the system that you used, you break down PGM into three main types, one, two-one and two, correct?
A. That is correct, yes.
Q. Are all PGM 1's -- well, take it back.
Do all PGM 1's contain the same protein?
A. No, sir.
Q. Do all PGM 2's contain the same protein?
A. No, sir.
MR. NEGUS: Can I have another piece of paper marked.
THE COURT: While you're preparing that, perhaps we could take the recess.
MR. NEGUS: Okay.
(Recess.)
THE COURT: Go ahead, Mr. Negus.
Q. (BY MR. NEGUS:) The proteins that we're talking about have varying numbers of peptide chains in them; is that correct?
A. That's true, yes.
Q. And on the basis of that, they are classified as monomers, bimers, trimers?
A. That is true.

Q. The -- in general, a monomer, bimer and a trimer, for example, will have different patterns in the heterozygote phenotype; is that correct?
A. That is correct, yes.
Q. Could you go to the board in Exhibit K-3 and draw, just as an example, the typical heterozygote pattern for a monomer, a bimer and a trimer.
A. Okay. As concerning -- do you want a specific enzyme in?
Q. Well, there's a general pattern that -- that's true for most monomers, bimers and trimers, are there not?
A. There is and there isn't. For instance, the pattern for PGM and AK, although they're both monomers, is slightly different.
Q. Okay. Well, let's -- let's start with, for example, in the monomer, start with -- with the AK, being the simple form.
A. (The witness marked the diagram.)
Okay. AK has -- I'll just put up all three types.
Q. Okay.
A. General types.
Q. And three basic types, the first one being a 1, which has a band closer to the origin; the 2-1, which has two bands; and the 2, which has one band.
Q. Let's take, then, a typical bimer, PEP A.
A. Either PEP A or esterase D, either one.
Q. Okay.

A. I'll put them both as the same. (The witness marked the diagram.) As far as either one of these patterns, there's also going to be three types, 1, 2-1 and 2, the 1 being essentially a single band, the 2 being essentially a single band, and the 2-1 being a combination of these, actually 3 bands with the 1 in the middle being the more intense.

Q. And a bimer, the intensity of the heterozygote bands will be approximately -- or, should be approximately 1, 2, 1; is that correct?

A. The bands, as I wrote them up here, they'll be -- the one towards the negative will be 1, and then, as you go up, it will be 2 to 1.

Q. Now, you say that PGM is a monomer but it has a different pattern than AK.

A. That is true.

Q. Why is that?

A. Because of the deamination product which occurs naturally within the body.

Q. And what does that -- what kind of pattern does that produce on PGM? If you could draw that on K-3 below the AK one.

A. (Witness complies.) Okay. Basically, again, PGM has three common types. The 1 will look -- will have two bands, one more towards the negative and then another one further up. The 2 will have one kind of in between

those and then again one further up. And then the 2-1 will -- will be a combination between the two of these.

(No omissions.)

Q. Will those patterns be the same in fresh blood or bloodstains?

A. Many times what you'll get with an older bloodstain, you'll get more of the pattern -- more of the secondary band up here, but generally they'll look like this.

Q. Can you distinguish blood cells in terms of their age when they're floating around in your body?

A. There are methods to do it, but I do not know them.

Q Will young red blood cells and old ones give the same pattern of PGM if you segregate them out?

A. No, they will not.

Q. Why not?

A. Basically, because as the enzyme goes along, more and more of the deamination product or the upper band will appear. The body is changing the enzyme, if you will.

Q. Do -- in your -- in your AK, all the 1's are the same protein; right?

A. Yes, they are.

Q. And all the 2's are the same protein?

A. Yes.

Q. The fact that you have different chemicals there in the PGM 1's, two different chemicals, and the fact that you have this deamination problem with it, can that -- can that cause any type of problems?

15-2

A. As far as calling it a problem, I wouldn't say it's really a problem, because it's predictable.
Q. How do you predict it?
A. Again, as I said, the lower bands will generally change and get weaker as time goes along; whereas, the upper bands will become stronger.
Q. Of the two chemicals that -- the two different types of chemicals that make up the PGM 1 band, do both of them change at the same rate?
A. I would say that generally the -- with the deamination of the 1, they will move more -- it will move into the secondary band.
Q. Well, let's -- when you -- when you do your Group I thing here, and you get this result which you've labeled as a 1, you don't know whether the protein that you have there is the 1 chemical which they call a 1+ or the other chemical which they call a 1-; correct?
A. That is correct, yes.
Q. So what I'm asking you is does this deamidation process take place at the same rate for PGM 1 pluses as it does for PGM 1 minuses?
A. That I do not know.
Q. How about for PGM 2 pluses and 2 minuses?
A. Again I do not know.
Q. You don't know anything about the relative rates of

15-3

deamidation of any of the PGM sub types?

A. The one thing I can say about the PGM sub type, it seems like the 1- and the 1+ do degrade at a faster rate than the 1+, 2+. As to whether they're actually breaking down or being deaminated into another product, I do not know.

Q. How about -- let's just then just in general terms of stability of the 1 pluses and the 2 -- and the 1 minuses and the 2 pluses and the two minuses, could you -- could you rank them in relative order of stability?

A. Not totally. I'd have to say that the 1's -- the 1+ and the 2+ are more stable than the 1- and the 2-.

Q. Generally, you'd expect in the aging -- in the process of just disappearing from the face of the plate, you'd expect the 1's to go before the 2's; is that correct?

A. Not really, no.

15b

Q. What order -- of these bands that you have drawn here under the PGM 2-1, what order do you expect them to disappear?

A. Okay. From the order that I've seen, you will many times see them disappear equally or have the upper bands disappear faster, but, basically, I would say equally.

Q. And you don't -- do you know what process, what chemical process it is that causes them to -- different

bands to disappear?

A. That would be the -- basically, the actions of peptidases on the bands or the protein themselves just simply ripping the protein apart or, as you mentioned, the deamination, which would essentially move the bands up into a further region up.

Q. Just arbitrarily writing on here in order A, B, C, D on the PGM bands, isn't it a fact that what happens as they -- as the deamination process gets underway is that the material that starts out in this what I've labeled as a little "a" band ends up in the spot where I've marked as a little "c"?

A. That is true, yes.

Q. And then the little "b's" end up in the spot where the little "d's" are?

A. That is true.

Q. And then the little "c's" and the little "d's" start migrating beyond where you stain or --

A. Or beyond where I would read the PGM, yes.

Q. You couldn't see these changes as causing any type of problems, though?

A. No, sir. They are predictable, and if I see something that's either a weak stain or something like that, I will not call it.

Q. Do you make an effort to try to find these bands on your plate that are sort of off scale, as it were?

A. I always look for any indications of degradation, yes, and I will develop further up than just the PGM sub 1 locus.

Q. What kind of wet state changes do you get with esterase D?

A. Basically, the production of what would be called storage bands.

Q. What causes that?

A. I believe it's deamination also. It may also be the combination of glutathione with the esterase D.

Q. The formation of mixed disulfide complexes which cause the bands to move more towards the anode?

A. Yes.

Q. The one is reversible by using the Cleland's reagent that you use; is that correct?

A. That's correct, yes.

Q. But the other one isn't?

A. No, it's not.

Q. When you look at your plate, can you tell the difference between a reaction caused by deamidation and a reaction caused by forgetting to use the Cleland's reagent?

A. That I do not know. As far as the standards, if I didn't use the Cleland's reagent on the standards, I could tell.

(No omissions.)

Q. What do you mean? The standards you're talking about you defined before as the -- as the bloodstains that you know what they are?
A. Yes.
Q. In general in -- in -- in coming to your typing results, is it possible to reach a reliable result without using standards?
A. I would say it is possible. However, it's much more reliable if you do use standards.
Q. If you -- well, if you do use standards and the standards don't come out, do you throw out your results?
A. I will, yes.
Q. If the standards come out to be something different than you think they are, do you throw out your results?
A. Generally, yes.
Q. How do you tell if you have deamidation on the -- on your unknowns other than --
A. The presence of storage bands.
Q. Well, you always get storage bands with EsD, don't you?
A. Yes, you do.
Q. What -- does the deamidation ever get to a stage where typing becomes unreliable?
A. It may get to the point where I wouldn't type it. But as far as unreliability, no. If I had something there, I'd say no.
Q. How -- where would it -- how would it -- how would you

recognize a situation where you couldn't type it?
A. If, for instance, you're looking at the thing and there was not a -- an esterase D 2-1 if there was not a 1 to 2 to 2 to 1 pattern, if there's a considerably more amount of storage bands than what would be expected, those two are ways I would tell.
Q. What is the difference between the different -- between the 1 allele and the 2 allele? What causes the allele difference in the esterase D?
A. I would assume that it's a modification of the protein itself.
Q. In what way?
A. I do not know.
Q. What sort of dry state changes does esterase D undergo?
A. Again, I would expect them to go under -- undergo the same things, only at a much slower rate.
Q. Are you aware of any discussion of that in the literature?
A. Not specifically, no.
Q. When the esterase D bands deactivate, do they deactivate at the same rate?
A. From what I've seen on the plates that I've typed, they seem to, yes.
Q. Have you read any discussions about that in the literature?
A. Not that I recall specifically, no.
Q. Do the different alleles of the -- of the esterase D vary in the rate at which they are synthesized?

A. That I do not know.
Q. Are there any hidden variations in esterase D?
A. I do not understand your question.
Q. I think we defined peptidase 1 versus peptidase 8 as a hidden variation.
A. Yes. Okay. If you're talking about the way that I do my systems on esterase D, you're probably talking about the esterase 5's and the 5-1's. Then, yes, there is some variation that I would not normally pick up on the Group I plates.
Q. Do you know what causes the difference between the 5's and the 1's?
A. Again, beside -- except for an actual structural difference, amino acids, no, I don't know the specific structural difference.
Q. Do they degrade at different rates?
A. That I do not know.
Q. In the -- in the acid phosphatase, are there any problems with reliable typing that can -- can develop in the course of the electroprophoretic run?
A. During the course of the electrophoretic run? Not that I'm aware of, no.
Q. How long have you -- how long do you -- how long is that run that you do?
A. Sixteen hours.
Q. Are you there throughout the course of it?

A. No, sir.
Q. Do you let it run overnight?
A. Yes, sir.
Q. With respect to the connection, I guess, the contact between the cooling plate and the glass, do you use -- follow the same procedures with the Group II as you do with the Group I?
A. Yes, I do.
Q. Is there any way that you can monitor amperage fluctuations in the run when you are not there?
A. No.
Q. Do you check the pH of the gel buffer when you get done?
A. No, sir, I do not.
Q. How long do you keep that particular -- those particular buffers?
A. Again, it's the same, five days or five runs.
Q. And there's no way that you can tell now on any given run whether it was the first of those five days or the last of those five days?
A. No, sir, I cannot.
Q. When the -- the protein is in the gel, it's in the wet state; is that correct?
A. Yes, it is.
Q. It's therefore liable to -- to wet state changes?
A. During the electrophoresis run?
Q. Yes.

A. I would say not as defined before, no, because you're simply removing all the -- you're taking the enzyme away from the substances which may cause the wet state changes.
Q. What about if it gets too hot?
A. Then you will have the problem with degradation.
Q. Same kind of degradation as you get with wet state changes?
A. No. I would assume with the too hot that you're looking at a complete denaturization of the protein.
Q. What degree is it not? I mean, you can get it so you can just burn it up or you can get it so you sort of speed up the rate of the changes, isn't that true, depending on how hot it is?
A. That's essentially correct, a question of degree.
Q. The -- the particular buffer that you use which contains the citrate acid, does that have any differential effect on the different acid phosphatase phenotypes?
A. As far as the citrate phosphate buffer as opposed to another buffer?
Q. Well, citrate acid --
A. Citric acid.
Q. C-i-t-r-a-t-e; is that right?
A. Citrate. That stands for citric acid.
Q. Does that affect any of the acid phosphatase phenotypes as far as degradation is concerned, one more than the other?
A. Not that I'm aware of, no, sir.

Q. Does it affect the electrophoretic mobility of any of alleles more than the other?
A. It can, yes.
Q. Which ones are those?
A. The fact you're using that buffer.

I do not recall specifically, but I do know that if you use different kinds of buffers you do get different patterns for EAP.

Q. But you're not sure which one is which?
A. I believe there's a diagram in Saferstein's book, but I do not know exactly which one will move differently.
Q. When you're talking about Saferstein's book, you're talking about a book edited by Richard Saferstein entitled Handbook of Forensic Science in which there's an article by George Sensabaugh?
A. That is correct, yes.
Q. What are the wet state changes that acid phosphatase can undergo?
A. Okay. Basically, the ones we stated before are possibly the, as you will, the formation of disulfide compounds and the deamination.

(No omissions.)

Q. Any others?
A. Not that I am aware of, no, sir, unless -- well, unless there's the presence of a bacteria which creates neuraminidase, the enzyme neuraminidase.
Q. What will that do?
A. That will change the intensity of some of the bands.
Q. Which one?
A. Basically all of them, in particular what is called the b and c bands.
Q. Under what sort of circumstances do you get the presence of neuraminidase?
A. I would expect the presence of neuraminidase to occur in, say, a body which has had some time to decompose or possibly a pool of blood which has not been allowed -- or has not dried for a sufficient -- or well enough so that you still have a good deal of water present which can breed the bacteria which produce a neuraminidase.
Q. Will that produce any shifting of the patterns that you get from the different phenotypes?
A. Not necessarily the patterns, but what I said the intensities, yes.
Q. With acid phosphatase, part of the pattern is the intensity, is it not?
A. When I was referring to the patterns, I was referring to just where the bands are themselves. If you are

including the intensities of the patterns, yes, I will agree with that.

Q. Well, the difference between the different types, like a "b" and a "c", for example, would be in the relative intensities of bands in the same spot; is that true?

A. That's true, yes.

Q So the neuraminidase can affect that kind of typing call?

A. It's possible that it can, yes.

Q How can you tell whether or not that's happened or not?

A. Basically from the origin of the stain, for one. If you have some good idea if the patterns and the stuff when you go to a crime scene, whether the stain looked like it was in a pool of blood or whether you got it from a corpse, for instance, or the other enzymes, for instance, or the serum proteins may tell you that you have the neuraminidase present.

Q. What other enzymes and serum proteins would tell you that?

A. The one in particular would be the transferrin, which has a very -- well, a peculiar pattern. When you have neuraminidase present, you get a multiple banding rather than usually the one or two bands, depending on the type.

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Q. When you have that particular problem present in the transferrin, you get an actual shifting of the bands?

A. You get a -- more bands than normal. You would get -- and these would be towards the negative or the cathode.

Q. And over time, will that gradually diminish so you just have one band again in a more cathodal position than you started with?

A. I would think that you are going to end up with a lot of bands just shifting, constantly shifting towards the cathode.

Q. And is that particular process in the transferrin referred to as desialidation?

A. Yes, it is.

Q. Are you familiar with any literature on that?

A. I know that there's an article. I can't give you the name of the author or the title, but I have read at least one article on that.

Q. In preparing samples for analysis in Group III, is there anything done to treat the samples to do away with that problem?

A. Basically, no.

Q. In the -- in the acid -- is acid phosphatase a monomer, dimer, what?

A. It's a monomer.

Q. Each -- in the patterns of acid phosphatase, the homozygote --

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THE REPORTER: I'm sorry. The what?

Q. BY MR. NEGUS: -- h-o-m-o-z-y-g-o-t-e will have more than one band; is that right?

A. Yes, they will.

Q. Why is that?

A. Because of other -- well, it's a post -- what's called a post-translational alteration. What happens after the protein is made, the body will create or change the enzyme into another type of -- or change some of the enzyme into another type.

Q. So in acid phosphatase you don't have, at least for all of it, a direct one-to-one correspondence between genotype and phenotype?

A. For one of the bands, no. It's modified at a later time.

Q. Does that fact of chemical -- of the chemical structure of the protein as you actually get it in bloodstains cause any typing problems?

A. Not really, no. Again, it's predictable. You are looking for patterns that are like that.

Q. As acid phosphatase ages, do -- well, strike. How many different alleles are there for acid phosphatase?

A. There are three basic alleles with more rare types.

Q. Just taking the three basic, the three basic types, do they all degrade at the same rate?

A. No, they do not.

Q. In what order do they degrade?
A. Basically, they are -- first of all, the three types are the A, the B and the C, and the ones that degrade first would be the A, then the B, then the C.
Q. Do they all -- do all the different alleles produce the same amount of protein?
A. That I do not know.
Q. When you -- when you type A, B and C, do you normally get as much banding -- "a" banding as you do "c" banding?
A. As far as the intensities?
Q. Yes.
A. I would say it would depend on the sample itself.
Q. Do you -- do all the -- do all the different alleles produce the same amount of enzymatic activity?
A. That they do not.
Q. How do they vary?
A. Okay. Again, the A is less than the B and the C.
Q. Does that get expressed in band intensity?
A. It can, yes.
Q. If it doesn't, does that show to you that there might be typing problems?
A. I do not understand your question again.
Q. If in fact there's more C activity than there is A activity and you get the -- in a sample you'd have the same amount of "c" bands as "a" bands, would that suggest to you there might be typing problems?

A. In, say, someone who's a CA or something like that; is that what you're asking?

Q. Right.

A. Either that, or a mixed sample.

Q. So there would be a problem with it?

A. I would question it, yes.

Q. What are the -- what are the rarer alleles?

A. This would be the R and the D.

Q. Do they show up at the same place as the -- as any of the other ones?

A. In the system I'm using, the D does not. The R can show up in -- around the same place as the B.

Q. How do you tell them apart?

A. Basically, you'll have to run them on another system. It's very hard to tell them apart on the system that I'm using.

(No omissions.)

Q. Are there any hidden variations in acid phosphatase?
A. As far as the subtype? Is that what you're asking?
Q. Right. Or -- or some type that you can't distinguish using your method.
A. Not that I know of, no.
Q. With respect to the -- to the ADA, is that a bimer?
A. ADA is a monomer.
Q. And what, do the bands vary in their longevity as to different alleles?
A. They can, yes.
Q. How do they vary?
A. I believe, and I may be wrong on this, I would have to refresh my memory on the article, that the two allele is not as active as the one.
Q. And what causes that variation?
A. I guess it's just the protein itself is not as efficient, if you will. But, again, I'm not sure if that's a function of the amount produced or the actual efficiency of the protein.
Q. What kind of wet state changes does ADA undero?
A. Basically the same ones. The deamination, where it will move towards the anode, you get storage bands up above that.
Q. Do you also get storage bands in the acid phosphatase?
A. Yes, you do.
Q. When -- when you're doing acid phosphatase and ADA, do

you again look for the presence of those storage bands?
A. Yes, I do.
Q. And if they are there, what does that tell you?
A. Okay. Basically I would -- most of the time I expect to see them. It's simply a part of the analysis. If they are extremely intense or something like that, then that will give me a clue as to a degraded sample. But if they are just a normal looking type of storage band, then I -- it's a reliable result.
Q. Well, you -- do you normally check to make sure that you don't have too intense a storage band?
A. Yes, I do.
Q. And that's for both acid phosphatase and ADA and any other?
A. Yes.
Q. Do you have to stain in a particular place on the ADA to -- to pick up those storage bands?
A. Basically you stain just all the way to the anodic side of the plate.
Q. What about with acid phosphatase?
A. Acid phosphatase you stain above or more anodic to where you expect the normal bands would be.
Q. And do you normally stain there in the area where the storage bands are present?
A. Yes, I do.
Q. So if you had a degraded sample, you would be able to

pick it up in your staining procedure?

A. Yes, you would.

Q. You normally also photograph it so that somebody else could come by and see that?

A. As far as I -- I try to photograph it so that you can see anything in there, yes.

MR. NEGUS: Could we stop for the day?

THE COURT: Sure.

It's my intent, to enable the dailies and to enable you people, to stop approximately four o'clock each day. I'll need sometime as well.

Counsel, before we break, however, I can give you copies of the transfer order to San Diego County, the original of which I order filed -- to be filed today. I'd like certified copies of the others which have to be sent to the Judicial Council. And it's all to take effect upon the conclusion of all of our motions that we handle in this county.

See you tomorrow at 9:30.

(Whereupon the proceedings for the day were concluded at 3:58 p.m.)