UNITED STATES
COURT OF FEDERAL CLAIMS

THERESA CEDILLO AND MICHAEL CEDILLO, AS PARENTS AND NATURAL GUARDIANS OF MICHELLE CEDILLO,	)	
Petitioners,	)	
v.	)	Docket No.: 98-916V
SECRETARY OF HEALTH AND HUMAN SERVICES,	)	
Respondent.	)	

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IN THE UNITED STATES COURT OF FEDERAL CLAIMS

THERESA CEDILLO AND MICHAEL CEDILLO, AS PARENTS AND NATURAL GUARDIANS OF MICHELLE CEDILLO, Petitioners,	)	
v.	)	Docket No.: 98-916V
SECRETARY OF HEALTH AND HUMAN SERVICES, Respondent.	)	

Ceremonial Courtroom
National Courts Building
717 Madison Place NW
Washington, D.C.

Wednesday,
June 13, 2007

The parties met, pursuant to notice of the Court, at 9:02 a.m.

BEFORE: HONORABLE GEORGE L. HASTINGS, JR.
HONORABLE PATRICIA CAMPBELL-SMITH
HONORABLE DENISE VOWELL
Special Masters

APPEARANCES:

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C O N T E N T S

E X H I B I T S

PETITIONERS' EXHIBITS:	IDENTIFIED	RECEIVED	DESCRIPTION
7	585	585	Hepner presentation
8	691	691	Kennedy presentation

E X H I B I T S

RESPONDENT'S EXHIBITS:	IDENTIFIED	RECEIVED	DESCRIPTION
3	806	806	Document entitled "Measles Virus Infection and Vaccination: Potential Role in Chronic Illness and Associated Adverse Events"

P R O C E E D I N G S

(9:02 a.m.)

SPECIAL MASTER HASTINGS: Good morning to everyone. To those of you at home, we're ready to start the proceedings this morning.

I'll start with just a housekeeping matter especially again for those people following along on the phone conferencing. I'll note that yesterday on our website appeared the list of the expert witnesses for both sides who will be testifying, including their names and specialties, so that's available on the Autism Proceedings portion of this Court's website.

I also note that the parties did give me yesterday further word on the expected, though no certain, witness order. Our understanding is that today will be the testimony of Dr. Hepner and Dr. Kennedy. Tomorrow we will have Dr. Byers and Friday Dr. Kinsbourne. Those are all Petitioners' experts.

Next week we will start the Respondent's experts, and the expected order is Dr. Fombonne and Dr. Cook on Monday, Dr. Wiznitzer, Dr. Fujinami and possibly Dr. Brent on Tuesday, on Wednesday Dr. Bustin, Dr. Ward, Dr. Zimmerman, on Thursday Dr. McCusker, Dr. Gershon and Dr. Hanauer, and on Friday the schedule of witnesses are Dr. Chadwick, Dr.

Fombonne once again, and on Monday of the third week Dr. Griffin. These again are subject to change, but this is the expected witness order.

Now let me then ask the Petitioners' counsel which witness did you want to start with today, Ms. Chin-Caplan?

MS. CHIN-CAPLAN: That would be Dr. Hepner.

SPECIAL MASTER HASTINGS: Dr. Hepner? Okay. Dr. Hepner? Could she take the stand at this point?

Thank you, Dr. Hepner. Could you raise your right hand for me, please?

Whereupon,

KAREN HEPNER

having been duly sworn, was called as a witness and was examined and testified as follows:

SPECIAL MASTER HASTINGS: Okay. And please, counsel and witness, please do speak up. Occasionally we're having still some comments from some of the people trying to listen in that it's a little bit faint, so do your best to speak up.

Thank you very much. Go ahead, Ms. Chin-Caplan.

DIRECT EXAMINATION

BY MS. CHIN-CAPLAN:

Q Could you kindly state your name for the

record, please?

A Karin Hepner.

Q And, Doctor, could you just give a brief description of your educational background from college onward?

A Sure. In 1994, I graduated from SUNY University, at which time I began working in a molecular diagnostics lab at New York Hospital Cornell in New York. In this lab I started working on PCR-based techniques and some of the techniques that we'll be discussing today.

Then I moved to southern California and worked in a lab, a pathology lab at UCLA, where I helped design a technique for in-situ aminos for chemistry looking at KSHC or Human Herpesvirus 8 in AIDS lymphoma patients.

I began graduate school at UCLA in 1997 where I got my Ph.D. in molecular biology and used many of the techniques that we'll be discussing today. I worked in a cancer research laboratory and studied tumor suppressor genes.

Since then I've worked with Dr. Steve Walker on a collaborative project looking at measles virus protection, looking at RNA measles virus protection in patient bowel biopsies.

Q Doctor, the techniques that were described in the Ulhmann paper, are you familiar with them?
A Yes, I am.
Q Have you worked with those techniques?
A Yes, I have.
Q For how long a period of time did you work with those techniques?
A Most of those PCR-based techniques I've worked with for over 10 years. Some of them are developed more recently, and I've worked on them more recently.
Q Doctor, you're here to discuss the Uhlmann paper with the Court, are you not?
A Correct.
Q And the Uhlmann paper utilized different types of experimental techniques, did they not?
A Yes, they did.
Q Before we go into the Uhlmann paper, I'd like you to run through the different types of techniques that the Ulhmann paper cited and explain to the Court how they are run.
A Okay. Do I have a laser pointer, or should I get it?
SPECIAL MASTER HASTINGS: All right. Ms. Chin-Caplan, let's mark this as Petitioner' Trial

Exhibit I guess we're up to No. 7. Okay.

I'll just note for the record that Dr. Hepner again will have some slides here to show us during her testimony, and we've marked the paper copy of those slides as Petitioners' Trial Exhibit 7.

(The document referred to was marked for identification as Petitioners' Trial Exhibit No. 7 and was received in evidence.)

SPECIAL MASTER HASTINGS: If you can as you go through these, Ms. Chin-Caplan, I see that the paper copy is marked with page numbers, so if you can refer to those it will help us later when we have to refer back to these.

MS. CHIN-CAPLAN: Certainly, Special Master.

SPECIAL MASTER HASTINGS: Please go ahead.

MS. CHIN-CAPLAN: Thank you.

THE WITNESS: So we'll be discussing a number of molecular biology-based techniques today, so I'm going to give a very brief and simple overview of molecular biology and some of the techniques specifically that we'll be looking at today.

So this is kind of the central dogma in molecular biology, and we want to look at some of the

molecule types that are going to be used or analyzed in this study.

We'll start here at the bottom of this very simple flow chart. This is a protein molecule, and proteins are important for carrying out all the functions of the cell. Proteins are specific, designed specifically for a specific function, and that specificity is critical.

How are proteins made? Well, there's actually a blueprint for that protein. The blueprint for this protein is found back here in the genetic code of the DNA. The DNA is a double-stranded helical molecule, and here's kind of a blown up version since we've now broken down the helix.

Just for simplicity I'll just refer to these things by their letter designations where we see As, Gs, Ts and Cs. Ts always bind As. Gs always bind to Cs. This is going to become important, so I'm pointing it out now.

These's a genetic blueprint for every protein found in a DNA molecule, in this double-stranded helical molecule that contains these what are called nucleotides, and these are the letter designations for those nucleotides. The blueprint here is specific for a specific protein, and that's

also very critical information we'll discuss.

Now, how is protein actually made? DNA resides in the nucleus. The protein is translated in the ribosomes, so there's actually this messenger molecule called RNA, and DNA is transcribed to RNA, which is kind of a reflection of the DNA. It's not identical to the DNA, but it reflects some information that is in the DNA.

The RNA then is translated into protein. It moves from the nucleus to the ribosomes where it can be translated into the components of proteins called amino acids.

We can go to Slide 2. Now, as I said, every piece of DNA, every gene is specific. It has a specific blueprint. What we're looking at here is a string of Gs and Ts and Cs and As and this code for a specific gene, which is a code for a specific protein.

What one would be able to do is to look in the database. You can just turn on your computer and look in the database and look for a specific gene corresponding to a protein of interest.

What scientists have been able to do. Excuse me. What scientists have been able to do is to use this information as kind of a blueprint, as kind of a fingerprint, like a detective uses a fingerprint, so

if someone wanted to determine the presence or

absence of a particular gene, whether it's a self-gene, a gene that we make ourselves in our own selves, or a foreign gene such as a viral gene, we have this information in databases, and we can look at the DNA to determine the presence or absence of a specific gene.

BY MS. CHIN-CAPLAN:

Q So, Dr. Hepner, just to be clear, is this database known and available to the entire scientific community?

A Yes, it is.

Q And are these genes specific to certain types of animals and plants?

A They're categorized by their host, so, or by the organism where they're found.

Q So if it's a plant molecule it would be found in plants, and if it's a virus it would be found within the viruses?

A What one would do if one had a nucleotide sequence that they wanted to find out what it was, they would simply insert a piece of the sequence, so let's say this line right here, Line 4. You would insert it into the database, and the database would then tell you the source of that particular gene.

Q Okay.

A So it would tell you plant gene and the name of that gene if it had a name and any information that is known about it and refer you to any information in any database that is known about it.

Q And this is computerized?

A Yes, it is.

Q And is it utilized by scientists everywhere all over the world?

A Everywhere all over the world all the time.

Q And it's standard practice at this time?

A Yes, it is.

Q Okay.

A We can go to Slide 3. So there's a particular feature that scientists have taken advantage of, so now what we need to do is say how are we going to visualize this DNA. This is the basis of the molecular biology techniques.

How are we going to visualize this particular gene, this particular stretch of DNA, in a cell? We can't just look at a cell and see it. We need techniques in order to amplify it or copy it multiple times so that we can visualize it.

Q So when you say amplification, that means that it's been copied?

A Yes. It's like a xerox machine. You would

put. You're

putting your template DNA or your DNA source in a copy machine. You set up certain parameters, and we'll discuss specifically what we do. And you copy it exponentially.

Q Okay. So just like a xerox copy, you put one copy down and you get two, and then you put two copies down and you get four. Is that what you mean by amplification?

A That's what I mean here, yes. Now, the feature that we take advantage of, that scientists take advantage of to copy this DNA is the feature of DNA replication.

Now, this is something that scientists do not make up. I'm showing you what actually happens in the cell. Scientists manipulate this in order to copy the DNA of interest.

So this is DNA replication. Here is our double-stranded molecule. It's not a helix here now. We have it straight so we can see it better. The strand starts to separate, okay? We have a separation of strands. This is separated by an enzyme called helicase.

Then there's an enzyme called a polymerase that comes and sits down here on the leading strands and adds the nucleotides one-by-one along the strand

to create the complementary strands.

So when we started out with one piece of DNA here we now end up as this continues along. We end up with two pieces of DNA. That happens a little bit differently on this side, but really I just want to show you the principle that with DNA replication there are specific enzymes that are used to replicate that one stretch of DNA.

SPECIAL MASTER HASTINGS: Just for the record, we're on page 3 of the trial exhibit.

Go ahead, Ms. Chin-Caplan.

BY MS. CHIN-CAPLAN:

Q Doctor, just to be clear here, this process is accepted within the scientific community?

A This is the way that DNA replication occurs, and it is something that everybody knows about.

Q There's no dispute about how this happens?

A No.

Q Doctor, when you talk about the helicase and the DNA polymerase, are those natural occurring substances?

A Yes, they are.

Q So the body automatically manufactures it?

A Those are proteins that exist in our body.

Q And the body knows when it's supposed to

replicate?

A Correct.

Q All perfectly natural?

A Yes.

Q No dispute about the fact that it occurs?

A No.

Q Thank you.

A Okay. Next slide, please? Slide 4? So now scientists. Again I showed you that slide about DNA replication to show you what scientists manipulate in order to detect our specific target genes.

This is a process called PCR, polymerase chain reaction, and this is what happens. You have your double-stranded DNA molecule. Remember, the first thing we had to do was we had to separate those strands. We don't add a helicase enzyme in this case.

We denature the DNA, which means we separate the strands using heat, so if you apply enough heat the strands will automatically separate.

Q And that's what you mean by denaturing? You separate the strand by applying some sort of substance?

A That's correct. Now, in this case we don't have the polymerase doesn't know where it is that we want to start amplifying or start copying, so we have

to jump

start this reaction using these little things here in red and green called primers.

Now, a primer is a stretch of DNA that is complementary to the ends of your target, so if this is the target gene right here you've separated the strands. We generated primers. Now, when I say generated, we designed the primers. And then company generates it for you.

You design the primers based on the nucleotide sequence of about 20 bases, and it is specific to this target, to the ends of the target.

Q Dr. Hepner, just to interrupt you, you said that you design the primers to be specific to the target. How do you know what target you are looking for?

A That's a good question. If you're looking for the presence of let's say measles virus RNA -- well, let's not talk about RNA yet. You're looking for Gene X.

You know that there's a specific region within Gene X that is important to you, one sort of regulatory region within that Gene X that you're interested in.

You have some idea roughly the size of the PCR product, the size of the DNA target that you want

to create. You have some idea about the secondary structure or the folding of this DNA molecule.

You have the database in front of you, and based on a number of variables, which we don't need to get into, you choose one or more primer sets that would give you the gene of interest, this stretch of DNA that you're interested in that contains the area of DNA that you're interested in.

Q So just to be clear here, Doctor, you know what you're looking for, and you tell the computer this is what I'm looking for. It brings up the gene that you're looking for. You're able to look at the gene as we see it on page 2. Is that it?

A Uh-huh.

Q And you look at the letters associated with it?

A Right.

Q And you match up the letters?

A You say I would like these letters to be represented in the product that I'm going to create in my xerox copy. This is the section of DNA that I would like xerox copied. These are the ends of that section.

Q Okay. And then when you get the ends of the section you say you develop the primers. How do you

develop the primers?

A You will develop the primers by looking at those ends, finding a suitable region using variables that are more complex than we need to get into.

You would find the ends, and you would basically write down those nucleotides, and you would put it in your computer and send it off to the company, and they will send you back a set of primers that correspond to what you have written and what you have selected.

Q So this is commercially available?

A Commercially available.

Q Purchased from someplace else?

A Correct.

Q That's utilized by any laboratory who wishes to seek this type of information?

A That is the standard way to do it these days.

Q Thank you.

A So now these primers that you've designed that are specific for your target, and there's one other thing that I want to say about these primers, and that is you need to be sure that you are amplifying what you think that you want to amplify.

That means that these primers need to be

specific for your gene and would not be represented -- this stretch of DNA would not be present -- in any other gene.

If you put down a set of primers that was more promiscuous or that would be found in other genes besides your target gene your primers would not be specific to your target, and you would amplify things other than your target.

You might also amplify your target, but you would amplify things that are also other than your target, and that would be a nonspecific primer.

Q Doctor, when you say a nonspecific primer, when you're seeking a target you try to make it as specific as possible to whatever you're looking for? Is that it?

A Correct.

Q And you try to avoid adding on anything that would be a potential match for that target?

A Right. One of the variables that you use when you choose those primaries and you send them off to the company is you take those primers, you take down the nucleotide sequence that you wrote down, and then you insert that in the database.

That cross checks it across the entire DNA database, and what you would want to see is that it

only corresponds to the gene of interest and it is not it does not correspond to any other genes in the database.

If it corresponds to your gene of interest and not to any other gene in the database, you have generated specific primers.

Q So the computer does all this calculation for you?

A Correct. Yes. We don't have that information in our heads.

Q Okay.

A Now we have these specific primers, which basically kick-start the reaction. There's an enzyme called a polymerase, and this is a thermal stable polymerase, which means it does not get deactivated by heat because, remember, we've had a heat step up here.

So we have a polymerase, which is this enzyme that basically comes along and copies the DNA strand, and so now it's copying along the DNA strand up here and a copy going down here. And we end up Where we started with one double-stranded piece of DNA, now we have two pieces of double-stranded DNA at the end of the first cycle theoretically.

This is theoretical because it doesn't always work in that first cycle, but at least in

theory I want you to understand the concept, which is

that you start with one and you end up with two.

The next slide? Slide 5, please? Now, we don't perform one copy. We don't do the copying once. We do it many times. In fact, you do a PCR usually on the order of 35 to 40 cycles, and what happens is in the first cycle you end up with two. The second cycle you take each two and you make two. The third cycle you take these four and you make two from each of the four. This is called exponential amplification.

An example would be if I give you a penny and then the next day I give you two pennies for your penny. The next day you have two pennies. You have I'm sorry. You have a penny. I give you a penny for your penny. Now you have two pennies. The next day I give you a penny for each of your pennies. Now you have four. Exponentially you are getting more money out of me.

This is exponential growth. You started with one theoretically with one piece of DNA, and by the end of the 35th cycle you have something on the order of 68 billion copies.

Q Doctor, what's the reason for amplification of this multiple copying?

A Well, I'm going to show you how we're going to detect it, and what you're going to see is that we

cannot detect very, very small amounts of DNA.

We need large amounts of DNA in order to visualize it. There are multiple methods by which we visualize it, but you need it to be in greater abundance.

Q This amplification process that you talk about, is that accepted within the field of molecular biology?

A Absolutely.

Q Is this the method by which every molecular biologist utilizes to study small amounts of material?

A Well, this is the conventional PCR, the solution-based PCR. There are amny. Ther are. Techniques that. This has been expanded, but this is the classic solution-based PCR that people use. We'll discuss some other techniques that are based on this technique.

Q And this is a standard that's accepted within the field of molecular biology?

A Correct.

Next slide, please? Slide 6? Now we've amplified our product, okay? We put it in the xerox machine. We've xeroxed it. Now we have 68 billion xerox copies.

Now what are we going to do with that, because all it looks like in the tube is just some

clear liquid. Now we need to see it somehow, so what

we do is there's this thing that we call an agarose gel, and this feels kind of like very, very hard jello.

It's made of agar. It's a very, very small matrix, and what you can do is take and there's little wells up in the top here. You take your DNA, you put it in the well, and DNA is negatively charged. You apply a current so it will run down to a positive pulse, so now we have DNA up in these wells that's starting to run down in each lane. This is a lane, this is a lane, this is a lane, and this is a lane.

It runs down at a rate that's inversely proportional to its size, which means that the smaller products will end up at the bottom. The larger products will end up at the top.

It's sort of like running through a very small, very narrow maze. If you're a small person you'll get through really quickly. If you're a larger person, it's going to take you longer to get through that very narrow maze.

Now, we still can't visualize it like that. We apply a stain called ethidium bromide which intercalates or basically inserts itself in between the bases and under UV light it fluoresces, so what we're looking at here are pieces of DNA that have been

amplified by PCR.

Now, what this thing here is is a DNA standard. It's kind of like a ruler. Each of these lines. This is something that is commercially available that you buy. Each of these lines here correspond to a different notch on your ruler. If this is 600 base pairs let's say, this is 500, 400, 300, 200, 100.

Now you have some idea of what the size of your DNA is, so if all of these here run around this size, run right here, you look at your ruler and this is 500 so it looks like it's running just slightly below 500. This would be something like 800. This would be something like 350.

What's important about this is that you've designed your primers, and based on the primers that you've designed you know you have a predicted size. So the first piece of evidence that your PCR is specific and that it works, that it's sensitive, it worked, is that you have amplified a product that is the correct size.

Q Doctor, gel electrophoresis. Is that a technique that has been utilized in molecular biology?

A Yes.

Q Is it accepted the way you described it as

standard practice within the field of molecular

biology?

A Yes.

Q Is there any question about the manner in which this would be conducted?

A No.

Q So it's accepted as the gold standard in the field of molecular biology?

A This is how you would look at DNA in the PCR product.

Q Thank you.

A Okay. Next slide, please? Slide 7? Now I'm going to take a little detour.

Because the subject in question is actually measles virus, I will not be discussing any issues related to virology today, but I do need to mention that there is going to be something slightly technically different so I need to take a slight detour and we'll come back to the technique.

So measles virus is actually a single-stranded RNA virus. The R cells have contained their genomic code in DNA. Viral DNA is generally single-stranded RNA, which is represented here. RNA for various reasons is not an appropriate template. It's not able to be xerox copied the same way, so we have to manipulate.

If we want to detect measles virus RNA, which is what we wanted to do- what they wanted to do in this paper that we're going to be discussing, we actually have to manipulate the RNA slightly in order to be able to apply this PCR technique, this xerox copy technique to it.

The next slide, please? Slide 8? Now, this RNA undergoes a process called reverse transcription, and what this is takes advantage and what we're attempting to do here I should say I should start. What were attempting to do here is to take an RNA molecule and make it look like a DNA molecule.

It's a reflection of that RNA molecule, but it needs to have the features of a DNA molecule. It needs to be double-stranded, and there's one base that is different between the RNA and the DNA, and we need to have that base- that DNA base inside this product.

We are now taking a single-stranded RNA and making it into a copy or a reflection of the RNA in something called cDNA.

Q Doctor, is it generally accepted that measles RNA is a single strand RNA?

A Yes.

Q And is it generally accepted also that it's unstable to study as RNA?

A RNA is a labile molecule and therefore unstable.

Q And is it accepted also that in order to study RNA molecules one must make it into a double-stranded molecule?

A Yes. One must undergo this- do this process in order to study RNA molecules.

Q So there's no dispute within the field of molecular biology at all that this is standard practice to study single-stranded RNA molecules?

A Correct.

Q Thank you.

A We're going back to 8, please. Where are we? Yes, 8. Thank you.

So we take advantage of a feature of RNA, which is this poly A tail, which is basically a string of As at the very end of the RNA. There's an enzyme called reverse transcriptase, and we use an oligo DT, which is a primer. Remember the primer? It's complementary to the target.

The target is now the As, and the Ts are the complementary primer. It binds to the As, and the reverse transcriptase essentially copies the complementary strand so it's now complementary to your starting material, to this starting RNA.

It copies it along, along here, so we can see that down here. It's copying along. Now we have

this complement to this RNA. It now copies the top strand and displaces the original RNA.

Now the original RNA is displaced, and we have a copy of what's now called cDNA that reflects what was in the original RNA.

Q Doctor, what you have just described, is there any dispute within the field of molecular biology that this is how what happens when the RNA is copied?

A No.

Slide 9, please? We went back. Thank you. Okay. So that was our little detour, okay, because I needed to let you know that in some cases your starting material is actually not DNA. It's RNA.

Now we're going to come back to the techniques that were used in the Uhlmann paper. If you recall, the last thing we looked at was this agarose gel, which was the pieces of DNA that were run through, the DNA product, the PCR product that was run through this matrix.

Now, I've shown you a more accurate version before. Here now we're going to look at a cartoon version of that agarose gel. Here's your ruler, your DNA standard, and here are your PCR products.

Now, what we're going to do here is the goal

of the Southern blot is to give us further indication that what we amplified is what we think we've amplified. The first thing we did was we ran it through a gel, and we used our ruler and said I knew it was going to be 150 base pairs. It is 150 base pairs.

That gave us some information, and that told us that we likely copied what we think we copied. Now what we want is more information. We want more confirmation that we amplify what we think we've amplified, what we had expected and planned to amplify.

The way this is done is you take this gel that you ran, this agarose gel, and here we're going to denature the DNA again. Remember, denaturing is separating the two strands. In this case we're not going to apply heat. We're going to put it in a specific solution that separates those two strands.

The strands are separated, and then you but we're not able to perform the type of experiment in that gel so we need to remove the DNA from the gel and put it in a filter. We do that simply by soaking them together. It's a filter like a coffee filter. It's called a nitrocellular filter. It's similar to any other kind of filter.

Now we have a filter that contains the DNA that was in our gel where the DNA is denatured. The strands are separated. Why is this important? Why did we separate those strands? We separated those strands because we're going to take advantage of the fact that DNA likes to bind to its complement. It has a strong need for. As have a strong need to bind to Ts, and Gs have a strong need to bind to Cs.

So what we're going to do is we're going to generate a probe, okay? We're going to take a piece of DNA and add some feature to it that's detectible. You can do this with a radioactive label, or you can do it with kind of a colorimetric or a color response. In this case they use a colorimetric label.

This complementary strand, we're going to now introduce this probe, this piece of DNA this size here, with this detection system. Now, if this piece of- this piece of DNA is designed to be complementary to your target gene. If this piece of DNA binds to something that's in here you will get a signal. If it binds, it tells you that its complement must be in here. If it doesn't bind, it tells you that the complement was not in there.

If you design the probe to be complementary to the strand and you know what your target gene is,

you will now know if this binds that you have amplified what you think that you have amplified, what you plan to amplify.

Q Now, is the Southern blot a check on whether or not you've picked the right gene?

A In this case the Southern blot was used to see if you have amplified the product that you expected to amplify.

Q And is the Southern blot technique utilized within the field of molecular biology?

A Yes.

Q It's accepted as an acceptable technique within the field of molecular biology to detect genes?

A Yes, it is.

Q And is it widely used within the United States as a standard to detect genes?

A Yes.

Q Thank you.

A The next slide? Slide 10? Okay. Now, there's another type of PCR-based technique that was used in the study. If you recall, this is called TaqMan PCR, and we're going to simplify it here. This is now here your DNA, okay, that's represented by these two lines.

Now, you should be familiar already with the

concept of a primer, so you have a primer going in this direction, a primer going in this direction. These are designed specifically for your target genes, and they will amplify from the ends of that target towards the middle, making another copy of that target.

What TaqMan PCR does, it does two more layers that are important. You also design in TaqMan PCR you design a probe which is complementary to one of the strands. Now you have a primer complementary to the strand, a primer complementary to the strand, and now we have a probe that's complementary to the strand.

Why did we do this? Because when this probe is complementary to the strand it -- I'm sorry. This probe gives off a fluorescent signal. However, it does not give off that fluorescent signal when it is bound to its target. It will only give off that fluorescent signal once it is displaced.

It will be displaced only if your primers are amplifying this gene because it will be displaced as this primer tugs along the strand. The probe gets kicked off, and it gives a fluorescent signal that is read by the computer.

Q So, Doctor, the TaqMan probe. Is it a more

specific way of finding your gene?

A Yes. It accomplishes two things. It is more specific because in addition to the primers, which are specific for your target, you now also have a probe that's specific for your target, and that probe will only give off a signal, appropriate signal, when it's displaced.

So now if you have a very small piece of DNA that you're trying to amplify, let's say 100 or 150 base pairs, part of that will be specific, so 20 bases are reflected here, 20 bases are reflected here and a certain number of bases are reflected here in the probe.

You've now pretty much found- been able to bind complementary DNA to a very large percentage of your target sequence, so it adds another layer of specificity.

Q So essentially it's eliminating the possibility that there's error or something else present other than the gene that you're looking for?

A Yes. It gives a higher level of confidence in the specificity of your primers and the specificity of your reaction.

Q Doctor, is TaqMan PCR an accepted technique within the field of molecular biology?

A Yes.

Q And is it an accepted technique nationally?

A Yes.

Q Internationally?

A Yes.

Q Thank you.

A The other piece of information that TaqMan PCR gives us is it can give us some quantitative information, which means it can give us some information about the amount of DNA that you actually started with.

The way this is done, and I will not go into great detail, is that the software essentially is able to detect the point at which the amplification is exponential. Remember, we have this exponential amplification.

In the first few cycles we don't actually get that kind of exponential amplification because there isn't enough target present. As time goes on, after 15 or 20 cycles we now start to get logarithmic or exponential amplification of your target.

The more you start with, the more starting material you have, the faster this reaction will enter that logarithmic phase, so if we go back to our penny example, if I give you a penny and then I give you a penny for every penny, it will take you a long time to

get to $1 million.

If I give you a dime and then give you a dime for every dime, it will take you a shorter amount of time to get to $1 million. If I give you $1 and give you $1 for every $1, it will take you less time, so -this- the faster that it gets to logarithmic phase, the faster that it gets to exponential phase, the more starting material you must have had.

Q Doctor, just to be clear, is that an indication that the greater the concentration is the sooner you'll see your result?

A The sooner you will detect, you will be able to reach logarithm phase, and the more amplified product you will have.

And the. This point at which you enter that logarithmic phase, that you enter this point of exponential amplification, is called the threshold, the cycle threshold. That cycle number is called the CT. It's the cycle threshold, and that cycle number is important for quantitation.

Q The cycle number is a standard? Is that it?

A The cycle number represents the threshold at which you are able to enter you are able to enter logarithmic phase.

Q So you now know that if you have five copies

let's say, you will enter logarithmic phase at Cycle 30. If you have 10 copies, you will enter it earlier. If you have one copy, you will enter it later.

Okay. The next slide, please? We can also If we're going back to PCR, the Uhlmann study the authors of the Uhlmann study also did something called in-situ PCR where they amplified directly in the tissue. The biopsy tissues were collected, put on a slide, and the PCR reactions in this case didn't take place in the tube, but it took place inside the tissue itself.

And you can This is not from that study, but this is just an example where you can get a colorimetric reading. The color represents amplification, and here there's no color, which represents lack of amplification.

Next slide, please?

SPECIAL MASTER HASTINGS: Now we're on Slide 12.

THE WITNESS: Correct. Yes.

SPECIAL MASTER HASTINGS: Go ahead.

MS. CHIN-CAPLAN: Special Master, let me ask just one question. Could we just go back to Slide 11, please?

BY MS. CHIN-CAPLAN:

Q Dr. Hepner, in-situ PCR. Is that an

accepted technique within the field of molecular biology?

A Yes, it is.

Q And is that a technique that is recognized both nationally?

A Yes.

Q And internationally?

A Yes.

Q Thank you.

A Slide 13. Immunohistochemistry is the last technique that we'll be talking about, and this is actually a technique that's used to detect protein.

This is not one that was used in great detail in the paper, but just to mention that this is a way to detect protein. Remember the bottom of that flow chart? This is a way to detect protein in a particular sample.

This is not an example from the paper, but this is a gene that I studied. This is also a colorimetric reading, so if you have a color you have the presence of that protein. The absence of color in these cancerous glands represents the absence of that gene. Protein. Excuse me.

Q Immunohistochemistry is an accepted technique within the field of molecular biology?

A Yes.
Q And is it practiced on a national level?
A Yes.
Q And is it practiced on an international level?
A It is.
Q And when somebody says that this was obtained by immunohistochemistry, is there any question about the technique that's utilized?
A Not in general. In detail perhaps.
Next slide? Last slide, please? This is Slide 13. Just to show you where it is how it is that all of this got started, you probably heard about the ileum yesterday and the small intestine.
Tissue is extracted from the ileum, and it can either be put on a slide to be subjected to things like the immunohistochemistry and in-situ PCR, or it can be stored in a tube that preserves the RNA for later extraction.
The RNA is purified and tested for RNA integrity. Because it's a highly labile molecule, you need to test the integrity of the RNA, and then it can be subjected to the experiments that we described.
That would be it.
Q Doctor, this is a description of the

techniques, the experimental techniques that are detailed in the Ulhmann paper? Is that correct?

A Correct.

Q Doctor, when you reviewed the Ulhmann paper how did you know that the results of that paper were valid?

A Well, the first thing you have to ask yourself is do the experiments that are used in this study, is does do they answer the question that you're trying to ask, that you are answering, trying to answer.

The question that is trying to be addressed here is is measles virus RNA present in the bowel biopsies of this cohort of patients, so that is the question. Do the experiments that were chosen help address that question? Do they address that question directly? The answer is yes.

Q Now, Doctor, in this paper the Uhlmann experiments were PCR?

A The first experiment that they used was conventional, solution-based PCR.

Q And that PCR, as you indicated, is accepted within the field of molecular biology as an appropriate experimental technique?

A Yes.

Q What was the next technique that they

utilized?

A The next technique they used was TaqMan PCR, which I described.

Q And is TaqMan PCR also an accepted molecular biology tool?

A Yes, it is.

Q Did they utilize any other experimental techniques?

A They used in-situ PCR for detection of measles virus RNA.

Q And is it appropriate in the field of molecular biology to use in-situ PCR to detect measles RNA?

A Yes, it is. Yes.

Q Doctor, when they ran these experiments and you were reviewing the paper, how did you know that the experiments that they were running were valid results?

A Okay. So like I said, we first asked the question were these experiments appropriate techniques, and the answer is yes. The next question is, did they perform these experiments properly? So, in order to first address that question, you want to ask did they use proper controls. Now let's talk about controls.

So controls really can be subdivided into two categories. There are controls for an individual experiment. So in an individual experiment, you would have something that would function as a positive control. So in the case of measles virus RNA detection, and again, when we talk about RNA, we are really talking about cDNA because we've had to create the complementary strand, the complementary cDNA in order to perform these experiments.

Q And that's accepted within the field of molecular biology?

A That's accepted. So I'm going to use -- I might use the terms interchangeably, but really what we mean is when we're subjecting these to these experiments, we are using cDNA, but it reflects the presence of an RNA.

So when we had selected controls, when controls were selected for this paper, the first thing they needed to do was to see if they -- to use a positive control. A positive control is a sample that necessarily contains the measles virus RNA, or the measles virus cDNA. Every time you run the experiment, that particular sample must be positive. If it is positive, then you have shown that your reaction or your experiment is specific for measles

virus RNA. If it's negative, then there is a flaw in your experimental design. You cannot get any information from an experiment where the positive control is negative.

The next thing you have to do is look at the negative control. The negative control is a sample where it is necessarily -- where measles virus RNA or cDNA is not present. If the negative control is positive, there is either a flaw in your experimental design -- like as if you're one example would be where your primers are not specific to that to your target -- or you have some level cross-contamination where measles virus RNA or cDNA contaminated your samples.

Q So the positives should always be positives and negatives should always be negatives, and that's how you know that at least that portion of the experiment that you are running is accurate.

A Correct.

Q And so Doctor, you indicate there were two levels of control. What's the second level?

A So the second level are the experimental controls. So you have your experimental group. Your experimental group in this case was ASD patients with GI bowel -- with this idiopathic bowel disease, and it's the GI biopsies from this cohort of patients.

That was your experimental group because that's the group about which you are asking your question.

Your control group, in this case, were developmentally normal children who underwent endoscopy and did bowel biopsies from that cohort of patients. So your control group has to be similar to your experimental group but differ by the variable of interest.

Q And Doctor, when you run those experiments, at that level, how do you know that they are valid at that level?

A Well, you are still open-minded when you ask the question. So when you ask the question, your hypothesis is that measles virus RNA is going to be present in the bowel biopsies of this cohort of patients. And when you run the samples, you should be you need to run them simultaneously and be open-minded about the possibility that there might be no difference.

If you see a difference, if you see that the developmentally normal children have MV RNA present at a frequency that is different, statistically significantly different, from the positive -- from the ASD group, then you have gathered information about the presence of RNA in this cohort of patients

relative to its controls.

Q How do you know you are not getting a false positive?

A The negative controls within your experiment will function as a control for contamination.

Q So are you saying that you run both the experimental specimen and the controls together?

A That is standard laboratory practice.

Q And if your positives are always positives and your negatives are always negatives and you control, then you have a certain level of confidence that that experiment is valid?

A Yes.

Q Now, Doctor, when you look at the Uhlmann paper, were their positives always positives and their negatives always negatives?

A Well, I can tell you what I see in the paper.

Q Okay.

A If we look at -- we kind of need to go through the experiments one by one to do that, because each one has its own series of positive and negative controls. Should we do that?

Q Yes, why don't we do that? Which one would you like to start with?

A Let's start with the solution phase PCR,

which is in Figure 2, for those who have it.

SPECIAL MASTER HASTINGS: So for the record here, we are now going to look at the article describing -- we are going to look at the Uhlmann paper, is that correct?

MS. CHIN-CAPLAN: That's correct.

SPECIAL MASTER HASTINGS: And for the record, I think that's -- it's in the record in more than one place. One place is at Exhibit 63, tab U, I believe. So, okay.

THE WITNESS: Okay, so if we look at Figure 2 --

SPECIAL MASTER HASTINGS: Figure 2 is on the -- let me see.

THE WITNESS: Do you have the --

SPECIAL MASTER HASTINGS: Yes, Figure 2 is on what's labeled page 2, okay, Figure 2 of the Uhlmann paper. Go ahead.

THE WITNESS: Figure 2 is the figure for the solution phase PCR reaction. And what they show here is that they had a positive control that acted true to designation, which means the positive control RNA consistently performed -- was positive. They also had a no-template control, which is a control that does not contain any nucleic acid, and therefore, is

necessarily negative for measles virus RNA. And that acted true to designation.

The experimental samples, some were positive and some were negative, so if you have a positive control that acts true to designation, a negative control that acts true to designation, you can now get information about the experimental samples. So that gives us confidence that the experiment is working.

BY MS. CHIN-CAPLAN:

Q And with respect to this experiment, was it working?

A It appears to have worked.

Q And Doctor, would you go on to the next technique that was utilized?

A Well, the next technique is actually kind of a sub-category of this technique, because they used Southern blot to confirm the specificity of this target gene that they amplified. So what they now are asking is, we have amplified a product of the size that we predict, that's what we saw in this agarose gel. So this A panel within Figure 2 is actually similar to that agarose gel that I showed you in our slides, and to the left is that DNA ruler.

Now, in panels B and C, what they did was -- oh, we should go back up to figure to panel A. The

left side,

the first seven lanes of this panel are using gene primers that are specific for a particular gene called the F gene in the measles virus. The right half of this panel, 8 through 14, used primers that are specific for the F gene of measles virus, as this actually adds another layer of confidence because we are actually seeing two panels two genes amplified, not just one.

Now, the Southern blots in Figures B and C are using probes that are specific either for the F gene in panel B or the H gene in panel C. And what this is showing is because we get a signal that this group has amplified what they believe that they have amplified, because they have used a probe that is specific for that gene.

Q Doctor, just to assist the Court a little bit more, could we go back to page 7 of your handout?

A Oh, my handout, yes.

SPECIAL MASTER HASTINGS: So page 7 of trial Exhibit No. 7.

THE WITNESS: Okay.

BY MS. CHIN-CAPLAN:

Q Doctor, this was a schematic of the measles virus RNA?

A Right.

Q Is that true?

A Yes.

Q And you were talking about the F gene, is that true?

A The F gene and the H gene. So the F gene and the H gene code for particular proteins in the cell, in the measles virus virion. And those particular components are not shown here specifically in the RNA, but there are portions of the RNA that you see in that single strand. So there are different, kind of, segments to the RNA, and one corresponds to an F gene and one corresponds to the H gene.

Q And they are both present on the schematic here?

A It's not detailed on the schematic, but it's implied.

Q Okay. So Doctor, after you reviewed this technique utilized by Uhlmann, did you have did you believe that the experiment was valid?

A Yes.

Q Uhlmann ran one last experiment?

A Well, they did a TaqMan PCR, which is the summary of the TaqMan PCR as found in Table 2. And they designed multiple primers and multiple probes to determine the presence of measles virus RNA and to get some information about the amount. This could be --

because remember, this is a quantitative experiment as well. So they get some information about the quantity of the starting material. Yes?

Q And Doctor, after reviewing the results of this technique, did you believe that the results were valid?

A Yes, they used a number of controls, which are listed in the text. They used a number of controls above and beyond what was used in Figure 2. They used a no-template control. They used a control that did not use- that did not have the addition of the reverse transcriptase enzyme, so it was basically RNA without any cDNA, which was not able to be amplified because you cannot amplify RNA. They used irrelevant target primers, which means primers that were not for our target gene, but they used a specific probe.

They used the probe but they did not use the primers. They spiked it with RNA, and they used a single primer and a probe. So they used multiple techniques to make sure that it couldn't have been caused by some other kind of technical flaw, that the results could not reflect some sort of technical flaw.

Q So these techniques acted as a cross-check on one another? Is that it?

A The TaqMan and the solution phase PCR?

Q Yes.

A Yes, the techniques ultimately give us the same information. TaqMan gives us more information, but they one really does validate the other.

Q So it gives you more confidence that the results that you are obtaining are valid?

A Yes.

Q Now, Doctor, how do you know that you don't have a false positive here?

A You don't have a false positive because your negative controls consistently act true to designation, and a false positive would really generally -- it would reflect two things. It would reflect the nonspecific primers, or it would reflect cross-contamination. If it's cross-contamination, your controls, your negative controls, should also be contaminated, because contamination would not preferentially contaminate one sample over another, at least not in multiple runs. So if you have --

Q And is that why you run the controls every single time --

A You have to run the controls every single time and if your negative control is consistently negative, and it is negative every time, then you simply can -- it is illogical to assume that you would

have contamination in all your samples but your negative control.

Q So, Doctor, are you aware of any criticisms of this study?

A Yes.

Q And what are the criticisms that you are aware of in this study?

A Well, the major criticism is that there have been labs that have attempted to reproduce some of these findings and have been unable.

Q And are you referring to specific studies?

A There are two studies that I am thinking of specifically. One is Afzal et al., and I don't know where you have that, and one is D'Souza et al.

(Pause.)

THE WITNESS: What do you want?

MS. CHIN-CAPLAN: Special Master, Afzal et al. is contained at Petitioners' Exhibit No. 63A.

SPECIAL MASTER HASTINGS: Right, okay. And just for the record, that's the 2006 article by Afzal, because there are more than one by that author.

MS. CHIN-CAPLAN: Right. And D'Souza et al. is contained at Petitioners' Exhibit No. 63L.

SPECIAL MASTER HASTINGS: F as in Frank?

MS. CHIN-CAPLAN: L as in Larry.

SPECIAL MASTER HASTINGS: Okay, thank you.

BY MS. CHIN-CAPLAN:

Q So Dr. Hepner, could you just address the criticisms of the Uhlmann study by Afzal et al.?

A Sure. Well again, when you attempt to reproduce a study, you need to reproduce all the variables of that study. Now, the study in question, the study that is being reproduced, if the study is attempting to show that measles virus is present in GI biopsy bowel-the the bowel biopsies of this specific cohort of patients, all reproductions of that study must use the same starting material and must use all biopsies from this cohort of patients.

What Afzal did, in Afzal et al., they attempted to reproduce these results, however, they neglected to use the same starting material. Instead of using GI biopsies from this cohort of patients, they used peripheral blood mononuclear cells from this from ASD patients. It actually was not even restricted to ASD patients with GI disease, so it was just ASD patients looking at the peripheral blood.

Q So, with respect to the Afzal study, are you saying that it's not comparable to Uhlmann because, first, it used blood cells as opposed to gut tissue?

A Yes, so, what I believe that they managed to

do was --

Q Okay, so they used blood cells as opposed to gut tissue, was one.

A Correct.

Q And they also, they used autistic children but they did not use autistic children who had GI problems?

A It was not restricted. That was not an inclusion criteria for the study.

Q So that's a potential flaw in that study?

A Yes.

Q Go on Doctor, I'm sorry.

A Okay. So, what I believe that they have done is they have tested actually a different hypothesis. The hypothesis that was tested there was, can you detect measles virus in ASD patients, in ASD children, in their peripheral blood? And it's a reasonable hypothesis based on the Uhlmann paper. It's a hypothesis that if it's in the GI tissue, it may also be in peripheral blood, and I would like to test that hypothesis.

And the answer was, it was not, it was either not there, or it was not detectable; it was there but not at detectable levels.

Q Now, Doctor, you also mentioned the D'Souza

paper?

A The D'Souza paper performs a similar study using peripheral blood, and was not restricted to ASD -- it was ASD patients, but not restricted to ASD patients with bowel disease.

Q So, essentially, the D'Souza group replicated the Afzal group, however, the Afzal group had two potential flaws in their study?

A Correct. May I add something?

Q Sure.

A Actually, the D'Souza study shows that they were able to use the primers that were used in the Uhlmann paper and were able to successfully amplify measles virus from positive control DNA -- RNA, or cDNA. So they actually show that the primers are specific. However, they were not able to detect it in their experimental samples because their choice of experimental samples was flawed.

Q So the positive that was supposed to be positive was positive?

A Correct.

Q And that indicates that the flaw is potentially with the study population and not the primers that was used?

A That's right.

Q Now, Doctor, there's been this question about detection limits. Could you? Is detection limits something that's standardized among all laboratories?

A Can you ask that question differently?

Q Sure. When you are speaking of results and everything, can we correlate the results of one lab with another lab?

A I'll try to answer that question. There is. I'll answer that question by pointing to another Afzal study, which was a collaborative study among seven into seven different labs, looking for measles virus RNA or cDNA in the same sample. So the same sample was divided -- multiple samples were divided up into seven different batches and sent out to seven different labs.

Each lab applied their own in-house technique to those samples, and the findings showed that there was about a thousand-fold difference in detection thresholds between different labs for different samples- for the same sample.

Q So Doctor, just to be clear on that, this one laboratory took this big batch of solution, is that it, and they divided it up into seven parts, and they sent it out to seven different laboratories to

test, and when those seven different laboratories tested it, the difference between the seven laboratories is a thousand times different?

A They didn't use one sample. They used multiple samples. Some were positive and some were negative. The only people who knew which ones were positive and negative were the people who conducted the study. The samples were sent out blind to these labs, and what they showed was that, while the RNAs that were present in high abundance were easily detectable, the RNAs that were detected in low abundance, there was about a thousand-fold difference in detection threshold, which means that that threshold is, what is the amount of RNA that needs to be present for my lab to be able to detect it?

In one lab, that's X. In another lab, I can't detect X. In fact, I can't even detect it until it's 1000 times X. And that would be the difference in those detection thresholds between the labs. And that reflects, most likely, it could reflect differences in operators, the people who are conducting the study, and it also reflects a difference in reagents and technical design.

SPECIAL MASTER HASTINGS: And just for the record, looking at your paper, Dr. Hepner, I think that study you were just talking about was the Afzal 2003 study?

THE WITNESS: Yes, my apologies. That was a

different study. This is 2003.

SPECIAL MASTER HASTINGS: Okay. Thank you.

Go ahead.

BY MS. CHIN-CAPLAN:

Q So Doctor, what does that mean when you have a situation where two laboratories are testing and they come up with different results?

A That means that either one of the labs -- what that tells you, if the first lab that found the results performed scientifically sound data scientifically sound experiments and generated scientifically sound data, that tells you that there was most likely something wrong with the ability of the second lab to detect it.

Q Doctor, during yesterday's testimony, Dr. Krigsman indicated that you had been a member of a team that presented a paper at IMFAR?

A Correct.

Q And can you just generally tell the Court what the substance of that paper was, or abstract?

A Sure. This was an IMFAR abstract.

Q Yes.

A So this is preliminary data. And the goal of this study was to create a highly reproducible, highly sensitive, very specific assay, or experimental

design, to detect MV RNA in the bowel biopsies of ASD

patients. And what we did was we generated multiple primer sets and tested them for their specificity and their sensitivity.

What we wanted to do, in addition to creating a very robust study, a very robust experimental design that hopefully ultimately would be reproducible in other labs, was we wanted to show that this that this RNA that we are detecting is vaccine strain-specific. So we designed the study so that it would show a vaccine strain-specific kind of signature in its DNA sequence -- in its RNA sequence.

Q And what were the preliminary results of your study?

A Well, the preliminary results essentially show that we were able to amplify MV RNA at a high frequency in many of the bowel biopsies that we tested, and there were multiple primer sets and they differed in their sensitivity, which we would expect based on what we know now. That's essentially what was shown. And we were able to confer vaccine strain specificity in some of those samples.

MS. CHIN-CAPLAN: Thank you.

SPECIAL MASTER HASTINGS: That's all of your direct?

MS. CHIN-CAPLAN: Yes, thank you.

SPECIAL MASTER HASTINGS: Mr. Matanoski, did you folks have any questions for this witness?

MR. MATANOSKI: Yes, we did, but, Your Honor, if I may have a brief moment to collect my notes here?

SPECIAL MASTER HASTINGS: Okay. How long are you talking about?

MR. MATANOSKI: Five minutes.

SPECIAL MASTER HASTINGS: All right. Go ahead.

(Whereupon, a short recess was taken.)

SPECIAL MASTER HASTINGS: All right, for those listening in, we are ready to go back on the record here, and we are going to now have cross-examination of Dr. Hepner. Mr. Matanoski, when you are ready, please go ahead.

MR. MATANOSKI: Thank you, sir.

CROSS EXAMINATION

BY MR. MATANOSKI:

Q Dr. Hepner, good morning.

A Good morning.

Q How long ago did you receive your PhD?

A Let's see. Three years ago. Three or four -- four years ago.

Q And you are the first author on one

published article and a coauthor on three?

A Correct.

Q One of those is in press right now, correct?

A Correct.

Q None of those articles deals with the detection of measles virus through PCR, does it?

A They do not.

Q Those articles generally concern cancer, correct?

A Correct.

Q Those articles don't concern the proper method for detecting viruses through PCR, do they?

A They do not.

Q What types of PCR have you worked with?

A I've worked primarily with solution phase PCR, RT-PCR, I've done in situ PCR, and TaqMan PCR.

Q About how many times have you personally used PCR?

A It's hard to really answer that question. I've done it so many times, it's hard to say. Hundreds.

Q Hundreds?

A Yes, well, my most recent study, which is in preparation, I generated over 20 mutant clones that required an inordinate number of PCR reactions.

Q Have you ever designed primers for the detection of viruses?

A I design primers for all of my PCR reactions.

Q And you've done those for viruses as well?

A Oh, for viruses?

Q Yes.

A I've done it for viruses, too. For measles virus.

Q Did you design the primers used in the work you are doing with Dr. Walker?

A I designed some of them.

Q Did your doctoral thesis explore how to properly use PCR for the detection of viruses?

A No, it did not.

Q You didn't offer a theory on how MMR alone or in combination with thimerosal-containing vaccines causes autistic spectrum disorders?

A I did not.

Q Do you believe that is proven?

A I do not believe that it's proven to a scientific certainty.

Q Do you believe it's proven more likely than not?

A I believe that it's a plausible hypothesis.

Based on the fact that measles virus RNA and protein have been detected in these GI biopsies, I believe that it's a plausible hypothesis.

Q It's a hypothesis?

A A plausible hypothesis.

Q Now, you believe the results in the Uhlmann paper are reliable and not subjective?

A I believe that they are reliable.

Q So --

A And not subjective.

Q Okay. So you also believe they are not subjective?

A I have no reason to believe they would be subjective.

Q What leads you to the conclusion that they would be reliable? What's the primary reason? I know you've gone through some and I don't want you to have to go through all your testimony again.

A Yes, sure. I mean, essentially, you look at the experimental design, you say, did they choose the right experiments and did they perform those experiments correctly? And I believe that they chose the correct experiments and performed them adequately.

Q You are basing that on published work?

A The published Uhlmann study?

Q Yes.

A Yes.

Q In your report, you took issue, and in your testimony today, you took issue with the conclusions of the Afzal paper, and the D'Souza paper, indicating that they weren't really specifically designed to replicate the same type of experiment that Uhlmann and his associates did. Is that correct?

A Correct.

Q Now, one of the criticisms that you have leveled is that they were comparing -- they were attempting to use a different material. They were attempting to use blood.

A That's right.

Q And why would that not be appropriate?

A Because we don't know -- the only thing that we have shown so far is that it's detected in GI tissue. It is fair to have the hypothesis that it could therefore also be detected in blood, but if this is a persistent infection in GI tissue that is not detectable in blood, these studies would not have been able to detect measles virus RNA in the blood.

Q Is there a reason to believe from a biological standpoint that if the measles virus was present in the gut tissue that it also would not be

present in the blood?

A Well, viruses are complicated, and there are different types of atypical viral infections, so while it is plausible hypothesis based on the literature, it is also plausible hypothesis based on the literature that it could not be there and that it could be persistent only in GI tissue and restricted to GI tissue.

Q So in your view, it's just as plausible that it just remains in the gut and never enters the blood?

A That is what the data is showing currently.

Q You mentioned in talking about the D'Souza paper -- I'm sorry, in one of your reports, one of your criticisms of both D'Souza and Afzal for using blood was that it might not detect low copy numbers of RNA?

A Many of the biopsies in the study were low copy. So even if we are working with a hypothesis that it could be in the blood, the viral copy numbers could be very, very low. So we really don't know at this point whether it's even in blood, or if it's in blood, if it is at a detectable level.

Q Okay. Didn't Uhlmann and his associates report high copy numbers as well?

A They reported a range.

Q And weren't some of those very high?

A Some of those were high. I don't know how many were high. Some were high and some were as low as one copy per cell.

Q And didn't you in your report say that you believe that they were so high that that was a reason why you would believe that it wasn't a subjective interpretation by Uhlmann and his associates that the samples were in fact positive?

A The high level of detection would suggest that it could not have been an artifact, that overall, the experiment is not designed to be -- the experimental design does not detect artifacts.

Q So if there were a high number of copies and the experiment was actually really truly finding them, shouldn't they also be present, if indeed it does make it to the blood, in the peripheral bloods?

A It's possible.

Q Isn't that likely?

A It's not what the data is showing.

Q Okay, so it's either not making its way into the blood, or it's not in high copy number.

A Correct. It's either not there or not detectable.

Q And it would not be detectable because it

was a low copy number?

A Correct.

Q Now, you mentioned in your report -- in your testimony, not in your report, you were asked a question about whether you thought the Afzal study was a flawed study, and you said yes. Is that what you really believe, that it's a flawed study?

A It's flawed to the extent that they did not -- their attempt was to reproduce the Uhlmann paper, the Uhlmann study. It is flawed to the extent that they did not use the proper reagents to do what they planned to do, which was to reproduce that study.

Q They didn't use the proper reagents?

A The starting material, the RNA source.

Q I see. They didn't use the proper RNA source. So it's your criticism about using blood as opposed to gut biopsy.

A That's right.

Q The study itself, from your standpoint, was it flawed?

A The only other flaw that I would say is that they also used in-house primers. They used primers that were not the same as the Uhlmann primers. So it would have been a good idea to use the primers that the Uhlmann study also used. Besides that, there is

nothing specifically that I can say is flawed.

Q And then the follow-on study by a different group --

A D'Souza.

Q -- who is D'Souza, they used the Uhlmann primers, so they addressed that --

A They addressed that question.

Q Again, they used peripheral blood --

A That's right.

Q -- which we've already discussed. Now, you've said that the D'Souza individuals that conducted that study showed that the Uhlmann primers were specific to measles virus. Is that really what they showed?

A That wasn't what they were attempting to show, but ultimately, that is what they showed, because they used the Uhlmann PCR primers and a measles virus template, a positive control template, and were able to amplify measles virus.

Q And what was the conclusion of the authors of that study? Did they say that their study showed that the Uhlmann primers were specific to detecting measles virus?

A That was not their conclusion because -- actually, it would be helpful if I could refer to that

--

Q Sure.

A Can you just point me in the right direction?

Q I think you were looking at it on your --

A Here, D'Souza. Okay. The D'Souza study.

Q Yes, the D'Souza study. I believe that came out in 2006.

A What they showed, I recall, is that, using the Uhlmann primers -- and they also used other sets of primers. They used a primer set by Kawashima, but the Uhlmann primers, they were getting a nonspecific band that they were saying showed that the primers were not specific. However, the amplification of measles virus in the positive control shows that they were sensitive to -- that those primers are sensitive enough to detect measles virus RNA, though there was also some nonspecific banding.

Q So in other words, nonspecific banding, it was also amplifying things that were not measles virus, correct?

A That's right. In peripheral blood.

Q You've criticized peripheral blood. Didn't -- in the Uhlmann paper, take a look at it. Didn't they use peripheral blood as one of their

controls?

A They used it, I believe, as a negative control, correct? And that would just be -- is that correct? Used it as a negative control?

Q Go ahead and take a look and see whether they believed that peripheral blood could be used as a control.

A "Negative control material included uninfected viral cells, human tissues, control RNA extracted from Raji cells, and normal peripheral blood mononuclear cells." So they were using it as a negative control, a source of RNA that should not contain measles virus.

Q If you don't have it introduced initially, correct?

A Right, and this was normal.

Q Isn't one of the uses of controls, the negative controls, is to detect environmental contamination in the lab?

A Yes.

Q So if you are using blood as one of your negative controls, you would expect that if there are contamination by measles virus, it would be in the blood.

A If there was contamination in the lab, yes,

it could be.

Q So it's not that measles virus can't be there in the blood if measles virus is present, correct?

A Measles virus could be in the blood if measles virus is present.

Q Now, in your expert report, you mention the melting curves in the Uhlmann study were consistent with measles virus amplification. Is that right?

A No, that was an error. It was related to a personal communication in which we were discussing a different method, and this was an editorial error.

Q So that was incorrect in your report?

A That was incorrect.

Q You have read the paper though?

A Yes, and I had access to other materials which were not admissible here.

Q They are not admissible here?

A I had discussions with people and I had heard that they had used a different method. I don't have record of that method, and it was just a personal communication, where they had used a SYBR Green method where you would use melting curves. And that should not have been in my report, and I deleted a part of the sentence and not the rest of it.

Q I see. How much of your testimony here today is based on this other information outside the report?

A All of my testimony is based on what's written here. Everyone has access --

Q In the Uhlmann paper?

A In the Uhlmann paper. And I believe I have only referred to things from the Uhlmann paper.

BY MR. MATANOSKI:

Q You characterized the results in the Ulhmann paper of the in-situ PCR and the real time RT-PCR as concordant. What do you mean by concordant?

A I mean that the experimental samples were positive at a frequency greater than controls.

Q Would you consider it concordant or disconcordant if the positive samples in the in-situ didn't match up with the results that you got from the RT-PCR?

A If the actual quantitative number, the number did not match up?

Q If what was deemed positive from in-situ, the numbers there, the number of samples that were deemed positive, didn't match up with the RT-PCR numbers.

A I would say in an individual sample it would

be discordant if the two samples did not match up, not the cumulative effect of the results.

Q The samples they analyzed by both in-situ and real time PCR. How many samples did they --

A I'll have to look. Can I refer to my notes?

Q -- analyze by both methods?

A Actually in the TaqMan PCR, according to the Table 2 summary of the TaqMan and in-situ PCR results, 70 out of 91 were positive for TaqMan, and 42 out of 57 were positive for in-situ PCR.

Q So 25 percent of the time they didn't get the same results using the two different techniques? Is that correct?

A I don't know if the samples overlapped. I don't know if these were the same samples. I don't have that information.

Q The information presented in the paper?

A Yes, the information presented in the paper. I don't know if all of those samples were subjected to the same experiment, so if Sample A was put through both TaqMan PCR and in-situ PCR.

SPECIAL MASTER HASTINGS: Let me ask both counsel and the witness. Your voices are dropping a little bit. If you can bring them up just a tad? Thank you.

THE WITNESS: May I add something?

MR. MATANOSKI: Yes.

THE WITNESS: I would not expect for the numbers to be identical. I would not necessarily expect for everything that's TaqMan positive to be in-situ PCR positive. That just has to do with sensitivity of a particular assay.

BY MR. MATANOSKI:

Q So there's variability across different PCR techniques that you use?

A Yes. Yes.

Q So you won't always get the same results?

A You won't always get the same results.

Q Sometimes it will be positive; sometimes it won't?

A Within a particular assay, a sample should be positive for that particular assay or negative for that particular assay in each run.

Across assays you might expect some variability based on the threshold, the detection threshold of that assay.

Q Now, in your report you also refer to the immunohistochemistry that was performed in combination with the in-situ PCR. Was this done?

A This was actually referred to. Yes. This

was not done in this paper. This was done in another study.

The immunohistochemistry done in this paper was looking at the region within the tissue where the RNA was detected.

Q So they weren't actually --

A No. In fact --

SPECIAL MASTER HASTINGS: If you're referring to a particular point, if you can help us by giving us the page number?

Where was the one that you were just asking about the discussion of the immunohistochemistry?

MR. MATANOSKI: It was in her report. It also was on page 87 of the Ulhmann paper.

SPECIAL MASTER HASTINGS: But you were asking about a discussion in her report. Can you tell me at what page of her report? That's what I'm asking.

MR. MATANOSKI: I believe that was page 3 of the report.

SPECIAL MASTER HASTINGS: Okay.

THE WITNESS: Yes. It was not done, and this was stated incorrectly. It was not done in this paper.

The immunohistochemistry in this paper was

looking at the particular subcellular localization of the measles virus RNA and did not detect the MV RNA or the MV protein.

BY MR. MATANOSKI:

Q So that was an error?

A That was an error.

Q But one of the things you were relying on in making your conclusion was that the presence of measles virus protein to your mind was highly supportive of their result?

A The presence of an MV RNA is very important information. The presence of an MV protein gives another layer of confidence to the hypothesis. Others have looked at this, but this was not done in this paper.

Q Ulhmann doesn't do that?

A Ulhmann does not.

Q So it's not supported there in the Ulhmann paper?

A That particular level of support is not here.

Q You've talked this morning and you also talk in your report that detection limits for measles virus in PCR-based assays can vary up to a thousandfold.

Wouldn't that variability of detection limit

make reproducibility of results that much more important?

A It certainly does.

Q You also stated in your report that as long as the original study which detected measles virus in clinical material was performed in a properly controlled and technically accurate study then you could have confidence in the result.

In terms of control, you mentioned that they listed a number of controls that they used. Did they provide data for all the controls that they used?

A No, they did not.

Q Hasn't one of the criticisms of the Ulhmann paper been that they have not presented their data?

A Yes, I believe it is.

Q And in response they still have not presented their data, correct?

A I am not aware of whether or not they've been asked to present those data. This is a peer reviewed study, and I don't know what information has been accessed.

Q But as you said, it's been criticized that their --

A It's a criticism, yes.

Q -- paper lacks essential data for evaluating

it?

A It lacks information. Some of the data that is described is not shown.

Q And some of the data that was described and now shown was the results that they had received with the controls, correct?

A Correct.

Q And it was important to you, at least to your thinking, that the proper result be obtained from those controls, correct?

A Yes.

Q That positive controls came back positive and negative controls came back negative?

A Yes.

Q If negative controls came back positive, would that shake you confidence in the report?

A Yes, it would.

Q Now, you talked briefly about your work with Dr. Walker.

A Uh-huh.

Q We were presented with the information from your poster yesterday so I haven't had a lot of time to digest it.

A Sure.

Q I have a number of questions to ask you

about that work.

A Sure.

Q Do you have the poster available?

A I don't believe I have it with me. Does someone have it? Do I have it in here?

Q It's trial Exhibit 3. It came in yesterday.

A I have it now.

Q I'm not going to start there, but I'd like to start with some general questions about what you've been doing with Dr. Walker.

A Sure.

Q In obtaining the RNA, how are the samples obtained? In what manner was the RNA extracted?

A The RNA was extracted by Dr. Krigsman and put in a solution called RNAlater that allowed the tissue to remain stable and the RNA to remain stable. It was sent to Dr. Walker, who extracted the RNA in his lab.

Q How was it stored?

A It was frozen on dry ice and then frozen in his freezer.

Q Dr. Walker did the RNA extraction?

A Yes, he did.

Q How did he do that? What method?

//

A He uses a standard protocol using an available type of cyogen kit. It's a specific kit that separates the RNA from the protein and all other exogenous materials.

Q Did you develop a protocol for how you were going to do the experiment?

A I'm sorry?

Q Did you develop a protocol for how you were going to do the experiment?

A From start to finish or that particular part of the experiment?

Q Actually from start to finish.

A We did not develop that protocol. The protocol for RNA extraction is a standard protocol for RNA extraction that we did not develop.

What we did was develop the reagent for the PCR or developed the primers for the PCR design.

Q And as far as a protocol for conducting the experiment, you've developed one?

A We've developed multiple primer sets and primer combinations.

Q Speaking more generally than just the primers, a protocol for actually conducting the experiment. What assays would you use? I've seen five different panels of assays used.

A Yes. Each primer set is in a way its own type of experiment because each primer set requires a different melting temperature or when it's suggested to the PCR thermal cycle or run.

Every primer set is slightly different. The PCR strategy is a standard strategy. You make alterations in that strategy based on the primers that you've designed.

Some of that requires some trial and error, but you design the primers and then you alter the strategy based on those primers.

Q Okay. So you're altering the strategy as you go along?

A That's standard practice, yes.

Q Okay. Do you have a general protocol that's been developed at this point to follow for these experiments?

A Yes.

Q How does the quality and the integrity of the RNA from the patient versus the control compare?

A In the Walker study?

Q In the Walker study, yes.

A As we said, this data is preliminary so we really have access right now to the experimental samples, and we don't have access to as many control

samples.

The large number of experimental samples makes it difficult for me to make any general comparisons, but I can say that all our RNA that we extract is tested for RNA integrity.

Q For the four controls that were postmortem specimens, was that degraded, the RNA degraded in those specimens?

A There was some slight degradation.

Q In terms of control, have you run negative controls at this time?

A No. We are in the process of procuring samples that would be appropriate negative controls. The problem has been access to appropriate materials, and that's why this data is so preliminary.

Q How about no negative controls. Have you run tests on those?

A Well, we run a no-template control with every sample, so that does not contain any template that's contained in the RNA.

We've also run irrelevant tissue, but it does not meet our standards for what would be an appropriate negative control.

Q I'm sorry. You're run irrelevant --

A Irrelevant tissue, which would mean tissue,

so it's a nucleic-based template, but it was not GI derived.

Q I see. And do you have results from those runs on the negative controls that are not GI samples?

A For what we have right now, those results show that they're negative.

Q Do you have the data?

A I don't have it with me, no.

Q But there is data?

A There is very preliminary data showing -- really the reason why that data is not useful at this time is because all it did was show primer specificity, but it did not give us any information yet about this cohort of patients relative to a different cohort of patients.

Q I don't mean to put you on the spot because I know you've said a number of times this is very preliminary.

A It is very preliminary.

Q I just am trying to get some more data because this has been presented yesterday.

A Yes.

Q I just want to get some more data about where you are and what you've been doing. How was the RT-PCR carried out in the Walker study?

A It was a two-step reaction, so the reverse transcriptase reaction to the generation of cDNA was carried out separately from the PCR. Once the cDNA was generated it was quantitated, and then it was subjected to PCR.

Q Okay. How much RNA was used?

A It was variable, but used on the order of five nanograms, one to five nanograms.

Q So it wasn't the same amount for each sample?

A Well, every run it was a comparable amount, but again we were testing. The goal of what we've done so far has been to test this assay, to optimize the assay.

Q Okay. So you're still trying to get the assay? You're trying to develop your assay at this time?

A We're optimizing conditions so that this will be an assay that can be used in every lab, and that requires a lot of manipulation.

Q How many cycles of PCRs do you conduct?

A Thirty-five to 40.

Q And how many cycles of nested PCR?

A Thirty-five to 40.

Q It's all nested?

SPECIAL MASTER HASTINGS: It's all what?

MR. MATANOSKI: Nested. I'm sorry, sir.

SPECIAL MASTER HASTINGS: Nested?

THE WITNESS: Nested.

MR. MATANOSKI: Nested.

SPECIAL MASTER HASTINGS: N-E-S-T-E-D?

MR. MATANOSKI: Yes, N-E-S-T-E-D.

THE WITNESS: The nested PCR was done in a second reaction, so you would have the primary PCR that was 35 to 40 cycles. Then you would take a certain amount of the primary PCR and do a nested PCR. That was also a 35 to 40 cycle PCR.

BY MR. MATANOSKI:

Q Could you describe what your technical controls were?

A For the nested PCR? For this PCR reaction?

Q For the PCR reaction.

A We would have a positive control. We would either have a positive control that was wild type, and the negative control at this point generally has been a no-template control because we don't have access to the right material.

Q Did you include negative environmental controls?

A What do you mean by environmental controls?

Q The controls that you would use to detect for contamination.

A The no-template control functions as a control for contamination.

Q What were your positive controls?

A At this time Dr. Walker only has a plasma DNA. We're trying to procure an SSPE sample that would be a wild type sample, and the reason why this would be preferable is because we are actually looking to confer vaccine strength specificity, and to confer vaccine strength specificity we sequence through an appropriate nucleotide so we look at the database and we look at a specific nucleotide that is only found in vaccine strength.

We want to show that we do not have cross contamination by using a positive control that's actually wild type, so if the positive control is wild type and the experimental controls are vaccine strength then we can show that the source of the RNA could not have been a contamination from the positive control.

Q But right now you don't have that developed?

A We don't have that at present.

Q And all the RNA for the samples, that is the patient samples, was extracted by Dr. Krigsman?

A The RNA was extracted by Dr. Walker. The biopsies were from Dr. Krigsman.

Q The biopsies.

A And then we are getting control biopsies from another source, so these would be pediatric IBD patients from another hospital.

Q When did you run the positive control?

A When?

Q How long ago did you run the positive control?

A Positive controls are run with each experimental run.

Q And where do you run them?

A Where in the thermal cycler or where on the gel?

Q Do you do it in the same lab?

A Everything is run in the same lab.

Q The positive controls are run in Dr. Walker's lab that he's testing other --

A Well, you need to run your positive control along with your experimental samples.

Q Why did you used gel-based RT-PCR and not real time?

A Why did we use solution-based RT-PCR and not real time?

Q Yes. I'm sorry.

A That was because we wanted to -- well, we could have done it, but RT-PCR is actually a less cumbersome assay to use, and if we're able to detect it using RT-PCR it's actually easier to do.

The Ulhmann primers do not span this vaccine strength specific nucleotide, and we wanted to generate different primers that would span that nucleotide that would confer vaccine strength viscosity, so we needed to generate a different assay and not simply reproduce what had been done.

Q In your poster, why did you show only 12 results?

A Twelve positive samples or 12 --

Q You talked about 12 in the poster.

A Well, I think that these were 12 representative samples. We note generally what one does is shows representative samples of your data.

Q Can you turn to the chart that you have?

SPECIAL MASTER HASTINGS: Are we on the poster right now?

THE WITNESS: Yes.

MR. MATANOSKI: We're still on the poster. Yes, sir.

SPECIAL MASTER HASTINGS: Would you bear

with me about 20 seconds while I get my copy?

Go ahead.

MR. MATANOSKI: Thank you.

BY MR. MATANOSKI:

Q Why did you use five different strategies?

A As I said, we were trying to optimize conditions, and three of those strategies reflect new strategies that have not yet been reported other than in this poster. Two of those strategies are actually the Ulhmann studies, and those are the F1-R, F2-R and those primers, those designations.

Like I said, we're trying to optimize conditions. If one has a difficult PCR to run, it is common to generate multiple primers and use multiple primer sets because there is variability and sensitivity between primers and primer sets, and a lot of that just takes time. You just need to test each primer set one-by-one.

The goal of this was to show at this point that an assay is being generated with the purpose of being a highly sensitive and highly specific assay for MV detection in this tissue.

Q You said F1-R and F2-R were the Ulhmann primers?

A Yes. Anything with an F1 or F2 designation

were Ulhmann, so F1-R is actually the F1 reverse primer. F2-F is the forward primer.

The ones that say F1-Outer, those are ones that Dr. Walker generated, so it had an F1, but the word Outer. That was actually redesigned by Dr. Walker.

Q He redesigned some of the Ulhmann primers?

A Yes, and then the other primers were ones that Dr. Walker and I generated.

Q You reported on the vaccine strain in two of the five strategies. You had four positive on one and two positive on the other.

A That's right.

Q Were those two in the same group of the four that you had --

A No. They were different.

Q They were different?

A Yes, so it's a cumulative number of six. We tried to separate them because I don't think it would be good scientific practice to lump all the results together.

Q Dr. Walker said his lab hadn't manipulated any wild virus vaccine strain measles virus prior to the study, this study that you're working on. Had he not worked with those viruses before?

A He had not, as far as I know.

Q Now, he had to introduce measles virus into his lab to do this study, correct?

A He did use some plasma DNA.

Q He'd need that for the controls?

A Uh-huh. And that is why we're trying to use only wild type so that we can distinguish it based on nucleotide designations.

We can distinguish it from the vaccine strain so that if there wasn't contamination from the positive control it would not be reflected in the experimental samples.

Q So really at this point since you've introduced virus into the lab to conduct the experiment you could say there's a low chance of contamination in the lab beforehand because you hadn't used it, but the contamination from actually conducting experiments is just as high, correct?

A A wild type contamination could be possible, but if we sequence through the vaccine strain nucleotide that will distinguish it from any kind of potential contamination source.

Q What were you using as your positive control?

A It was a wild type plasmid.

Q I'm sorry?

A A wild type plasmid.

Q Okay.

A A wild type piece of DNA.

Q I thought I heard you say that you were still trying to develop that as your control.

A We were trying to get a source, an SSPE source, so a brain source. This was a plasma, which is an artificial laboratory construct.

Q Okay. So it's an artificial construct?

A Yes.

Q That's your control?

A Yes, but it contains the proper sequence that's the positive control. All we want to do with the positive control is to show that we can amplify what we say we amplify.

Q Ms. Chin-Caplan spent a great deal of time asking you about whether it was acceptable scientifically to use PCR.

I'm not here to dispute that. I just want to find out though. PCR, if it's used, you have to conduct the experiment properly, don't you?

A Yes.

Q And the results of the experiment themselves, you have to look at how the experiment is

conducted to determine whether those results are reliable, correct?

A Yes.

Q And the results can be affected by changes in study design, right?

A Changes in what?

Q Study design.

A Changes in the design of the experiment?

Q Yes.

A Yes.

Q And they can be affected by variations in the calibration of the machinery used, correct?

A By calibration you mean what?

Q Is the PCR actually working properly.

A The PCR machine?

Q Yes.

A Certainly. If the machine isn't working well, the experiment won't work.

Q The results can be affected by whether or not you're blinded to whether you're working with a negative or a positive control or the sample in question, correct?

A Theoretically it's always good to have a blinded study. That's always preferable.

However, if you treat the sample, the

experimental and the control samples, in an identical fashion from start to finish there really should not be any variation in your results.

Q There is some interpretation though that's involved in the data, correct?

A Yes. In some of the experiments, yes.

Q And so blinding would be preferable because then you wouldn't know which ones you were working with?

A Yes.

Q And the reliability of the results of the PCR will also depend on how specific the primer is to detect the target, correct?

A It depends on both the specificity and the sensitivity of the primer.

Q The reliability of the results would depend also on whether there's contamination present?

A Certainly.

Q The reliability would be affected by whether or not your positive control consistently came up positive, correct?

A Yes.

Q And whether your negative controls consistently came up negative?

A Yes.

Q And if any of these things were askew in some way -- let's say your negatives didn't consistently come up negative or your positives didn't consistently come up positive -- that would cast doubt on the reliability of the results, correct?

A Yes. You would need to apply some level of logic here. If you consistently run a PCR and your positive controls are always positive and your negative controls are always negative, I think that your results are interpretable.

If in a particular PCR reaction at one time your positive control was negative, I think that you can say that since your assays are generally reliable something must have occurred at that one particular time.

In general, you know, if most of the time, if nine out of 10 times, your positive control acts positive and your negative control acts negative, you can say you have a reliable assay.

Q In doing your experiment right now with Dr. Walker, if you run two tests on the same sample and the first time it comes up positive and the second time it comes up negative, does that make you wonder about the reliability of the run that you've just done?

A That's a good question. If the first time it comes up positive and the second time it comes out negative, I am concerned and I would not call that sample positive until I confirmed that in a more reliable way.

Q And if you ran it the first time and it came up negative and the second time it came up positive, would that also give you concern?

A It's the same, really the same type of circumstances.

Q The same?

A If you were getting a positive result and then you get a negative result, or you get a negative result and a positive result, the question is which one is correct.

If you are hovering around your detection threshold, if you are dealing with a starting material that's in low abundance, you might be able to say and you can say that it's possible that the problem is that you are hovering around your detection limit.

That still would not be reason to call that positive at that time, but it would give you -- you know, you think logically through the steps. You just think logically.

Q It might make you want to do it again?

A It would make you want to do it again.

Q Or maybe check how you were doing your experiment?

A Yes.

Q You said you constantly need to change how you're manipulating the --

A We want to optimize conditions so that happens less.

Q What's the gold standard for PCR specificity?

A For specificity it would be -- I think if I understand what you're asking, the gold standard would be to sequence the nucleotide, sequence the results.

Q And Ulhmann didn't do sequencing, did they?

A No, they did not.

MR. MATANOSKI: I have no further questions.

SPECIAL MASTER HASTINGS: All right. Any questions for this witness?

Go ahead.

SPECIAL MASTER CAMPBELL-SMITH: I have just a few questions, Dr. Hepner.

You indicated in terms of your experimental group that you're looking at the material that we were provided in Petitioners' Trial Exhibit 3 indicates that some history was gathered on these patients.

THE WITNESS: I'm sorry. Is that the poster?

SPECIAL MASTER CAMPBELL-SMITH: Yes, that's the poster.

THE WITNESS: Okay. I don't know what exhibit number.

SPECIAL MASTER CAMPBELL-SMITH: It's the printout poster.

THE WITNESS: Okay.

SPECIAL MASTER HASTINGS: It talks about we recognize how the sort of division of labor was broken down, but if you're talking about what your comparisons are you indicated -- let me be clear -- that your positives are all only wild virus content, your positive controls.

THE WITNESS: The positive controls are wild virus at the moment.

SPECIAL MASTER CAMPBELL-SMITH: Are only wild virus?

THE WITNESS: Uh-huh.

SPECIAL MASTER HASTINGS: Your negative controls, which are the postmortem specimens, have some form of IBD, are nonautistic and have or have not received the MMR vaccine?

THE WITNESS: Okay. Let me go back.

SPECIAL MASTER CAMPBELL-SMITH: Okay.

THE WITNESS: First of all, our positive control is actually not an RNA source. It's a plasma DNA source, which is an artificial laboratory construct.

I just want to make that clear that there is a virus that we're using to extract the source. It's a laboratory construct.

SPECIAL MASTER CAMPBELL-SMITH: Okay.

THE WITNESS: Now, the controls that were used in this assay so far, some of these were postmortem patients. They were not GI necessarily. I don't actually know. All I know is that they were postmortem patients. We don't actually know anything about -- I don't know anything about the history. These were not GI biopsy tissue.

As I said, these data are preliminary because we do not have a proper control source. We are procuring samples and we have now a number of tissue specimens which are developmentally normal children without ASD with GI symptoms, with IBD symptoms.

SPECIAL MASTER CAMPBELL-SMITH: Have these children been vaccinated or not specifically with the MMR vaccine?

THE WITNESS: The information is available, but I don't know it.

SPECIAL MASTER CAMPBELL-SMITH: Okay. And that's your control or that's your experimental group?

THE WITNESS: The control group will be when it's done the developmentally normal children.

SPECIAL MASTER CAMPBELL-SMITH: Okay.

THE WITNESS: The developmentally normal children with inflammatory bowel disease-like symptoms. Their MMR status will be known. At this point I don't have those samples, and I don't have those records.

SPECIAL MASTER CAMPBELL-SMITH: And for your experimental group, which are the ASD kids --

THE WITNESS: Yes.

SPECIAL MASTER CAMPBELL-SMITH: And you have at least one diagnosis of that. They have an IBD problem, and they have received the MMR vaccine?

THE WITNESS: The ASD patients have IBD-like symptoms, the specific kind of idiopathic bowel disease, and their MMR vaccine status is known. They are not all vaccinated. They are mostly. Like most children, they are vaccinated, but they are not all vaccinated.

SPECIAL MASTER CAMPBELL-SMITH: Okay. Thank

you.

One other question I had, and I'm just trying to get a visual. I know that part of the purity of the laboratory method is important, and you indicated that when you run an experimental you also need to run a positive control.

THE WITNESS: Uh-huh.

SPECIAL MASTER CAMPBELL-SMITH: Do you run that in the same equipment, or do you have sort of side-by-side? I've not been to one of these labs.

THE WITNESS: Yes. What you need to always do when you run an experiment is you need to run the positive control and the negative control and the experimental samples and the control samples in the same run.

SPECIAL MASTER CAMPBELL-SMITH: Okay. So that's all on the same equipment?

THE WITNESS: It must all be on the same equipment at the same time.

Let's look at what would happen if you didn't, okay? If you ran your experimental samples with a positive control and no negative control, let's say, and you got 90 percent positive.

Well, I would need to know what that negative control showed because I would need to know

in that particular time, in that particular space, in the particular way that I did this, I performed this experiment, did I cross contaminate my sample. If I don't have a negative control then I can't know that.

The same thing with the positive control. I would need to know in a particular time and the particular way that I did this on this particular day on this particular instrument. If I got negative results I would need to know that some sample in there could have been positive had there been a positive sample.

So I cannot say anything about negative results if I don't have a positive control. I can't say anything about positive results if I don't have a negative control.

SPECIAL MASTER CAMPBELL-SMITH: Thank you very much.

THE WITNESS: Okay.

SPECIAL MASTER HASTINGS: Any questions?

(No response.)

SPECIAL MASTER HASTINGS: I just had one. I wanted to follow up on a question you answered for Mr. Matanoski about the dimension of the melting curve.

I think you stated that there was a sentence that you had incorrectly put into your report and then

you took part of the sentence out, but not the whole thing.

THE WITNESS: I should have said standard curve, not melting curve. Melting curve refers to a different type of experiment.

SPECIAL MASTER HASTINGS: Okay. Can you help me? Now, I see at the bottom of page 4 -- do you have a copy of your report?

THE WITNESS: I'm opening it up right now.

SPECIAL MASTER HASTINGS: At the bottom of page 4, four lines from the bottom is a mention of melting curve. Is that where we're at?

THE WITNESS: That's what we're talking about, yes. This is inaccurate.

I should not have written this because the Ulhmann group performed other types of experiments which were not in this study using a different method called CyberGreen, and in those types of experiments you generate a melting curve that allows you to see if it is consistent with what true measles virus amplification would be.

It gives you some information about -- that curve is informational. It tells you what MV amplifications should look like. This was not done in this study because they did not employ this technique

in this study, and I should not have referred to it here. It was an editorial error.

SPECIAL MASTER HASTINGS: So the full sentence there basically should be stricken out?

THE WITNESS: Yes.

SPECIAL MASTER HASTINGS: The sentence that begins with, "However..."?

THE WITNESS: That's right.

SPECIAL MASTER HASTINGS: Okay. Fine. I don't have anything further.

Any redirect for this witness?

MS. CHIN-CAPLAN: No redirect, Special Master.

SPECIAL MASTER HASTINGS: All right.

MR. MATANOSKI: Excuse me, Your Honor. Based on the questions, I have just three brief questions on the Walker study.

SPECIAL MASTER HASTINGS: Go ahead.

FURTHER CROSS-EXAMINATION

BY MR. MATANOSKI:

Q On the Walker study --

A Yes. I'm opening it up again.

Q Actually, I think it's pretty easy.

A Okay.

Q You may have answered it. I just want to

make sure I'm clear. You've run no-template negative controls?

A Yes.

Q And you're still developing the other negative controls, correct?

A We're still trying to get the controls for the experimental samples.

Q For the experimental samples themselves.

A Uh-huh.

Q And you weren't blinded in your study, right?

A In this study that was done here?

Q Yes.

A Well, there would be no point in being blinded because there was only in this case only experimental samples.

When I did this study, because what I did was I worked on helping to develop these assays, and I worked in a separate lab from Dr. Walker. He sent me samples that were blinded. He didn't tell me the designation. I was going to work on optimizing conditions for that assay.

For a very, very small portion of this whole assay development there was a blinded component, but those that are reflected here were not blinded.

Q And you're still in the data collection phase of this?

A Absolutely.

Q We can't draw any conclusions yet from what you've reported, correct?

A The only thing that I would say that one might of why this is actually interesting and why it was presented as a poster of preliminary data is that we believe that we have developed some assays to detect MV in this cohort of patients in biopsied tissue that is vaccine strain specific.

So really this is more of a technical accomplishment at this point because I certainly wouldn't draw any conclusions about the biological significance.

MR. MATANOSKI: Thank you.

SPECIAL MASTER HASTINGS: Any redirect, Ms. Chin-Caplan?

MS. CHIN-CAPLAN: No, Special Master.

SPECIAL MASTER HASTINGS: All right.

(Witness excused.)

SPECIAL MASTER HASTINGS: Counsel, what do you suggest? We go into Dr. Kennedy's testimony?

MS. CHIN-CAPLAN: Could we just have perhaps a five minute break?

SPECIAL MASTER HASTINGS: Okay.

MS. CHIN-CAPLAN: A five minute break.

SPECIAL MASTER HASTINGS: Let's take a five minute break. We're going to take a five minute break at this point.

(Whereupon, a short recess was taken.)

SPECIAL MASTER HASTINGS: All right. We've concluded our break now, and we're ready to go back on the record.

We have Dr. Kennedy in the witness chair. Ms. Chin-Caplan, when you're ready go ahead.

MS. CHIN-CAPLAN: Thank you, Special Master.

SPECIAL MASTER HASTINGS: I'm sorry. We need to swear the witness. Would you raise your right hand please, sir?

Whereupon,

RONALD C. KENNEDY

having been duly sworn, was called as a witness and was examined and testified as follows:

SPECIAL MASTER HASTINGS: Very good. Go ahead, Ms. Chin-Caplan.

MS. CHIN-CAPLAN: Thank you, Special Master.

DIRECT EXAMINATION

BY MS. CHIN-CAPLAN:

Q Dr. Kennedy, could you kindly give the Court

a brief description of your educational background from college?

A I graduated from high school, received a Bachelor's degree in Microbiology from Rutgers College in New Brunswick, New Jersey, received a Master's degree and Ph.D. degree in Microbiology with a specialty being immunology, either immunochemistry or immunogenetics, from the University of Hawaii in Honolulu.

I performed postdoctoral fellow studies at Baylor College of Medicine in Houston in the Department of Virology and Epidemiology with a specialty being viruses and my area of interest being combining viruses and immunology, which ultimately resulted in looking at issues related to vaccine development and looking at new approaches to develop vaccines.

I became an assistant professor while I was at Baylor College of Medicine. I followed by moving to a nonhuman primate facility in San Antonio, Texas, called the Southwest Foundation for Biomedical Research, where I was an assistant scientist, moved up through the ranks to scientist.

My academic appointment was assistant professor to full professor at the University of Texas

Health Science Center in San Antonio in the Department of Microbiology and Immunology.

While in San Antonio I was awarded an NIH Center for AIDS Research grant. I became the director of the Center for AIDS Research while I was in San Antonio, the area being pathogenesis persistence of HIV infection in nonhuman primate models to look for developing new vaccines and therapies.

After I left San Antonio I went to the University of Oklahoma Health Science Center in Oklahoma City as a professor of microbiology and immunology and obstetrics and gynecology. There I directed a baboon breeding colony with the idea of using baboons as a relevant model for human situations.

From Oklahoma I left and went to cross back over the Red River to Lubbock, Texas, where I'm presently professor and chair of the Department of Microbiology and Immunology at Texas Tech University Health Science Center.

Q Doctor, as chair of the department can you describe to the Court what your duties and responsibilities are?

A From a standpoint of being chair, yes, a lot of times I just babysit my faculty, but I think

realistically 25 percent of my duties are administrative; 25 percent are research oriented, so I still have an active research program; say 20 percent are teaching, and I teach both undergraduate students in advanced courses, graduate students and first and second year medical students in aspects related to microbiology, virology and immunology.

I have a service component that's institutional based which is five percent. I serve on a number of institutional committees, including the Biohazard Committee where individuals use biohazard agents, and we want to make sure that they are doing correct procedures for that. I also sit occasionally on medical student admission committees.

Then I have a 25 percent component which is what is termed national service where I sit on review panels both for the National Institutes of Health, for the Department of Defense, National Science Foundation and a number of foreign countries and their scientific review panels in my areas of expertise.

Q Doctor, you've indicated already that you have teaching responsibilities?

A Yes.

Q And you teach medical students, as well as Ph.D. candidates? Is that true?

A As well as Bachelor's students, students obtaining a Bachelor's degree, but that's the advanced courses, the 300/400 level advanced immunology type courses or microbial pathogenesis.

Q Doctor, have you authored any peer reviewed articles?

A Yes. Last count I think I've got over 240 peer reviewed scientific publications.

Q And your peer reviewed articles, do they involve the topic of viral persistence?

A Yes, they do.

Q Doctor, have you done vaccine work at all?

A Yes.

Q Could you describe for the Court your vaccine work?

A A lot of my vaccine work was early on and it was looking at hepatitis B virus and the hepatitis B surface antigen vaccine.

The group that I was at when I was a postdoctoral fellow at Baylor College of Medicine had licensed a number of patents to Merck & Company, at that time Merck, Sharp & Dohme, and we were looking at possibilities of improving on the existing vaccines that were coming out so I was involved in a number of studies that looked at new and novel approaches to

developing vaccines.

During that time point an immunodeficiency syndrome came up in a population that was unknown, thought to be a viral etiologic agent, and it was thought that it had characteristics similar to hepatitis B virus.

I started studying initially that field and that agent, and that turned out to be HIV. I then focused a lot of my efforts unsuccessfully on developing HIV related vaccines.

Q And are you currently researching actively in HIV still?

A I still do HIV stuff. A lot of my efforts have shifted more towards cancer, cancer vaccines, but I still do hepatitis B and C work. I still do some HIV work, Simian Virus 40, cancers that are caused by viruses that persist.

Interested in a lot of those activities and still do some work related to vaccines because of the nonhuman primate models that I've worked with.

Q Doctor, do you hold any patents?

A I have a number of U.S. patents, and if we include U.S., Canadian, European and Warsaw I have over 10 patents that have been issued and probably another 10 to 15 that are pending.

Q Have you done any work with MMR or measles?
A I've done some work with MMR and measles.
Q And have you authored any articles on measles?
A Yes, I have.
Q Your current work with the primates. Do they involve any sort of measles-related activity?
A Yes, they do. We're trying to develop what's called a specific pathogen free colony of baboons.

Essentially what we're trying to do is breed baboons selectively to remove all potential infectious agents that may be immunosuppressant, so that includes the HIV, human immunodeficiency virus, Simian immunodeficiency virus group, all of the Simian retroviruses, a lot of the persistent Simian viruses that infect, and in that group is the morbilliviruses, measles virus being one.

Q Now, Doctor, we're going to turn to your slides here. Could you kindly describe to the Court what the immune system is?
A Okay. The immune system is essentially a two component system. The first component is called the innate immune system. The second component is the adaptive immune system. They both function together,

and they cross feed.

The innate is really the first responder. If the innate does not take care of the foreign invader then it's up to the adaptive to provide protective and constructive responses that should resolve any situation of a foreign entity invading the host and wanting to cause disease or infection.

SPECIAL MASTER HASTINGS: Before we go on, Ms. Chin-Caplan, let's mark this as Petitioners' Trial Exhibit I believe No. 8.

(The document referred to was marked for identification as Petitioners' Trial Exhibit No. 8 and was received in evidence.)

SPECIAL MASTER HASTINGS: I note that Dr. Kennedy was just talking about page 2 of that exhibit.

Go ahead, Ms. Chin-Caplan.

MS. CHIN-CAPLAN: Thank you, Special Master.

BY MS. CHIN-CAPLAN:

Q Would you kindly describe the characteristics of the innate immune system, as well as the adaptive immune system to the Court?

A Sure, if you go to my next slide. Thank you. The innate immune system -- I actually have it

memorized.

The innate immune system is essentially a nonspecific response. It's fast. It contains natural barriers. For instance, the skin is a natural barrier. Mucous that's secreted is a natural barrier. It also contains specialized cells, which are white blood cells that are specialized to react immediately and in a nonspecific fashion.

In general terms, these cells are called phagocytes. There's a specific lymphocyte population that's called a natural killer cell, and these are important components in that they react immediately to a foreign entity or foreign pathogen. There's also a number of secreted molecules that are produced. Interferons are among these, and there are a number of cytokines that are produced.

One thing that is unique about the innate immune system is it is predominantly responsible for the inflammation, the fever that's caused when an entity breaks through the innate immune system.

The pattern recognition molecules are essentially a key to the innate immune system in that particular things like viral RNA and viral DNA can activate through these pattern recognition molecules the innate immune system to fire up, produce inflammation, and this then sets up the adaptive

immune response, which in turn differs from the innate in that it is very specific.

It is much slower, so it's not an immediate. It's not the first responder. It's slow. It takes days to evolve. The adaptive immune response also contains memory where the innate does not, and memory means if you're exposed once you produce adaptive immune responses, and when you're exposed a second time the adaptive immune responses take hold. They're much faster, they're much broader, and they're much more successful at defeating the pathogen that they may encounter.

From a standpoint of the adaptive immune response, the molecules that are involved are specific white blood cells referred to as macrophages and lymphocytes. You'll hear a specialized cell, an antigen presenting cell called a dendritic cell, which is very important in the adaptive immune system.

In addition, the adaptive immune system also has secreted molecules such as cytokines that essentially determine the type of response that will be induced as a result of exposure to a specific pathogen and the characteristics of that pathogen.

Also, the adaptive immune response contains what's called antigen recognition molecules. These

are on the surface of the specific immune cells of the adaptive immune system, and these allow for specificity.

Q Doctor, just to go back a little bit here, with respect to the innate immune system you indicated that one of the components is a pattern recognition molecule. What do you mean by that?

A If the innate immune system specifically sees a sequence of either protein or a sequence of nucleic acids, it has molecules that have specific receptors, and these are called toll-like receptors. These are based on findings of the common fly, a family of members that are analogous to what occurs on specific phagocytes.

These toll-like receptors recognize these pattern recognition molecules, become activated and induce inflammation. By doing this, they set up the adaptive immune response to kick in a little faster than it might normally.

SPECIAL MASTER HASTINGS: Now, Doctor, are you saying toll-like, T-O-L-L?

THE WITNESS: T-O-L-L like Toll House cookies.

SPECIAL MASTER HASTINGS: Go ahead.

BY MS. CHIN-CAPLAN:

Q Doctor, to be clear, the immediate response comes from the innate immune system when you're exposed to a pathogen?

A Correct.

Q And if the innate immune system is unable to eradicate the pathogen then the adaptive immune system kicks in?

A Correct.

Q Doctor, if we look at your next slide, which is on page 4?

A This essentially is how the innate and adaptive immune system cross talk to one another, and from a standpoint of pathogens --

SPECIAL MASTER HASTINGS: Can I interrupt?

THE WITNESS: Yes.

SPECIAL MASTER HASTINGS: What's showing on the screen is not the same as what I have for Slide 4.

MS. CHIN-CAPLAN: Special Master, could I just have a moment to try and straighten this out?

SPECIAL MASTER HASTINGS: Okay. Yes. Certainly.

THE WITNESS: Good, because the one on the screen is not the one I want. Hopefully the next one you have has a lymph node on it.

SPECIAL MASTER HASTINGS: Yes, that's what

we have as No. 4.

(Pause.)

MS. CHIN-CAPLAN: I think we straightened it out.

SPECIAL MASTER HASTINGS: Okay. Yes. That looks right. Okay. Go ahead.

THE WITNESS: The other two slides weren't identical, but they were close enough. I believe No. 2 was identical. The third one, the characteristics are very similar to what I had. I just put in a more specific situation where the products I specifically give products like tumor necrosis factor alpha, interleukin-1 B, IL6, as being part of the innate immune system.

Then for adaptive immunity things like antibodies, cytokines, are essentially the products that are produced.

BY MS. CHIN-CAPLAN:

Q So, Doctor, when we move to page 4 of your slide is this a depiction of what happens when the body encounters a pathogen?

A This is what happens when the adaptive immune system kicks in. Essentially the initial response is in a specialized lymphatic organ referred to as lymph nodes, and within the lymph nodes it is an

area that allows specific lymphocytes, in particular T lymphocytes and B lymphocytes, to communicate in close proximity with one another and to efficiently evoke an adaptive immune response that hopefully would be protective.

What you're seeing in the lymph node is that the pathogen comes in contact with a particular lymphocyte population, which is called a T lymphocyte. When it hasn't seen the pathogen before it's in a naive state. When the pathogen is encountered and presented by a professional antigen presenting cell, which is called a dendritic cell, it will take this naive lymphocyte and activate it such that it allows it to become an effector T lymphocyte.

That effector T lymphocyte has now been activated. It knows what it's looking for with the specific pathogen that the dendritic cell presented to it. That effector T lymphocyte then moves from the lymph node -- and I'm sure you've all had swollen lymph nodes. That's when your immune system is cranking up and getting ready to send a lot of effector T lymphocytes to the site where the infection may be occurring.

It's at that site that allows the effector T cells in this case to eradicate the infection or

resolve the infection. It's the effector T cell in this case that is responsible for providing immunity.

Q Now, Doctor, at any point of this process could the immune response be affected?

A Yes.

Q So to summarize on page 5 innate immunity?

A Okay. So the innate immune response is always on. It responds in minutes, so it's fast. It's the critical first response to microbial pathogens. It's fairly nonspecific, but it does have the capacity to recognize the pattern recognition molecules. Pattern recognition can be found on things like viral RNA and viral DNA.

The innate immune system is composed of natural barriers, skin, we talked about mucosa, sweat is part of the innate immune system, the lining of the gastrointestinal tract. The cells involved are white blood cells, macrophages, dendritic cells. These cells are phagocytizing cells. We also talked about a specific lymphocyte population called an NK or natural killer cell.

Essentially the innate immune response is responsible for producing inflammation and in particular inflammatory cytokines such as tumor necrosis factor alpha, which is TNFa, IL6 and IL,

which is interleukin-1 beta.

Q And, Doctor, when the innate immune system is unable to clear the inflammation that triggers the adaptive immune system?

A Correct.

Q And when the adaptive immune system is triggered what components would be activated?

A So the adaptive immune system has evolved with time essentially to look at the type of pathogen that it needs to respond to. There are two different components of the adaptive immune system. One I refer to contains B lymphocytes and CD4+ cells. The other one I refer to contains T lymphocytes and CD4+ T cells. They have different functional molecules, different cells involved, different cytokines and they respond to different forms of pathogens.

So when B cells are involved this sort of adaptive immune response is the result of a pathogen that is free. It is associated not with a cell, it hasn't infected a cell. So these are pathogens, things like polio virus, hepatitis B virus. These are early components that respond to what we call a group of pathogens who have the ability to infect without being associated with an infected cell.

And this differs from the next type I'll

talk about which targets specifically infections that are inside a cell.

Q So the B lymphocytes, Doctor, are they the immunoglobulins?

A The B lymphocytes are the cells that are eventually responsible for producing immunoglobulins, and the functional molecules in the B cell responses are referred to as antibodies. Antibodies are serum proteins which are in general terms referred to as immunoglobulins. Within humans there are five classes. The predominant ones for producing effective immune response against pathogens include IgM, which is really the first response, it sees the first set of infections.

The second one is IgG, and that is the one that is responsible for not only turning on later as the result of first exposure, this is the one that's responsible for memory. So when you get a second exposure IgG is there ready to go, and when you get a third exposure IgG is ready to go. For an IgG to provoke protective immunity it has to have certain characteristics, and I list some of those characteristics there.

That really depends on the type of pathogen that an IgG must have those characteristics to resolve

the infection. Some of the terms such as opsonization, compliment activation, antibody dependent cell mediated cytotoxicity. In neonatal immunity IgG is very important because it's transferred from the mom via the placenta to the neonate.

It's also important from a standpoint of passive immunity. For instance, if you happen to eat some bad shellfish and they can't give you a hepatitis A vaccine they'll give you immune gammaglobulin, and that's an example of passive immunity where they provide IgG. The most important thing from a standpoint of measles virus and for viruses in general that have what's called a cell free cycle is neutralization.

The ability to neutralize the virus IgG must have to prevent and subsequently resolve the infection. The important thing to note from a standpoint of the CD4+ T cells that are invoked in a B cell type response is that they are referred to as a TH2 type, and TH2 type cells secrete specific cytokines including IL4, IL10, IL13, among others.

These are what drive the B lymphocytes to produce the best quality of antibody that should be available to resolve infection if everything is

functioning normally.

Q Doctor, if any of the immunoglobulins are affected would it affect the ability of a person to fight off infection?

A Absolutely. Within the IgGs there are actually four subclasses in humans. There's IgG1, 2, 3 and 4. Each one has specific components. There are a number of examples in the literature where individuals are deficient in one particular subclass, and they are more prone to infection because they are lacking that subclass.

Q Now, Doctor, the next slide, which is page 7, is this a depiction of what happens with the adaptive immune system and T lymphocytes?

A Yes.

Q Is that the other arm of the adaptive immune system?

A This is the other arm. From a standpoint of immunology we refer to the B cell arm as humoral, H-U-M-O-R-A-L, and the CD8+ T lymphocyte side as the cell mediated. This component is very important for killing pathogens and resolving infection of pathogens that infect cells. So this component is the one that goes after pathogens who are within an infected cell.

Essentially what happens is that exposure to

the microbe in an infected cell will activate these CD4 cells, which are also called T helper cells, and these CD4 cells with the proper cytokine driven response will expand and differentiate into a TH1 type cell. The TH1 type cell produces IL2, gamma interferon.

These TH1 type cells will assist the CD8 cell which has acquired the specificity so it recognizes the pathogen on its receptor that is being provided by the infected cell, and it allows the expansion of the CD8 cells into effector cells, which are called cytotoxic T lymphocytes or CTLs. The CTLs are the cells that once activated they destroy the infected cells.

So from one aspect it's antibodies that is the effector molecule, in the other aspect it is the cytotoxic T lymphocyte which is the effector molecule. Both are important in resolving infection, and both are important with regards to inducing immunity as a result of vaccination.

Q So, Doctor, if any of the components of the cellular immunity system is affected would that affect the person's ability to fight off infection?

A Absolutely.

Q Now, Doctor, do these systems talk to one

another?

A They absolutely do.

Q Could you describe for the Court how these systems talk to one another?

A Essentially they regulate one another, and if I can have the next slide it shows it a little clearer, that there's cross-talk. In other words, you want to put your best effort forth. If you have a cell free pathogen there is no need to make a TH1 type response to kill infected cells. Vice versa, if you have an infected cell although antibodies may be important later on at regulating the response you really don't need that response to kill the infected cells.

Essentially what happens is the naive CD4 T helper cell depending on the signals it receives will become a TH Type 1 and secrete gamma interferon, IL2, tumor necrosis factor, that activates that cell mediated immune arm with eventually the cytotoxic T lymphocyte being the effector cell that destroys the pathogen within an infected cell.

Now, the secretion of IL2 and gamma interferon essentially suppresses the ability of that CD4 T cell to move toward a TH2 type, so you're focusing the immune response where it needs to be.

Alternatively, if you have a situation where you have a cell free pathogen the naive T cell will become a TH2 type cell. That cell will secrete IL4, IL10, IL13, and will focus the response towards the production of specific antibodies.

It will also shut down the response of the TH1 cell and focus the immune response toward antibody production and away from activating cytotoxic T lymphocytes. The interesting aspect of this, I can demonstrate, if things are not in a proper manner it's difficult to resolve an infection, and not only that, the infection can get worse.

I'll give you an example of an infection that occurs in mice but mimics the human disease of Leishmaniasis, which is caused by the parasite Leishmania. It's common in Central America, it's common in South America, common in Africa, a little bit in southwestern United States. You don't hear much about it, but essentially it causes a skin disease and a disseminated disease that can be fatal if not treated.

If you infect specific inbred strains of mice who are genetically prone to produce only a TH1 type response they will recover from the Leishmania infection saying that the TH1 type response, and

Leishmania does infect cells, is responsible for attacking the infected cell that expresses the Leishmania, and killing it and allowing the mouse to recover.

If you take mouse strains that produce predominantly a TH2 type response and you infect them with Leishmania what happens is you get disseminated infection, it doesn't resolve and the mice eventually die. So that's an example of how you want the immune system to work. If it works in the manner it should everything is fine, you recover, you resolve infection.

If it works in the manner you don't want it to then problems exist. A specific instance occurs in humans with the agent that causes leprosy, mycobacterium leprae.

In individuals who are exposed to mycobacterium leprae if they invoke a TH Type 1 response, and mycobacterium leprae again infects cells, those patients that produce a TH Type 1 get a very mild form of leprosy which is called the tuberculoid leprosy, meaning that they are skin test positive much like TB, but they don't have the symptoms of the melting flesh, the disseminated infection that one sees or thinks about when you think

about leprosy.

On the other hand, if those individuals have a defective TH Type 1 response and they have a dominant TH2 they get a form of leprosy which is called lepromatous leprosy. That's the one where the skin melts away, it's systemic destruction, it causes major issues. These individuals are highly infectious. This is what Father Damien eventually got exposed to when he was on Molokai was the lepromatous form, the most infectious.

Tuberculoid form, less infectious and essentially is attempting to resolve the infection.

Q So, Doctor, would it be fair to state that if you have an imbalance of TH1 or TH2 that it's going to affect the course of whatever infection you encounter?

A Absolutely.

Q Doctor, would environmental exposures affect the course of TH1 and TH2?

A It could, yes.

Q Now, Doctor, are there known immunodysfunctional conditions?

A Yes. Actually, they're called immunodeficiencies, and that's what I get to lecture the medical students on is immunodeficiencies and how

they come about. What is the difference between a primary or a congenital versus secondary or adoptive?

Q And could you describe to the Court exactly what you would tell your medical students?

A Okay. I can do it by memory now, but it is a slide.

SPECIAL MASTER HASTINGS: We're now on Slide 9. Go ahead.

THE WITNESS: So immunodeficiency or other terms that are used are immune dysfunction or immune disregulation. Essentially what it means is that the immune system is not functioning the way it normally should. There are two types, a primary or a congenital, or secondary or acquired. With the primary immunodeficiency this is the result of genetic defects. It's an inherited disorder. It can cause biochemical or metabolic deficiencies.

Probably the most common primary immunodeficiency you're familiar with is SKIDS or severe combined immunodeficiency syndrome. These are the children who are born without any functional immune system, and essentially live in a bubble type environment. It's a genetic defect, it's inherited and these children have a very shortened life span and quality of life.

That's one extreme. On the other extreme is immunodeficiencies that can occur in approximately one in 500 individuals in the U.S. So primary congenital immunodeficiencies occur in quite a few individuals. Are there a lot of deaths from those? No, because essentially it is an issue where something is working but other things are functioning normally.

An example would be of the most common immunodeficiency in the U.S., it's referred to as common variable immunodeficiency. What happens in this situation is that there are specific holes or certain defects in components of the immune system that does not allow essentially one to resolve specific infections rapidly or efficiently.

Well, shouldn't that kill them? The answer would be yes, unless we had antibiotics, unless we had antivirals, unless we had other means of treating these infections, because the immune system has a hole or defect in its ability. I'll give you an example of one of the common variable immunodeficiencies that I talk to medical students about.

It's called a hypo IgG subclass deficiency. In these individuals they have one particular subclass of IgG that they're deficient in, but in general they have normal levels of the other IgG component.

They show no problems, no signs, but one characteristic of these individuals, if they receive something like the pneumococcus vaccine they produce great IgG levels to the vaccine, however, when they're exposed to pneumococcus, because they're lacking this one particular subclass, which is important for functional activities and it's particularly important in something called opsonization, which was on the previous slide, these individuals are susceptible to pneumococcus even though all their other IgG levels are fine.

So that's an example of a common immunodeficiency. Does the child die of pneumococcus? No. How do you treat it? Antibiotics, et cetera. But it's still an example of an immunodeficiency that doesn't cause serious consequences. There are also secondary or acquired immunodeficiencies. These can result from certain environmental triggers or environmental factors. Malnutrition is an example of something that can cause an immune dysfunction, immune disregulation.

Heavy metal exposure, mercury, plutonium, polonium. We've heard the recent thing about polonium, and if they hadn't given it at such high toxic levels to kill him rapidly they could have

killed him slowly over a period of time by suppressing his immune response. Infection of cells of the immune system.

There are agents that cause immunosuppression. The classic acquired immunodeficiency syndrome is AIDS, so the human immunodeficiency virus is an acquired immunodeficiency syndrome. Human T cell leukemia virus 1 is also immunosuppressive. Measles virus surprisingly is an entity or a virus that can cause immune suppression or immunodeficiency.

Chronic malaria infection is another example, among others. Ones you're probably more familiar with, cancer. During the course of radiation and/or chemotherapy you are producing immunosuppression or an immunodeficiency. There's also examples of intentional immunosuppression.

When one receives a kidney transplant, when one receives a bone marrow transplant they are intentionally immunosuppressed with drugs such that they don't reject the transplant which essentially functions, if it's not from an identical twin, the same as one would expect a foreign pathogen. Trauma, severe burns, can cause immunosuppression.

The one that's bothering me right now is

age. Both neonates, infants, are not immunologically mature to respond effectively to all scenarios. As you get older you become more immunosuppressed, immunodysfunctioned. That's why they keep telling us every year get a flu vaccine, get a flu vaccine.

If we're in our 20s the flu vaccine that we got, you know, five years ago would be fine. Now, they tell us get a flu vaccine. So age is also a factor that can cause immunodeficiency or immunodysfunction.

BY MS. CHIN-CAPLAN:

Q Now, Doctor, you mentioned that you could have holes or defective ability to clear pathogens. Would people be able to exist with a hole in their immune system and not know about it?

A Eventually it would come to light at some point. Would they necessarily be examined for it? Absolutely not. If this was an ideal situation prior to giving any vaccine it should be recommended that you test the child to see if they have any sort of indication of an immune dysfunction or some sort of hole in their immune system, and there are ways to do that.

However, they're very expensive, very costly and the risk award is not worth it.

Q Now, Doctor, you mentioned a secondary or acquired immune deficiency, measles virus. Can you tell the Court generally what measles virus does to the immune system?

A Measles virus can affect a number of things. It can cause disregulation of things like dendritic cells, it can suppress T lymphocyte responses such that they're not as stringent as they should be, it can delay the production of specific antibodies, the level of antibodies or titer of antibodies produced can be much lower and when you have a number of environmental factors it can be additive.

Q Doctor, on page 10 of your slides is this a measles virus that we're depicting here?

A Yeah. This is a cartoon of what the measles virus looks like, and I thought this might be important because we keep hearing about F gene, H gene, N gene. What does that mean? From a pictorial standpoint of the measles virus if you look at measles virus by electron microscopy its size is approximately 150 to 300 nanometers. You need an electron microscope to look at it, so it's small.

You can't see it with the naked eye, you can't see it with a normal microscope, you need an electron microscope. Measles virus has a number of

interesting characteristics. First of all, it contains a negative single strand RNA, and I'll get into that in a minute. It's nonsegmented, which is also important. The measles virus carries its own RNA transcriptase, and that's an enzyme that is very important for the virus to replicate.

It also contains a number of structural proteins that are important for the measles virus to produce new measles virus progeny. These include the phosphoprotein, the nucleoprotein, the matrix protein and important for infection in general the measles virus contains an envelope which includes the H protein, which is also referred to as hemoglobin, and also an F protein or the fusion protein.

So the ones on the envelope are the outer surface. That's the H and the F, round circle and oblong circle. You can see the matrix protein surrounds the internal nucleoprotein, and the red is the negative stranded RNA.

Q Doctor, does the vaccine strain appear differently from the wild type?

A Yes. It's been attenuated.

Q And when you say attenuated you mean the ability of it to infect is lessened?

A Yes. So if you take a wild type measles

virus and you take measles, mumps, rubella vaccine, or just a measles virus vaccine one of the ways they look to see if attenuation has occurred is they take measles virus and they inject it into susceptible strains of mice or they may even inject it into the brain of a mouse.

If it kills the mice at certain doses then it obviously hasn't been attenuated. If it doesn't kill the mice then it has been attenuated, which means it's less lethal, should be less capable of causing disseminated disease.

Q Doctor, does the vaccine strain also contain phosphoprotein?

A To a degree, yes.

Q And does it contain nucleoprotein?

A Yes.

Q Matrix protein?

A Yes.

Q And does the envelope carry both the F gene and the H gene?

A Yes. Q So the structure is essentially the same. And it contains the negative strand RNA.

Q And the structure is essentially the same?

A Yes. In fact, that's why one attenuates a vaccine. They want it to look like the normal,

natural pathogen. They just want to make it such that it doesn't replicate as violently as it should.

Q Now, Doctor, does the measles virus belong to a certain type of family?

A Yes, it does. I'm going to focus on the measles virus genus rather than the overall paramyxovirus family in general. So the family is paramyxoviridae.

SPECIAL MASTER HASTINGS: Now we're on Slide 11.

THE WITNESS: Yes. That says morbillivirus. Within the paramyxoviridae there are a number of subfamilies and genus that differ from measles virus. For instance, in the same group with similar characteristics is the mumps virus.

But realistically the genus which measles virus belongs to is called the morbillivirus genus, and within the morbillivirus genus are a number of other viruses that are very similar to measles virus except they infect different hosts. They have different host strain specificity is the term that we use.

Canine distemper virus is a morbillivirus whose predominant host is dogs. It can also infect closely related species like fox, minks, wolves.

Canine distemper virus causes a neurologic manifestation, a neurologic disorder, distemper, meaning that the dog doesn't have a real good temper, other terms that have been used are deranged, and within canine distemper virus for instance you can find in the cerebral spinal fluid and the brain of diseased dogs canine distemper virus, so it's isolated and found at those places.

Phocine distemper virus was a virus that was discovered in seals that were dying off the North Sea. Similar scenario. It is neurotropic and causes neurologic manifestations, and actually was responsible for a large seal kill. Dolphin morbilliviruses infect dolphins and porpoises. One thing that all these viruses have in common is that they can be found in the cerebral spinal fluid and can cause neurologic manifestations.

Now, why is it easier to find canine distemper virus in the brain of a dog? I think when one does an autopsy on a dog people don't feel so bad about taking brain tissues to the extent that they might in a human. So from an experimental standpoint it's easier to work on other species than humans to look for specific aspects. But all these viruses share a similar characteristic.

BY MS. CHIN-CAPLAN:

Q And, Doctor, would that include the vaccine strain of measles, also?

A From the standpoint of?

Q Of causing the same type of adverse reactions that you would see with the wild measles virus, the canine distemper, the phocine distemper virus and the dolphin morbillivirus?

A It's possible. I wouldn't anticipate it in a host that is affective at generating immune response.

Q So in an individual who has a proper functioning immune system there should be no problem in clearing any sort of measles virus?

A Correct.

Q Now, Doctor, let's get into a little more specific about the measles virus itself. You indicated earlier that it's immunosuppressant?

A Yes.

Q And could you tell the Court exactly what you mean by immunosuppressant?

A So it essentially causes situations where the immune response doesn't function normally.

Q And from studies that are published is there any indication of how long that dysfunctional immune

system remains after exposure to say a wild type measles virus?

A Three to six months.

Q And would that be the same for a vaccine strain as well?

A Yes.

Q You give a range. What is the reason for the range?

A Usually it takes a while for things to repopulate and get going, and I would assume that at some point that measles virus infection was not persistent and resolved. If it's resolved you would still have some time before the immune system becomes competent again.

Q So are you saying that the total number of immune cells is affected initially, and then it's able to regenerate?

A Yeah, from bone marrow. It's the same thing that one would expect if you gave a bone marrow transplantaation patient. The individual is not immune competent for a while until the bone marrow regenerates the population of cells necessary. They go to the thymus, they go to the spleen, they go to the lymph nodes, they differentiate, they become naive cells, they're exposed and they need to be retaught

memory.

Q Once that population regenerates is it fully functional?

A It would depend. In an ideal situation, yes. In reality, probably not.

Q Doctor, if the immune system was not fully functional within that period of time would it be possible that an individual could contract something that he would have been able to clear if the immune system was fully functional?

A Yes.

Q When that occurs, how does that affect the immune system of the individual?

A It's another environmental trigger that adds injury to insult.

Q And would it be added with respect on the immune system?

A It could be. Yes.

Q Now, Doctor, you had indicated that measles virus affects certain portions of the immune system. Is that true?

A Yes.

Q Can you tell the Court what types of cells are affected in the immune system?

A It affects the professional antigen

presenting cells such as dendritic cells. It also affects T lymphocytes. So T cells that are both involved in the humoral and cell mediated immune responses.

Q So measles virus initially would affect the immediate ability of the body to respond to an infection?

A Correct. However, to a new infection.

Q To a new infection.

A It may slow down the memory response because those memory cells are still there.

Q So the memory cells would take a longer time to regenerate? Is that it?

A Yes, or to invoke.

Q So that leaves the person more exposed to contracting the potential infection?

A Correct, or not controlling the infection in a timely and efficient manner.

Q Doctor, does that also apply to the measles vaccine?

A From what perspective?

Q From immunosuppression standpoint.

A If the measles vaccine is immunosuppressive, yes.

Q So is the vaccine strain

immunosuppressive --

A It can be. Yes.

Q And for the vaccine strain is this period of immunosuppression three to six months as well?

A The studies that have come out suggest indeed it can be three to six months.

Q Now, Doctor, have you read the Ulhmann paper?

A Yes.

Q Just very generally since we had a discussion about the Ulhmann paper today can you tell the Court what that study was designed to do?

A To show the presence of measles virus in gut biopsy samples from patients that had an intestinal disorder, which was called IBH.

Q IBD?

A Whatever. Some hyperplastic condition that normally you don't find in the ileum.

Q Now, Doctor, from that study do you know were they studying measles specifically?

A Yes, they were looking for measles.

Q In their results did they recover some portions of measles virus?

A They were using quantitative PCR technology, and they reported recovering products that were the H

gene and the F gene.

Q And the H gene and the F gene, is that contained on the envelope of the measles virus?

A Yes.

Q And that you had shown earlier on the slide?

A Yes.

Q Doctor, what is the likelihood that the F gene and the H gene are false positives?

A If you'd let me get through my slides I'll show you the possibility that it's probably not.

Q Okay. All right. Let's talk about replication, Doctor.

A Okay. Good. Because it's important to understand replication before you look to see why look for H gene and why look for F gene products?

SPECIAL MASTER HASTINGS: So we're going to Slide 12, right?

THE WITNESS: Yes.

SPECIAL MASTER HASTINGS: Okay. Go ahead, Doctor.

THE WITNESS: So essentially for replication the H protein on the envelope attaches to the host cellular receptor for measles virus, which is referred to as CD46 for cluster of differentiation antigen 46, so the receptor for measles virus has been identified.

Replication takes place in the cytoplasm. The negative strand RNA serves as a template for messenger RNA synthesis.

Messenger RNA synthesis is a positive strand RNA, and this is the part that will be translated into viral protein. Also, the negative strand serves as a template so one can synthesize additional positive strand RNA to then make new negative strand RNA to produce new virus. The virus contains an RNA transcriptase used to generate the messenger RNA, the positive strand.

SPECIAL MASTER HASTINGS: Now we're going to Slide 13?

THE WITNESS: Slide 13.

SPECIAL MASTER HASTINGS: Go ahead.

THE WITNESS: The RNA transcriptase is packaged into the infectious virus particle, and again you need to generate more positive strand RNA to produce the negative strand that is actually the entity that is packaged into the measles virus particle. You've got M protein involved, and that's important for assembly and for the virus to release itself from the cell it's infecting.

The F protein is responsible for fusion, and it's important in the entry process of the virus into

the infected cell following the attachment of the H protein. The F protein allows fusion with the host cell membrane and entry of the virus particle in encoding. N protein, which is a nucleoprotein, functions in viral replication and is further involved in allowing the genome to be packaged and processed.

SPECIAL MASTER HASTINGS: His last testimony was about Slide 14.

MS. CHIN-CAPLAN: That's correct, Special Master.

THE WITNESS: Correct. Slide 14.

BY MS. CHIN-CAPLAN:

Q So, Dr. Kennedy, you've just described how a measles virus multiplies. Is that true?

A Yes. How it replicates. How it infects, and how it replicates, and how it can potentially persist and how it can produce new virus particles.

Q Now, Doctor, why is this important?

A Well, it's important for a number of aspects. So the next slide will be 15, and I don't think we can do -- back one. Sorry. So this is Slide 15 entitled Replication. It's a cartoon, and unfortunately I don't think the Power Point presentation is working because it's animated, but I'll take you through it anyway.

What I want to show from this is the process of what it takes for a measles virus to not only produce the transcripts that are picked up by quantitative RT-PCR, but also what it takes to produce protein from those transcripts and have that protein been assembled with additional negative strand RNA to form new virus particles to go on and infect other cells.

So if you take a look at the red line up there, that is the initial negative strand RNA. We use the term nonsegmented. Now, how does that become separated from the rest of the virus? When the virus attaches via the hemagglutinin to the CD46, which you see up top, the F gene product then comes into play and allows the entire encapsidated measles virus to enter the cell.

So it goes through a phase of encoding. When it encodes two things happen. That negative RNA becomes free and the viral polymerase, the RNA transcriptase, the enzyme that the measles virus carries, becomes free and active and can go and do its work. The first thing it does is from that negative strand RNA it produces a positive strand RNA.

That positive strand RNA becomes the messenger RNA. You see the messenger RNA contains

specific gene products in a particular order.

SPECIAL MASTER HASTINGS: Let me stop you, Doctor, because maybe you're going a little too fast for me or whatever, but is this an important --

THE WITNESS: Yes.

SPECIAL MASTER HASTINGS: I sense this is an important part of your presentation.

THE WITNESS: Yes.

SPECIAL MASTER HASTINGS: Why don't you start with this slide over again, and I think I'm following the segment, but let's see. Let's start with this slide over again and watch me. If I put my hand up and stop you --

THE WITNESS: Hold up your hand and then -- okay.

SPECIAL MASTER HASTINGS: Go ahead.

THE WITNESS: The important thing is that the measles virus when it causes infection in a cell it is an orderly process that occurs to ultimately produce more measles virus to then go on and infect additional cells, so it's a self-regenerating, self-procuring process with the ultimate goal of producing more measles virus particles.

SPECIAL MASTER HASTINGS: Got that.

THE WITNESS: The process is defined, it's

characterized and it's very orderly, and it occurs in a very orderly fashion using not only a specific set of enzymes that the measles virus carries with it, those enzymes then allow the ability to produce the necessary components to then produce new measles virus that can then go on to infect another cell and do the same process over.

So what I'd like to point out as the important components, the red strand. That is the nonsegmented negative strand of measles virus.

SPECIAL MASTER HASTINGS: The red strand at the top?

THE WITNESS: The red strand at the top. That will serve as a template for the virus enzyme to produce the blue strand. That blue strand is positive, so negative goes to positive. The positive RNA is what's referred to as messenger RNA, and that messenger RNA contains defined genes in a specific order. Those genes will then go through a cell machinery to produce proteins, and those proteins are the green and different green colored things down below.

So at No. 2, that is the cell machinery. Things called ribosomes, things called ribosomal RNA, transfer RNA, bring amino acids that allow the protein

to be produced.

SPECIAL MASTER HASTINGS: What do you mean by the cell machinery? I'm not following that. Which cell machinery?

THE WITNESS: The host cell. So measles virus takes over the host cell and uses its synthesis machinery to assist it in producing its protein. So it just says your protein is not being made, I'm making my protein.

SPECIAL MASTER HASTINGS: One minute.

SPECIAL MASTER VOWELL: I have a question, Dr. Kennedy.

THE WITNESS: Yes?

SPECIAL MASTER VOWELL: This cartoon is essentially a cell.

THE WITNESS: A cell. Yes.

SPECIAL MASTER VOWELL: And so at the upper right-hand corner when you have the schematic cartoon you've been using to show the measles virus you show the measles virus entering the cell.

THE WITNESS: Right.

SPECIAL MASTER VOWELL: And then at the bottom you show the measles virus exiting the cell.

THE WITNESS: Correct.

SPECIAL MASTER VOWELL: So I understand your

cartoon.

THE WITNESS: Okay.

SPECIAL MASTER VOWELL: Thank you.

THE WITNESS: Yes.

SPECIAL MASTER Vowell: Okay. That is helpful to me.

THE WITNESS: Okay. The important take away point is if you look at the blue, the blue has N, P, M, F, H, L from left to right. Those correspond to genes that will be involved in making those proteins and from a standpoint of the quantitative PCR that's what is being detected, those products.

You have to make N before you can make P, you have to make M before you make F, you have to make F before you make H, and you have to make the whole thing to get all the proteins necessary for what's called a productive infection. A productive infection means that new measles virus is produced and released. Does that make sense? So it's an orderly fashion.

You can't find N message sequences- I'm sorry- You can't find H message sequences without being able to find F, you can't find F without M, you can't find P without N. So it's a process. N is made first, P follows, M, F and H. This is going to become very important later on. The other thing to note is going

from the negative, the red, to the blue there is

an amplification process.

When that occurs replication has occurred. So infection starts once that negative red starts becoming blue.

SPECIAL MASTER HASTINGS: And that occurs naturally in the infection process?

THE WITNESS: Absolutely. Now, protein. We heard issues related to protein. Can you detect protein? Protein is a later process. So you detect the blue first, and then you detect the green later, and then once everything is together it's organized and put together in a particle and that particle is released from the cell. Now, that is a productive infection.

Persistence doesn't necessarily mean that you have to produce more virus. Persistent means you're at some stage of this process. So you can be blue, you can be green, but you don't have to produce new virus particles. Does that make sense?

SPECIAL MASTER HASTINGS: I'm not sure exactly what that means. You're saying for persistence that the virus doesn't have to keep reproducing itself?

THE WITNESS: Correct. You don't have to have that.

SPECIAL MASTER HASTINGS: Those virus particles, or is it the whole virus that just remains living indefinitely?

THE WITNESS: It's pieces of the virus. Depending on the situation at some point it may be able to reactivate and produce new viruses, at other points it may not. It really depends on the host cell that is persisting it.

SPECIAL MASTER HASTINGS: So you can have persistence of portions of the virus?

THE WITNESS: Correct.

SPECIAL MASTER HASTINGS: For an indefinite period?

THE WITNESS: For as long as that cell stays around. Or you can have persistence where you're getting new infections occurring, but it's at a very low level.

SPECIAL Master Campbell-Smith: Let me ask you, Dr. Kennedy, that calls a question into my mind. You made a point about that in this sequencing they can only occur in a particular sequence.

THE WITNESS: Correct.

SPECIAL MASTER Campbell-Smith: But when you refer to persistence is it possible that you can have portions of that sequence that remain and others are

not there?

THE WITNESS: No. Actually, you could have a situation where you could get N but not necessarily P, M, F and H, but it would stop there. So it would tend to stop at a particular stop point.

SPECIAL MASTER Campbell-Smith: So what had been generated would be all that would persist?

THE WITNESS: Right.

SPECIAL MASTER VOWELL: Thank you.

THE WITNESS: So is that fairly clear?

SPECIAL MASTER HASTINGS: I think up to the point you're at. Right.

THE WITNESS: Okay.

BY MS. CHIN-CAPLAN:

Q Okay. Now, so, Doctor, just to be clear if one finds F gene then N, P and M must have existed before F gene to appear?

A Yes.

Q And if one finds H gene then N, P and F has appeared before it?

A Yes.

Q So is that an indication of replication?

A Yes.

Q Now, Doctor, you also just generally talked about persistence, that the presence of these genes

indicate the viral persistence. Would the presence of these genes cause harm to the cell?

A They could, they may not. Persistence usually means you don't harm the cell. If you harm the cell then you no longer persist.

Q So if it persists it's potentially harmful, and it depends on the host's immune system?

A A persistent infection to the general host can be harmful, to that cell probably not.

Q Thank you.

A Does that make sense? So in other words to persist it has to stay in that cell. If it harms the cell and the cell is lived then it's not persisting in that cell. Now, its persistence in that cell, that's now an infected cell so the host wants to try to get rid of it.

SPECIAL MASTER VOWELL: Let me ask two question, Dr. Kennedy. Do we know anything at all about the length of time these genes can persist within a cell? In other words, the cell makes N, and then stops for a while, and then goes back and something triggers it and it makes P. Do we know anything about the length of that process?

THE WITNESS: About the individual ones? No.

SPECIAL MASTER VOWELL: Okay. Another question. If that cell is infected with a persistent but not actively replicating virus so the cell doesn't die can that cell's particular function in the body be impaired by the presence of the virus?

THE WITNESS: Yes.

SPECIAL MASTER VOWELL: And in what way?

THE WITNESS: Metabolic. It can be a target for neighboring cells to be damaged or destroyed as a result of a developing immune response.

SPECIAL MASTER VOWELL: So the body is responding to the infection in that particular cell but other cells are --

THE WITNESS: Are hurt. Yeah. A bystander.

SPECIAL MASTER VOWELL: Okay.

SPECIAL MASTER HASTINGS: Go ahead.

MS. CHIN-CAPLAN:

Q Doctor, to summarize your report here?

A Okay. So this is the next slide.

SPECIAL MASTER HASTINGS: Number 16. Go ahead.

THE WITNESS: So measles virus and close-related viruses in the same subfamily and genus are neurotropic and can cause neurologic disorders and sequelae in humans and other species. Measles virus

and other morbilliviruses can persist and cause persistent infection and have been detected in the CSF and the brain of individuals that exhibit neurologic disorders. Measles virus infection and replication requires the presence of measles virus RNA.

Measles virus is produced from measles virus RNA, excuse me measles virus protein, is produced from measles virus RNA and is required to produce new measles virus progeny.

"Immune dysfunction" is a term that encompasses problems associated with the normal function of the immune response.

SPECIAL MASTER HASTINGS: Now you're shifting to --

THE WITNESS: -- Slide 17.

SPECIAL MASTER HASTINGS: -- Slide 17. If you could, let us know when you're shifting, for the record, and it will make it easier for us to follow this testimony later on when we reread this, which we will be doing.

THE WITNESS: Okay. I apologize. "Immune dysfunction" is a term that encompasses problems associated with the normal function of the immune response.

Viruses, in general, do not persist in a

host because the host has an effective immune

response. Viruses, in general, persist in a host because of an ineffective immune response, and this would be Slide 18.

A number of contraindications for administering the measles/mumps/rubella vaccine are described in the Physician's Desk Reference. Adverse events involving the measles/mumps/rubella vaccine have included neurologic disorders. Next slide.

BY MS. CHIN-CAPLAN:

Q Doctor, can we just stop here for one minute?

A Yes.

Q You're moving on into the Uhlmann paper here, are you not?

A Yes.

Q Doctor, we've heard a lot about the Uhlmann paper today. You've reviewed the Uhlmann paper?

A I have.

MS. CHIN-CAPLAN: Could we just go back to Slide 15, please?

BY MS. CHIN-CAPLAN:

Q Doctor, the Uhlmann paper detected measles genes in the gut tissue. Is that correct?

A Correct.

Q Could you tell the Court which measles genes

the Uhlmann paper detected?

A The H and the F.

Q Doctor, from your prior testimony, before one can reach F, it must have gone through N, P, and M. Is that correct?

A Correct.

Q So, for F to exist, does that mean that there has to be replication prior to F?

A Yes.

Q And you indicated also that they had recovered H gene. Is that correct?

A Yes.

Q So to recover H gene, they would have to have gone through N, P, M, and F gene.

A Yes.

Q And is that an distinction of replication as well?

A Yes.

(Discussion held off the record.)

BY MS. CHIN-CAPLAN:

Q So, Doctor, if there is recovery of F gene and H gene, this is an indication that the virus is replicating, you said.

A Yes.

Q Is that an indication also that the virus is

persistent?

A Not necessarily.

Q Could you kindly explain that?

A Okay. The fact that it's replicating means that it's there. How long is it there? That's what determines persistence. If it's there for a period of time, three to six months, in that site where it causes bystander effects to occur in nearby cells as a result of an ineffective immune response, then it persists. It's there.

Q Doctor, if we could go to Slide 19, you were continuing your summary of your report here. Are you familiar with the lab of John O'Leary and Dr. Orla Sheils at Trinity College in Dublin?

A Yes, I am.

Q Are you familiar with their reputation in the community as molecular biologists?

A Yes, I am.

Q Could you tell the Court what that reputation is within the community?

A Well, if you look at it just in general terms, in the past two years, the laboratory has published 12 peer-review publications in excellent scientific journals in the area of PCR and molecular-based diagnosis. It's primarily been in the area of

cancer, cancer diagnostics and cancer detection, but they are highly productive and highly qualified to perform these types of analyses.

Q Doctor, they used PCR technique. Is that true?

A Correct.

Q Is PCR a generally accepted standard test to detect measles RNA?

A Yes. In this decade, yes.

Q In previous decades, no? Doctor, if we could go on to the next slide, please.

A Okay. So this is the --

THE WITNESS: Next slide.

SPECIAL MASTER HASTINGS: Number --

MS. CHIN-CAPLAN: -- 20.

SPECIAL MASTER HASTINGS: -- 20. Correct?

THE WITNESS: -- 20. Okay. So the detection of measles virus RNA from several measles virus structural gene products; we didn't talk about it, but the high levels of measles virus RNA detected in the intestinal samples of Michelle Cedillo at a time point when an effective MMR vaccine-induced immune response should have cleared the measles virus indicates that measles virus RNA has amplified as a result of replication and persists in part of her body

where it is not expected to be found.

The detection of measles virus protein in intestinal tissue samples from autistic enterocolitis children further supports measles virus replication and persistence.

BY MS. CHIN-CAPLAN:

Q Doctor, there was previous testimony about the copy number that was found in the gut tissue of Michelle Cedillo. Are you familiar with the copy number?

A Yes.

Q And the copy number that was found; would characterize it as high, medium, low?

A Higher than one would anticipate.

Q Higher than one would anticipate --

A -- with a natural measles virus infection.

Q And is there a reason for that?

A Yes. In my opinion, this is evidence that replication occurred. There was amplification. The only way you get higher amounts is if that process that I showed you in that cartoon slide occurred. There was amplification of that red strand into blue.

SPECIAL MASTER CAMPBELL-SMITH: Can I just interject? Dr. Kennedy, you said higher than you would expect to find with a natural measles virus.

THE WITNESS: With a natural measles virus.

SPECIAL MASTER CAMPBELL-SMITH: So even beyond what you would expect to find for someone who had been vaccinated, you're saying --

THE WITNESS: Correct, correct.

SPECIAL MASTER CAMPBELL-SMITH: Thank you.

BY MS. CHIN-CAPLAN:

Q Doctor, did you also look at an immune workup that was done on Michelle Cedillo?

A Yes.

Q Was that workup performed by Dr. Gupta?

A Yes.

Q Do you recall what the values of that immune workup was?

A Not specifically, but I remember certain things that I thought were relevant.

Q And what were those things that you thought were relevant?

A An elevated IgG-2 response.

Q What's the significance of an elevated IgG-2 response?

A It would be indicative of some sort of immune dysfunction. It would also suggest that there was a skewing toward TH Type 2 response because, if you look in humans, IgG-1 and IgG-3 are evidence of a

TH Type 1 response. IgG-2 and 4 are evidence of a TH 2-type response. The fact that IgG-2 was elevated would suggest to me that, based on subclass characterization, that there was a skewing of the response.

Additionally, I noted that Dr. Gupta did not perform any sort of serology for functional activity of measles virus antibodies. The IgG that he looked at; he did not look to see if there was any neutralizing activity or any functional activity that would tell me that that is evidence of a protective immune response.

So, based on those two things, the fact that there appears to be an ineffective immune response that lacks any sort of viral clearing activity and elevation of a particular subclass that is indicative of a specific type of response, a TH Type 2, that there is some sort of immune dysfunction.

Q What is the significance of the TH Type 2 elevation?

A TH Type 2, as I showed in previous slides, can be very problematic, from a standpoint of resolving an infection. If you skew the response in the wrong direction, you can make a situation worse instead of better.

Q And in this situation, if you had a TH-2 response, would she have been able to clear the measles infection?

A I wouldn't be able to speak to that unless additional assays were performed, but I would suspect that since those assays were not available, that she had a difficulty of clearing it. That elevated IgG-2 didn't help her and may have hurt her.

Q Doctor, do you have an opinion as to whether the measles virus results that were found in Michelle Cedillo's chart is indicative of replication in the gut?

A I would say yes.

Q Do you have an opinion whether the presence of the F gene and the H gene, as reported in the Uhlmann paper, would indicate persistence of that virus?

A Yes, with all of the other caveats that I mentioned.

Q Doctor, what is the basis of your opinion?

A The immunology and virology workup and the only plausible explanation, in my mind.

MS. CHIN-CAPLAN: Thank you, Doctor.

SPECIAL MASTER HASTINGS: Doctor, before we break here, could we back up to the previous slide,

Number 20?

THE WITNESS: Sure.

SPECIAL MASTER HASTINGS: Now, there's two points here. The first one talks about the detection of the MV RNA, and in that, you're talking about the Uhlmann paper.

THE WITNESS: Correct.

SPECIAL MASTER HASTINGS: Then the second point there, the detection of MV protein; now there are you talking about the research described earlier today by Dr. Hepner that was contained in the poster?

THE WITNESS: No.

SPECIAL MASTER HASTINGS: No.

THE WITNESS: I'm talking about additional information that I have from attending a meeting where Dr. Sheils presented work from the O'Leary and Sheils Laboratory.

SPECIAL MASTER HASTINGS: Is this published anywhere?

THE WITNESS: To my knowledge, no. This would be a personal communication, actually, a personal observation.

SPECIAL MASTER HASTINGS: Of who, Dr. Sheils?

THE WITNESS: The O'Leary-Sheils group. Dr.

Sheils is a pathologist, so he does a lot of immunohistochemistry, and I'm aware of the types of specific antibodies that they used. I attended a meeting where the data was presented from a standpoint of both detection of the F gene and N gene products by PCR and also the detection of the nuclear protein by immunohistochemistry in gut tissues from autistic enterocolitis children that was positive for immunohistochemistry.

Part of this came up as a result of a report by a former student in -- I don't remember the specific report -- that was criticizing the immunohistochemistry that was being used using a polyclonal antibody, the measles virus. Do you remember which report that is, Counselor?

It was a graduate student of Dr. Wakefield who submitted a rebuttal letter. If you'll give me a second to look --

SPECIAL MASTER HASTINGS: Done by Dr. Chadwick?

THE WITNESS: Dr. Chadwick. Thank you. So Dr. Chadwick talks about immunohistochemistry studies in there, and I attended a meeting that Dr. Chadwick was not present at that Dr. Wakefield was not present at, but had the O'Leary-Sheils group there, and they

presented this data.

SPECIAL MASTER HASTINGS: Is there any record of this meeting that you're aware of?

THE WITNESS: Just the individuals that were there would be the record.

SPECIAL MASTER HASTINGS: Where and when did this meeting take place?

THE WITNESS: It took place in the U.K. in 2002.

SPECIAL MASTER HASTINGS: Why don't we take a lunch break right now? You're done with your direct, I assume.

MS. CHIN-CAPLAN: I am, Special Master.

SPECIAL MASTER HASTINGS: Let's take a lunch break of about one hour, and we'll be back here, and there probably will be some cross-examination.

(Whereupon, at 1:00 p.m., a luncheon recess was taken.)

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A F T E R N O O N S E S S I O N

(2:04 p.m.)

SPECIAL MASTER HASTINGS: All right. We're ready to go back on for the afternoon here. So, I believe, next we were going to move to the cross-examination of Dr. Kennedy. Are you ready, Mr. Matanoski?

MR. MATANOSKI: Yes, sir.

SPECIAL MASTER HASTINGS: Go ahead.

MR. MATANOSKI: Thank you, sir.

CROSS-EXAMINATION

BY MR. MATANOSKI:

Q Good afternoon, Dr. Kennedy.

A How are you?

Q Doctor, you're a Ph.D. Correct?

A Yes.

Q You're not an M.D.

A Ph.D.

Q So you're not a clinical immunologist.

A Not a clinical immunologist, no.

Q You mentioned you're published on measles virus.

A Yes.

Q How many times?

A I have one peer-review publication.

Q You said you published on MMR.

A I stated that, in that publication, it discusses MMR.

Q So the same publication that talks about measles virus is the one that talks about MMR.

A Correct.

Q Have you ever testified in court before?

A No. This is my first time.

Q Welcome.

A It's loads of fun.

Q Have you ever participated as an expert witness or a consultant for litigation before?

A Yes, I have.

Q How many times?

A Patent, probably three or four over patent issues, from a standpoint of I'm doing one relative to an environmental toxin. How about less than five, other than patent?

Q Other than patents.

A Other than patent.

Q Of these five, one, you said, was on an environmental toxin.

A Yeah. It involves an environmental toxin, something called acrylamide.

Q Okay.

A The other was an HIV needle-stick situation. There was the U.K. report on the measles/mumps/rubella issue and another one relative to these annuity companies wanting to cash in on AIDS patients' life insurance policies early and to essentially give them a lump sum at a discount to kind of -- what was the status of their HIV/AIDS infection. So I believe that's it.

Q That's four.

A Four? And this is five.

Q Okay. You didn't participate in litigation involving a claim that MMR, other than this litigation.

A The U.K. one; I was an expert witness.

Q Other than those two, you don't recall any others.

A No. I honestly don't like being in court, so I try to avoid it.

Q Well, neither do I.

In 1999, you were invited by Representative Burton, Dan Burton, to speak before the House Committee on Government Reform. Do you recall that?

A Yes.

Q In your report, you mentioned that particular instance, and you said that when you were

preparing for your testimony there, you were surprised to find that there were some contraindications for MMR vaccine.

A Yeah. Yes.

Q You don't prescribe medications.

A No, I don't, but I can read the Physician's Desk Reference.

Q Now, aren't there contraindications, though, if you read the Physician's Desk Reference, to just about every single medication?

A From the standpoint of vaccines, I would say it varies on the type of vaccines, that the live, attenuated group, of which MMR is, has more complications than, let's say, a subunit or a recombinant derived or a glycoconjugate type of vaccine, such as the HiB, and with the HiB vaccines, there are different conjugate forms, depending on which carrier protein you use, or whether you use a synthetic oligopolysaccharide. So it's really vaccine dependent.

Q Okay. If I were to take a look at, say, Tylenol, would I find contraindications to that?

A Yes.

Q Do you think there may be more for a live vaccine? Is that what you're implying?

A I would say a live vaccine has special issues and contraindications that you wouldn't find for other vaccination forms.

Q Now, you were in front of Representative Burton to talk about Hepatitis B vaccine. Correct?

A Actually, I was there to talk about issues related to safety considerations and informed consent and essentially educating physicians on what they should tell parents relate to providing vaccines.

Q For Hepatitis B vaccine. Correct?

A For Hepatitis B, yes.

Q Hepatitis B vaccine is not a live vaccine, is it?

A No. It's a subunit: early subunit, later recombinant form.

Q Now, I know this is 1999 when you were testifying, but in your report you profess surprise at these numbers of contraindications. Does that mean, up to 1999, you really didn't know much about MMR vaccine?

A I would say I knew more about Hepatitis B vaccines, and I knew the most about failed HIV vaccines, but certainly I looked at live attenuated approaches as one of the approaches to develop new types of viral vaccines in my laboratory. So I was

certainly aware of anything that was a successful vaccine and certainly anything that was licensed. I believe I used the MMR vaccine in my first medical school lecture in 1989.

Q In 1999, you didn't know what the contraindications were before you prepared --

A I was surprised at the level.

Q And the contraindications are that you shouldn't give it to someone who has a neomycin sensitivity or allergy.

A That's one. There's a number, and it's developed with time. So if you look at the various Physician's Desk References, the various volumes over the years, so Volume 51 differs from Volume 54. So it's developed as things have become clearer.

Q Is there any contraindication there to administering MMR vaccine in someone who has autism?

A To my knowledge, no.

Q You mentioned that you thought that, ideally -- to be fair, you said it wouldn't be practical, and the cost-benefit wouldn't justify this, but you thought that everybody, ideally, should have an immune workup before they got any vaccinations.

A I think that they should not only have an immune workup; they should be tested prior to and post

because there's issues, from a standpoint of transplacental transfer of immunity. So if mother has been exposed and transfers IgG antibodies to the infant, that can affect the vaccine's efficiency, its ability to induce an immune response. There are a number of conditions that can be measured prior to decision when is the optimal time to give the vaccine or when the vaccine should not be given.

Q Has any public, scientific body advocated such an approach?

A Not to my knowledge.

Q Now, when you were giving your testimony to Congressman Burton, did you state, at any time during that testimony, that you believed that MMR vaccine was causing problems?

A To the best of my recollection, I was there to talk about the Hepatitis B vaccine, and I talked about, in general terms, vaccines and adverse events and reporting systems and VAERS in very general terms.

Q And did you mention any problems with MMR vaccine?

A At that time, I didn't specifically mention, to my knowledge, anything relative to MMR or DTaP or any other vaccines. I was more present to discuss the immaturity of the neonatal immune system.

Q You didn't advocate, at that point, that there should be a choice with regard to whether to vaccinate with MMR vaccine. Is that right?

A I'm not a health policy person, so, no, I didn't.

Q But you were advocating that there be a choice for Hepatitis B vaccine.

A Hepatitis B is a certain situation. For instance, Hepatitis B is transmitted by infected blood products, via sex, and, in utero, from a chronic carrier mother to its infected infant. I don't know too many newborns or one- or two-year-olds that are IV drug users, that are sexually promiscuous, or if mother is chronically infected, that the physician or pediatrician or OB/GYN is not aware of that prior to.

Q So you were willing to comment on health policy, as far as that vaccine.

A I believe I didn't comment to those direct terms -- that was 1999 -- but I would be happy to comment on it now.

Q I think you just have.

A Yes.

Q Now, measles vaccine, or measles virus, I should say, has never been the focus of your research, has it?

A Actually, it is.
Q It is the focus of your research.
A One aspect of a lot of my research activities.
Q And you have yet to publish anything --
A Correct.
Q -- other than your one paper.
A The one paper.
Q Anything in press?
A No.
Q Any studies that any data has been generated from?
A Yeah. Actually, we're trying to develop a specific, pathogen-free baboon colony that is free of all pathogens that potentially cause immunosuppression. So what we're doing is trying to select females and males that are characteristically free, using qualitative PCR and some serologic diagnosis of a number of infections, of which we're examining measles virus, or measles virus-like entities, along with Simian Virus 40, the retroviruses that are present in human and Simian species; Herpes B virus, so a number.
Q You're using measles virus to help develop a certain subspecies or subset of primate for use in

research. Correct?

A Correct.

Q So the focus of your research is developing that primate, is it not?

A Correct. But I use measles virus, among others, to develop that primate model.

Q And that's your research.

A One of them. I do other things.

Q None of which that have been published.

A No. Actually, I have eight publications this year.

Q On measles virus.

A No.

Q I'm sorry.

A I was talking about the other things.

Q Okay. So you have other research going on.

A Yes.

Q But that's not involving measles virus.

A Correct.

Q And you're not researching mumps or rubella either. Correct?

A No.

Q And neither at Texas Tech, nor at any of your previous academic positions, were you researching measles virus or MMR. Correct?

A Could you restate the question?

Q Neither at Texas Tech, nor at any of your previous academic positions were you researching measles virus or MMR, and let me qualify that, other than to the extent you've talked about, with this study to try to develop the primate --

A "Research," do you mean physical, hands-on, my laboratory is working on it, or "research," do you mean that I am competent in reading the literature and being aware of --

Q I mean research that you're carrying out with your laboratory --

A Laboratory. Okay.

Q -- as part of your academic research.

A No.

Q This is just a point of clarification. You mentioned that you've been working with HIV, what turned out to be HIV, an unidentified virus at the time, and don't take this question the wrong way; I just want to make sure that it's clarified. You weren't suggesting that you had discovered HIV.

A Oh, no, not at all.

Q I want to make sure I understand your theory of causation here. Can you please state it as simply as possible, step by step, what your theory of

causation is?

A With regards to?

Q Why are you here to testify, in a sense? How do you think the MMR vaccine led to Michelle Cedillo having an autistic spectrum disorder?

A Well, if you look at my Slide 20 and 21, I think it's pretty clear there, if we could have that, my last two slides. I didn't get a chance to memorize my slides, just my lectures. I don't have a copy.

Q I guess I could start by saying, does this encapsulate your theory?

A Yes. Do you need me to read it again?

Q No. I don't need you to read it. I need to go through it to make sure that I understand.

A Okay.

SPECIAL MASTER HASTINGS: Wait. I'm not sure what the question was. He said "this." Are you referring to the summary of the expert report?

THE WITNESS: Yes.

SPECIAL MASTER HASTINGS: When you answered yes, you were talking about the entire summary of expert report, which goes from 16 through 21?

THE WITNESS: No. I would just say the summary, which are the last two pages, 20 and 21, relative to the last question.

SPECIAL MASTER HASTINGS: Twenty and 21.

THE WITNESS: Right.

SPECIAL MASTER HASTINGS: Okay. Go ahead, Mr. Matanoski.

MR. MATANOSKI: Thank you.

BY MR. MATANOSKI:

Q This would be Petitioners' Trial Exhibit No. 8, I believe, on page 20.

A Yeah.

Q And you have that before you, don't you, Doctor?

A Yes.

Q Now, it starts out, and it says: "The detection of MV --" measles virus "-- RNA from several measles virus structural-gene products," and I won't go further. Where is the immunosuppression? How does immunosuppression play a role in this? I heard a lot of testimony about immunosuppression.

A I gave essentially basic aspects related to immune dysfunction, immune disregulation, and immune deficiency, and one of the contraindications for MMR is an individual that is immunosuppressed. So I really didn't discuss aspects relate to immunosuppression.

I think it's best addressed by the clinical

immunologist, but certainly I provided aspects that this is how immunosuppression, immunodysfunction, and immunodisregulation can occur; what are the environmental triggers; what are factors; and what is the difference between a congenital or primary versus a secondary or acquired?

Q In your hypothesis, do you need immune suppression?

A I would say it definitely would help, yes.

Q So you do need it. Is that a "yes," you do need it, or, in some circumstances, you don't need immune suppression?

A I would say that immune suppression is one of the environmental requirements or factors that are necessary to allow persistence of the virus, and the virus must persist for the scenario to occur.

Q So, in the absence of immune suppression, in your postulate, or your hypothesis, you wouldn't hold to that.

A I would say it's less likely, yes.

Q Then are you still saying it's going to happen?

A Have you ever heard a scientist say anything is a hundred-percent certain?

Q I'm not asking for 100.

A Okay.

Q I just want to make sure I understand your theory, that's all. So do you need immune suppression, in your theory, to be of the opinion that, more likely than not --

A Yes.

Q -- that MMR vaccine caused ASD.

A That measles virus persisted, and this caused problems associated with ASD, yes.

Q What level of immune suppression do we need?

A From my assessment, I think that's probably best left for a clinical immunologist.

Q I understand from your testimony that there are different kinds of immune suppression. What kind of immune suppression do we need?

A I would say that anything that would affect the induction of an effective vaccine, MMR-induced, protective immune response.

Q Let's break that down.

A Okay.

Q What would prevent an effective vaccine immune response?

A It could be holes in the innate immune system. It could be holes in the adaptive, the humoral immune response. It could be holes in the

cell-mediated immune response. It could be holes, from the standpoint of the induction of any of the products necessary. It could be a skewing of the response that would not provide normal protective immunity. It could be the lack of production. It could be the overproduction.

Q So any of them may play a role. If any of them are present, would you find that it's more likely than not?

A Yes.

Q So it doesn't matter which one of them.

A No.

Q And you don't need more than one. You can have any of them.

A You can have any of them, or you can have several of them.

Q What if you have some of them working and others not? Does that make it less likely that there is immune suppression that would lead to viral persistence of ASD?

A No. That's why the term "immune dysfunction" or "disregulation," meaning not everything is operating properly.

Q Okay. So just any at all.

A Yes, but there are probably specific ones

that one would anticipate, based on the biology of the measles virus, that might be occurring.

Q As I understand it, your testimony is about somebody who does not mount an effective response to the vaccine.

A Correct.

Q How do you measure an effective response to the vaccine?

A There are a number of serologic, virologic, and immunologic assays that can be used.

Q Do you want to name those assays?

A Viral neutralization assay.

Q Any others?

A Indirectly, a cytotoxic T cell assay measuring the ability to lyse infected targets with an MHC, Class 1, matched target.

Q Any others?

A Indirectly, an ELISA specifically for the neutralizing epitopes found on the H gene product.

Q Any others?

A There's more complex ones. There's T cell assays based on flow cytometry, the induction of proliferative responses specific to a protective epitope that is MAC Class 1 matched to one of the measles virus proteins that are components thereof.

These tend to be very specialized assays, not routinely used.

Q What's the one that we most commonly use to test whether there has been an effective immune response to a vaccine?

A We really don't measure that. What we tend to measure is an ELISA where you use the whole virus components, and you're measuring the antibody response to it.

Q Antibody response.

A Antibody response. But we don't know if those antibodies are protective or not.

Q And that would be a sign that there has been an effective immune response to the vaccine.

A No. That would be a sign that there is an immune response to the vaccine.

Q Now, why is it that you believe that there is immunosuppression when someone is introduced to an MMR, or when MMR is introduced to someone?

A There's citations from the literature. There's a number of citations that deal with measles virus being immunosuppressive. I pretty well think that if it occurs in medical and graduate student textbooks, that it's immunosuppressive, that it's pretty much considered dogma by a majority of people

learned in the art and field.

Q You heard that my question was about measles Vaccine virus -- I think I said "vaccine virus." I'll clarify.

What support is there that measles vaccine virus causes immunosuppression.

A There's some publications in the literature.

Q Could you name the most recent?

A No, because I stuck with textbooks and older ones, but I can certainly name one that I cite in my report.

Q And what one is that?

A Fireman, 1969, I believe, but I would have to get a copy of my report to verify that.

Q Was that MMR?

THE WITNESS: I would have to get a copy of my report. If you can bear with me.

(Pause.)

THE WITNESS: In 1969, it would be very difficult for it to be measles MMR vaccine, wouldn't it? So that would be measles virus, the Fireman article. I apologize.

BY MR. MATANOSKI:

Q Measles vaccine virus. Right? Is that what it's about?

A The measles virus.

Q Measles virus itself?

A Yes. A 1969 publication would, most likely, not deal with MMR being immunosuppressive. Would you agree?

Q My question was to you, and I thought it a little surprising when you --

A Measles virus. I stand corrected.

Q Any other literature support for the notion that measles vaccine virus causes immunosuppression?

A Yeah. I would go ahead and cite the chapter from the Plotkin and Mortimer Vaccine, 1994, the chapter on measles MMR vaccine. Do I know the most recent? No.

Q Anything else that you can think of today?

A That would be it right now.

Q Do either of those references tell us what level of immunosuppression there would be after measles vaccine virus?

A No. They really don't. This is with the vaccine. They really don't discuss what levels.

Q Do either of them say whether those levels of immune suppression are clinically relevant? Do you know what I mean by that term?

A No.

Q Do they have any meaning for the host, for the body that receives them, of any significance?

A I would counter that any level of immunosuppression has some clinical relevance. Has the clinical relevance for that been defined? I would say no.

Q Does the immunosuppression that you say are in these references; do they indicate that the body, or the host that receives the vaccine, is unable to mount a response to the vaccine virus itself?

A In some of the instances, it is not clear what sort of vaccine-induced responses were generated.

Q I don't think I followed your response.

A So, in other words, the studies were not done in a careful manner to address those questions. So, in other words, they looked at total ELISA, but they didn't look at responses to specific epitopes. They didn't measure T cell responses from a specific measles virus, recall response. They looked at things like general overall immune function.

Q What did they find when they looked at general overall immune function? Any problems with clearing measles virus from the vaccine?

A They did not specifically examine to see if the measles virus persisted anywhere. What they did

was they correlated the presence of an immune response with that being an effective immune response, and, therefore, getting rid of the measles virus.

As far as the normal situation in a healthy host, the measles virus is cleared.

Q So these two references don't support the notion that immunosuppression plays a role in persistent measles virus infection in the body.

A These references actually do mention, actually state, that immunosuppression, as the result of exposure, can result in a persistent infection.

Q A persistent measles virus infection from the vaccination.

A Okay. You're tripping me up.

Q No. I'm not trying to, Doctor.

A Okay. So from the MMR, does it persist as the result of immunosuppression? That really has not been addressed or looked at.

Q So they don't support that notion.

A They don't support that because the technology, at that time point of that reference I gave you, was not there to be able to look at it.

Q Is that what they said?

A No. That's what I said.

Q You see, because it seems that important to

your theory is that it not just immunosuppress but that it immunosuppress in such a way that the vaccine virus continues to persist. Correct?

A But I'm not saying that it's necessary for the vaccine to cause the suppression. I'm saying anything can cause the suppression, any environmental trigger, and if you go back to the material I presented, I mentioned that measles virus is an environmental factor that can cause immunosuppression.

My premise does not deal with MMR being responsible for immunosuppression; my premise deals with any environmental trigger that can result in immunosuppression can set up the potential for an ineffective immune response that then leads to persistence.

Q So the MMR vaccine itself, the immunosuppression involved in it, is not integral to your theory.

A No, but if it happens, it helps.

Q But we don't have support that it happens.

A We have support in that chapter that says that --

Q -- that it allows the vaccine virus to persist.

A -- that it can cause immunosuppression.

Q Of any clinical relevance to the vaccine virus.

A Of clinical relevance to the vaccine virus? So do you mean, is it clinically relevant to --

Q -- to the persistence of vaccine virus?

A That is not stated in there, no.

SPECIAL MASTER HASTINGS: All right. Just for the record, before we leave this topic, the two references that Dr. Kennedy cited -- the Fireman article and the excerpt from the Plotkin text -- are both cited at page 10 of Dr. Kennedy's report, and the articles actually are in the record at Exhibit 112, Tabs D and N. Go ahead, Mr. Matanoski.

MR. MATANOSKI: Thank you.

BY MR. MATANOSKI:

Q I have some further questions on the vaccine virus. The MMR contains an attenuated measles vaccine.

A Yes.

Q I'm sorry. Attenuated measles vaccine virus. Right?

A Right.

Q And you mentioned briefly what was meant by "attenuated." Could you explain to me what process is involved to attenuate that virus?

A So, essentially, they take what was a wild-type measles virus and passage it multiple times in host cell tissue culture that allows it to replicate, and the more that you allow it to replicate, it becomes less virulent, less pathogenic, and attenuated. It doesn't replicate as strongly.

Q What kind of host cell do they use?

A They usually use some sort of a monkey kidney fibroblast as standard, hopefully not contaminated with other viruses like SV 40.

Q You said it doesn't replicate as well.

A Right.

Q So if I'm understanding that part of your testimony -- I'll take a step here -- it wouldn't replicate as well as the wild virus.

A Right, as the wild type.

Q So it wouldn't proliferate as readily in the body.

A It should not, no.

Q Turning back to your theory, after this immunosuppression, there is an introduction of the virus. What happens next?

A As far as --

Q -- your theory.

A -- this case or --

Q You've gotten the vaccine. What happens next?

A The virus persists.

Q How does it persist?

A It's not killed by the normal host immune response, and innate and adaptive immune responses come into play. They are not effective at resolving the infection, but they can be effective at causing other problems affecting bi-standard cells, causing inflammation, causing tissue destruction, causing tissue damage, causing inflammation, so standard things for an innate and adaptive immune response.

Q And this will happen in anybody who has any kind of immune dysregulation because you had a whole broad array.

A Right. It's individuals that have no functioning immune system, it would be lethal. In those that have a pretty good functioning immune system, it may not be as severe.

Q If we were to take, we've got about 50 people, total, in this courtroom, I would say, a rough guess, and if we were to perform an immune test on them now, take all of these functions that you were talking about, Do you have an estimate of about how many would have some abnormality and some immune function?

A I would kind of need to know other factors like how much alcohol they had last night, what their liver functions were. So, for me to make a real good guess, I would need some other information.

Q So any of these could affect it.

A Any environmental trigger.

Q And they could --

A Did they eat a heavy meal? Can I get the lymphocytes necessary to do the assay? I mean, there's a lot of confounding factors. If you're asking a statistically general question, in an adult population that has an income over $75,000 a year and has good hygienic scenarios and lived past the age of 35, I would say that you probably wouldn't find anybody who exhibited immunodysfunction.

Q They wouldn't have any IgG-2 elevation amongst these 50 people.

A Well, you're looking, in general, at kids, you know, one in 500.

Q One in 500 for what?

A For any sort of evident immunodeficiency.

Q By all of the tests that you were talking about, it would be one in 500.

A About one in 500 for congenital.

Q For congenital?

A Right.

Q Okay. Because you said any environmental.

A I was referring to congenital. Okay? From the standpoint of the secondary or acquired, it's really not known.

Q Is it fairly common, or is it fairly rare?

A It really hasn't been looked at in a lot of detail, from the standpoint of a large population. I don't know how many people in here are HIV infected. If they are HIV infected, there is a good chance that they have some sort of immune dysfunction. I don't know if people have Hepatitis C. So these are confounding factors.

Q I just want to know, is that the level of immune dysfunction you're talking about to be significant for causing this problem?

A No. It could be anything. I thought I gave you the example of pneumovax, where just a slight alteration affects the ability to resolve an infection.

Q Amongst these 50 people, do you think that maybe -- I can tell you, all of my paralegals are below the $75,000 range in their --

A You guys are underpaid. I would demand more.

Q Amongst these people, do you think every single one of them is going to have every single panel absolutely normal?

A No. There is going to be variation. There is going to be some that are high and some that are low, but will they fall in a median, plus or minus standard error? I would need a biostatistician for that, but I would say there is a good chance that they would. They would fall into some sort of normal range.

Q Now, we give MMR to -- I guess we've vaccinated just about over 90 percent of our children. Do you think every single one, except for the autistic spectrum disorder, the kids who eventually would have autistic spectrum disorder, would develop that, whether or not related to MMR, have normal -- every single immune panel is normal when they get that MMR vaccine?

A No. If you're giving me neonates versus adults, that's a different question.

[Pause.]

MR. MATANOSKI: Fortunately, I'm moving rapidly through my questions here, Doctor, so this pause is good news for you.

THE WITNESS: I would say, take your time,

but please don't.

MR. MATANOSKI: It's good news for you.

BY MR. MATANOSKI:

Q Now, in your report, you mentioned thimerosal as part of your thesis of immunosuppression. I didn't see that in your conclusion, your summary here, and I didn't hear it in your testimony this morning. Is thimerosal a part of your theory?

A I just mentioned mercury as being an agent that is responsible for the immunosuppression. I mentioned heavy metal exposure, some mercury.

Q And is it necessary to have exposure to thimerosal, in your view, to have this immunosuppression or immunodisregulation that could cause a persistent measles virus infection?

A I would say that anything that would be an environmental trigger that could cause immunosuppression would be a reasonable part of the immune dysfunction that's necessary for my -- actually, it's not really my hypothesis; it's more my plausible explanation on the events that occurred.

Q What do you mean, it's not your hypothesis?

A As a scientist, my hypothesis would be stated differently, so it's not a hypothesis. A

hypothesis is developed before you want to address a question. I had the information, and from that information, I came up with a plausible scientific explanation.

Q Okay.

A I wouldn't call that a hypothesis.

Q So you were actually working from a result and working back to how that result could happen.

A Correct.

Q So the result was autistic spectrum disorder after exposure to measles/mumps/rubella vaccine.

A No. Was measles virus in the gut at a timeframe when I wouldn't anticipate that it would be there if the child mounted an effective immune response to the MMR vaccine?

Q And resultant autistic spectrum disorder.

A And that, subsequently, based on what measles virus and morbilliviruses can do, could lead to neurologic manifestations.

Q So you reasoned back from those facts to an explanation that you think is plausible.

A Correct. I didn't have an overriding hypothesis when I looked at this.

Q As far as your explanation, is thimerosal necessary?

A No, but it is helpful. Anything that is an environmental trigger that causes --

Q So we could take out, in this instance, we could take the thimerosal-containing vaccine -- assume that Michelle Cedillo never got it, and your conclusion would be the same.

A Mercury and thimerosal is not my area of expertise. So the only thing I know is from the textbooks, that heavy metals cause immunosuppression, and apparently thimerosal is composed of mercury. So as far as relying on what she got at what time point and how that affects, that's not my area of expertise. I would leave that to other experts.

Q Okay. So you have no idea what level of immunosuppression you could expect after exposure to thimerosal.

A No, I don't, but I know the level that you can expect on general exposure to heavy metals.

Q You said in your report, "a potent immunosuppressive." Was it potent in this case?

A Potent immunosuppressive from a standpoint of this child? I think I just used it in a general context of thimerosal containing mercury, in general, heavy metals, are potent immunosuppressants. I don't think I used it specifically to say, for this

particular individual.

Q At what level, in your view, what level of exposure to thimerosal would be necessary for it to be a potent immunosuppressive?

A As I stated, I'm not an expert in inorganic chemistry or in heavy metals, per se. The information that I obtained to state that heavy metals are a potent immunosuppressant are textbooks and general knowledge of the field that these do cause immunosuppression, to point out that this is another environmental factor or trigger that could cause it.

Q I don't want to belabor the point. So I can take it, then, that what you did is you read some textbooks in preparing your report. Is that right?

A Actually, I took my lecture notes from medical school, graduate school, and read some additional textbooks, and made some slides from that.

Q So, in preparing your report, in preparing your slides, you took some notes that you had taken back when you were in medical --

A No, I wasn't in medical school. No. I teach medical students.

Q So, in terms of your statement -- I was just dealing with your statement on potent immunosuppressive -- that's taken from some textbook

references.

A Yeah, and general knowledge that heavy metal exposure is a potent immunosuppressive, much like chemotherapy is a potent immunosuppressive, much like steroids to prevent transplant rejection are a potent.

Q Steroids are potent immunosuppressives? I'm sorry. Did you say --

A No. I didn't say steroids. I said, immunosuppressive agents used to prevent transplant -- corticosteroids. Sorry.

Q Now, I want to make sure I'm clear. You don't feel that you have expertise, though, in the area of heavy metals. Is that right?

A From the standpoint of how they function and work, no.

Q As an immunologist, do you feel like you have expertise to address how they work on the immune system?

A A little bit.

Q Can you answer, then, how much, from that standpoint, how much exposure to thimerosal would be necessary to produce a potent immunosuppression?

A From a body standpoint, I couldn't. From a test tube standpoint, I could.

Q And from a test tube standpoint, could you

tell us now how much --

A Yeah. You can get down in nanomolar ranges in test tube to exposure to cause an aberration of an immunologic function assay.

Q What nanomolar range?

A In the low nanomolar range like 10-to-100 range.

Q Per what?

A So nanomolar is a volume. So nanogram --

Q Expressed as what?

A As the amount added to the culture. So nanomolar is nanogram per measure of volume.

Q Okay, exposed to culture.

A Exposed to culture.

Q And what's this based on?

A Publications in the literature that show specific dendritic cell dysfunction.

Q Can you tell me right now what those are?

A No. I would have to refer to notes that I didn't bring with me or a literature search that I performed, or I'm sure you could ask the clinical immunologist, who would be more in tune to looking at this. This is just based on studies that we've done from immunosuppressive agents in general in the laboratory.

Q "We've done"? You've done these studies?

A I've done certain ones, not with mercury but with other heavy metals.

Q These are published somewhere?

A No.

Q Why were you doing those studies?

A We were actually looking at proteins on HIV that were immunosuppressive, which we did publish on. We didn't include those heavy metal controls because we were interested in what were the proteins of HIV that were causing immunosuppression.

Q What heavy metals? I'm sorry. Go ahead and finish.

A So we were using essentially lead because that was extremely toxic to the culture, and we had to have some sort of control as a baseline to show that everything was dysfunctional, everything was potent, so we used that as a control, but we never described that in the publication because our interest was looking at HIV, and these were in culture, so in test tube culture.

Q Any other heavy metals that you were using in this experiment?

A Lead was the easiest one we could get, and we just threw it in there as a control.

Q And you knew that this was going to be toxic.

A Yeah, at levels that we were also measuring our HIV proteins at, and we found a wide array of dysfunction, including affecting NK cells, lymphocyte-activated killer cells, general immunosuppression, and I have published those, if you go to my C.V., 1987-1998, although if you asked me the details, my memory is not --

Q Do you have any idea how long the immunosuppression would last after thimerosal?

A In a person who is exposed, it would depend on the amount, how often they got it, the additive effects, age --

Q You've given me a number of factors --

A -- body fat. The answer is no, not without those factors, and I wouldn't be the one to calculate that. I would ask an expert in that area to calculate that. But there are a number of factors that determine.

Q So it's dependent on a number of variables.

A Yes.

Q And you're not competent, in your area of expertise, to comment on this.

A No.

Q Now, it's important to your theory that measles virus RNA be detected in the host that is supposed to be harboring the persistent measles virus.

A Yes.

Q Where do we need to find that measles virus RNA for purposes of your explanation of --

A I would say PCR is a good start.

Q Not how. Where?

A Where?

Q What part of the body?

A I would say --

Q What material from the body?

A I would say, in this particular instance that we're focusing on, I think a gut sample, gut tissue, gut biopsy, from the area of where the inflammation was noticed, so at the site.

Q Why the gut?

A Because that's where the problems were caused. That's where they did the biopsy, and that's where they found it, and that's where I would not expect it to be found.

Q Why wouldn't you sample the brain?

A Pardon?

Q Why wouldn't you sample material from the brain?

A For ethical reasons. It's pretty tough to do a brain biopsy.

Q I understand that. I understand that.

A But if you do it on dogs, you can find it in canine distemper.

Q If your theory is that the virus is causing autism, where would you expect the virus to persist to cause a problem?

A I think the fact that it persists and causes inflammation in a specific area and that the immune response is a global, systemic issue, that problems in one area can manifest themselves as problems in other areas.

Q So, in your theory, then, if you have a problem in your gut, you're going to have a problem in your brain as well.

A You could, yes, because these are neurotropic viruses.

Q But that means that they are in the brain.

A That's neurotropism, yeah.

Q Yes. So you need the virus to be in the brain to cause the problem.

A I didn't say that. I said they are neurotropic viruses. I'm saying if --

Q I'm just trying to understand your theory.

If it's just in the gut, and I'm trying to figure out how now that's going to be infecting -- giving a problem that's going to affect -- in the brain.

A Inflammation, the inflammatory response, can be local, but it can also be systemic. The immune response can be local, but it also is systemic. If you didn't get a systemic immune response, then you wouldn't be able to clear the MMR vaccine because it's found in multiple places as it goes through the process of inducing an effective immune response.

Q So, just to understand, in your construct, we just have the persistence in the gut, but it creates an immune effect that is felt throughout the body.

A That's one possibility.

Q Is this your explanation, though?

A No.

Q What's your explanation?

A My explanation is that if it's found in an unlikely place, such as the gut, there is evidence that suggests that it can be found in other places, like the CSF and the brain, and then that can cause more neurologic manifestations than one may see just as a result of the systemic immune response gone haywire.

Q So, in other words, really, your explanation is that it's not just in the gut, but it's, at least, throughout the body to your brain.

A What I'm saying is this virus, and group of viruses, has the capability of doing that.

Q And do you think that that's what's, more likely than not, happening, as far as your explanation?

A Without having the information and data on Michelle, from a standpoint of the presence or absence, I can't say that specifically, but I know of instances where that can occur, and that can result in neurologic disorders.

Q Now, we'll turn to those instances, and I'm still just trying to figure out, to make sure that I understand your theory. Is your theory, overall, that the virus has disseminated through the body? Is that what you think is happening, more likely than not, after this immune immunosuppression or immune dysfunction that permits the virus to persist?

A That's a plausible explanation but not an absolute explanation.

Q Is that your explanation?

A My explanation would be dependent on what information was available. The information I have

available suggests that it's a plausible explanation.

Q If I take that way, if I tell you that it's only in the gut, and it's nowhere else in the body, do you still feel that it's, more likely than not, that the persistent measles virus is causing autistic spectrum disorder?

A I would say, yes.

Q And what's the basis for that opinion?

A The immune response and the systemic inflammatory response. I'm saying it's a possibility. Is it the most likely possibility? No. Is it more likely than not? Yes.

Q So, for you, just recovery -- if I could show you that it wasn't in the brain, it wouldn't change your opinion, if you could recover measles virus from the gut.

A I would say that there is a possibility that that set up the overall systemic response that caused --

Q More likely than not, would you say that it caused the autistic spectrum disorder, if I told you that I could not recover measles virus, or I had no evidence that it was in the brain?

A More likely than not, I would say yes, but if you gave me measles virus in the brain, then I

would say, "Guess what? I'm right."

Q So you still would say, even if there was no evidence that it was in the brain, you would say it's, more likely than not --

A There's a lot of things that can go on from the presence of having foreign viral RNA activation of toll-like receptors. There's all sorts of inflammatory conditions in cytokines that can be secreted that cause systemic issues, just from a standpoint of having the presence of foreign RNA, viral RNA.

Q Doesn't that happen in numerous occasions?

A But in normal people, it's not an issue. In people that have a skewing immune dysfunction, then it can become an issue.

Q Okay. Do you have any examples of viral persistence of measles virus, other than when you've recovered it from the gut biopsy tissue? Are you aware of any? Are there other examples?

Let's say you don't have any recovery of measles virus from gut biopsy tissues. Are there other examples of persistence of measles virus?

A Yes.

Q And you were talking about the other morbilliviruses.

A Yes.

Q Are there examples of persistence in those instances?

A Yes.

Q In those instance, turning to the morbilliviruses, what's the outcome when the virus persists?

A Usually, from the standpoint of a dog, it has a temper problem, issues. It's deranged, and it's usually put to sleep.

Q And if it weren't put to sleep, what would happen?

A Probably those neurologic manifestations would result in death.

Q And in the other instances, the phocine?

A That was found in seals that had died.

Q And in the morbillivirus that was affecting dolphins, what happened?

A Again, found in dolphins that died, but, again, you usually don't do autopsies on live animals. So if they are floating around in the ocean, it's kind of hard to know what the level of infection is. There are examples of North Sea seals that recovered from exposure to morbilliviruses.

Q Is it persisting when they have recovered

from it?

A They actually resolve the infection.

Q So when they have resolved the infection, there is not a problem.

A I'm not a behaviorist, from the standpoint of what the long-term effects would be on dolphins and other animals like that, but I can imagine that, in some instances, there may not be issues; in others, there may be issues. The brain is a very touchy thing.

Q What do you know about measles virus, what it does on a cellular level?

A Quite a bit. It activates cell-mediated immune responses. It activates T cells. The studies that have been done that really well characterize measles virus have been done in experimental animal model systems, such as mice, and when they have set up the infection in mice to examine what is required for protective immunity, they having injecting measles virus at doses that cause CNS disease, and they eventually have to euthanize the animal, and they use some sort of scale on when to euthanize the animal, because the legs are dragging or some other issue. But certainly it's shown clearly that cell-mediated immune responses to measles virus are important for

protective immunity.

Q How long will it take for this persistent measles virus to begin damaging the brain?

A I don't know. In a human? In a mouse, I know.

Q I'm sorry?

A In a mouse, I know. I don't know in a human. Ten days is standard in a mouse, depending on the route. You can get it faster, if you want to give a higher dose.

Q And how long before the mouse has to be sacrificed because of the level of neurologic damage?

A Actually, it's really dependent on the institution and what is required by their institutional animal care and utilization committee for pain and suffering of animals. So it's pretty much dependent. Some people can --

Q What's the longest amount of time?

A Depending, if you give a low dose, and you want it to persist, you can bring it out to 40 days. If you want to give a high dose and want it to manifest quickly with CNS disease, 10 days.

Q Ultimately, the course for those mice would be death soon after --

A Yes, convulsions, death, yeah.

Q -- whether you sacrifice them or not.

A Whether you euthanize them or not, yeah.

Q You can't tell me how long the process would be of neurotropism in a human.

A No, I can't.

Q Do you still have your expert report in front of you? I'm sorry, not your expert report; your slide presentation?

A Yeah. I sure do.

Q Could you turn to page 15 of that? You're talking about PCR and recovery of measles virus. When we're doing the PCR, what part are we amplifying here, the red, the blue, the green?

A The blue.

Q And that's the blue at the top line would be numbered right with the red at number one.

A Yeah.

Q And you said that that was positive.

A A positive strand.

Q In your view, as an immunologist, is a single test enough to determine whether or not there has been an immune disregulation?

A Yes.

Q And in your view, just having the IgG-2 is enough for immune disregulation, that that will permit

persistent virus.

A Not only the IgG-2, but the fact that it still persists tells me that whatever immune response was made wasn't effective.

Q If I take away the evidence that there is still persistence, if I were to take that away, and you just had the IgG-2, would you think that there is still persistent measles virus?

A If you took away the persistence, no, then there wouldn't be persistence measles virus.

Q If I took away the recovery of measles virus genomic material?

A I would have to know if that IgG-1 and IgG-2 are viral neutralizing. If they were viral neutralizing, then I would say there is not a problem.

Q So you said the IgG-2 level was off in this case.

A Was elevated.

Q Was elevated. If you didn't have recovery of measles virus genomic material, if that was called into question, would you still be of the opinion that there is persistent measles virus?

A So if you don't have measles virus RNA there, and you had no evidence of measles virus being there, but you have an elevated IgG-2A response, which

I'm not even sure is specific toward measles virus, would I still think that's persistent?

Q That's right.

A No. If you don't have measles virus RNA there, why would you anticipate that it has anything to do with measles virus?

Q So you really do need recovery of measles virus RNA.

A Yes.

Q Now, in the one article that you published in 2004 on MMR vaccine -- again, that was your first article on MMR and measles virus. Right?

A Correct.

Q At the time you published that, you were a consultant in the U.K. litigation, MMR litigation. Is that right?

A No. That was over with.

Q Okay. So you had been a consultant before then.

A Correct.

Q Was the litigation over with, or was your involvement with it over with?

A My involvement was over, and I think -- I don't remember the timeframe, but I think the litigation was over with. I would have to refresh my

memory.

Q When did your involvement end?

A When I filed my expert report.

Q When was that?

A Again, I would have to look, but I would say the timeframe would be 2002-2003.

Q You took parts of that report that you published and used it in your expert report in this case. Is that right?

A I used information that I obtained to do my expert report to provide certain aspects from my input and also from the other co-authors, their input, to provide that article, yes.

Q I'm talking about the expert report. You took parts of your article in 2004 and placed them into the expert report that you filed in this case. Correct?

A The article in 2004 was actually published after the expert report.

Q I'm sorry. I can see the confusion. Not your expert report in the U.K. litigation, your expert report here in this case.

A Actually, to be honest with you, I couldn't find any reprints of that paper because my secretary had moved my office. So I had to go without having

access to that paper to do this. So --

Q So you didn't take parts of your 2004 paper and use them in this report.

A I didn't have access to it because the reprints that I had were in a file somewhere, but I had excerpts that I used to essentially develop. So, to answer, did I use that manuscript, the published paper, word for word, I used excerpts that I had, which were probably from formulating the opinion or opinions or information from those, for instance, the section on morbillivirus.

Q You used those word for word. Right?

A I would have to look to see if I used it word for word. Hopefully, I would have changed it little bit. I think the common premise would be identical, that they are neurotropic.

Q In your paper in 2004, you had parts in there that you didn't use in your report here.

A Right.

Q Actually, I guess we can show you, if you would like, side by side, word for word, where parts of your paper are repeated in the report here.

A Is that illegal? I mean, it's my reports.

Q No, it's not illegal, sir. Actually, it's the next point that I want to make that this becomes

important because it's the parts of your paper that you left out that I think we want to focus on now, that you left out of this report.

In your paper, you acknowledge that, based on your review of published research --

MS. CHIN-CAPLAN: Would you give him a copy so he could follow along?

THE WITNESS: Do you have a copy? I don't have a copy. How long was the paper? How many pages was the paper? Twenty-eight?

MS. CHIN-CAPLAN: He is reading from the publication.

MR. MATANOSKI: Actually, I'm reading from my notes.

(Pause.)

SPECIAL MASTER HASTINGS: Mr. Matanoski, is this paper in the record?

MR. MATANOSKI: I believe it was submitted.

THE WITNESS: It's cited in my expert report.

MR. MATANOSKI: It would have been a Petitioner exhibit.

(Pause.)

MR. MATANOSKI: We're going to pull it up on the screen so that you can take a look at it there.

BY MR. MATANOSKI:

Q In your paper, you acknowledged, based on your review of the published research, population-based studies had not been able to detect a link between MMR vaccination and autistic spectrum disorder. Is that right?

A Correct.

Q That didn't find its way into your report here.

A Was it relevant to my report? I don't believe my report says anything about autism, does it?

Q That's the issue that we have to decide here.

A Well, I'm the immunologist and the virologist, so I focused on the immunology and the virology. I agree to that statement. That statement is supported by the literature and by the IOM/NAS report, so I agree with that statement.

Q So do you hold an opinion as to whether or not MMR vaccine is causing autism?

A Yes. I have an opinion.

Q Is it?

A I don't know. Is it plausible? Yes. Is it absolutely? No.

Q Is it, more likely than not, causing autism?

A It would have to be examined on an individual-to-individual instance, if that makes sense.

Q So you believe it is.

A I believe it might and can, under the appropriate conditions. Do I believe it is in all instances? Absolutely not.

Q You believe that it is, or do you believe it's not?

A On an individual-to-individual situation, that it could.

Q So that it could. Is it, more likely than not, doing it?

A Yes, in an individual-to-individual situation. Do I say MMR causes autism? Absolutely not. Can it, in an individual that has the following considerations: persistent measles virus infection, present in the gut? Yes, it could.

Q So, in your opinion, is MMR responsible for an epidemic of autism?

A No.

Q In your opinion, is it responsible for 5,000 cases of autism?

A I'm not an epidemiologist, so I can't --

Q In your opinion, would it be, then, you only

could tell on a case-by-case basis?

A I would say that you would need to look at instances on a case-by-case basis.

Q Would it be rare?

A You know, I'm not really an expert in neurology or autism, so I don't know what you mean by "rare."

Q Well, you're offering an opinion that, on a case-by-case basis, it can.

A Yeah.

Q How often will we see it?

A It would depend on the conditions of that case. You would have to find measles virus. They would have to have an immune response to the measles virus, and that immune response didn't clear it.

Q So you would have to find a measles virus in the individual.

A You would have to have a set of characteristics and criteria that I would need to state that, yes, it's possible for, in this situation, measles/mumps/rubella vaccine to cause neurologic disorders.

Q In your view, would one of those criteria that have to be fulfilled be recovery of measles virus genomic material from the individual?

A I would say --

MS. CHIN-CAPLAN: Special Master, I think Dr. Kennedy has answered this question several times in different manners and reformulated and reformulated it again. It's been asked and answered.

SPECIAL MASTER HASTINGS: I'm not confident that that's the case, so I'll let them go on.

THE WITNESS: So, yes, you need to find measles virus. RNA is a good start.

BY MR. MATANOSKI:

Q Is it necessary to do that, in your opinion, to reach the conclusion that MMR caused autism spectrum disorder?

A In my opinion, from the standpoint of measles virus vaccine being directly involved, yes. Indirectly, I can't say that.

Q I'm not sure I understand you, sir.

A So all of the other circumstances that we've talked about -- the presence of immune dysfunction, the presence of nonspecific inflammation -- I would have to look and see how did the individual respond to the vaccine, how much other factors were involved.

So you're asking me to give a global statement that I can't give. The best that I can say is, on an individual basis, that direct evidence is

the best.

Q It's just an easy question. Do you need it, or do you not need it?

A You need it for direct evidence. Is it possible that it could occur in another manner, indirectly, through inflammation, et cetera? Yes. It's also possible that way.

Q How does the inflammation occur without persistent measles virus?

A Through a number of mechanisms.

Q And this would not be related to MMR, then, without persistent measles virus.

A Persistent measles virus is one form that can cause inflammation. MMR that doesn't persist, when it was resolved, could have set up an inflammation, could have set up a fever, could have set up an issue, been resolved late, if no longer persistent, but it set up a scenario that allowed the immune system to get haywire, so that's another possibility. Less likely, but it's still a possibility, indirect cause.

Q Is it more likely than not?

A I would say it's less likely. That's the opposite of what you're saying.

Q So it's not more likely.

A It's not more likely. The indirect --

Q It falls below the 50-percent threshold.

A Correct, but it's still possible.

Q So in your view, MMR -- it's possible that MMR can cause autistic spectrum disorders even if the virus doesn't persist?

A In some circumstances, it's possible. But, again, it's the -- that it's not the most likely than most, though, the low side?

A It's below 50 percent?

Q Yes.

Q Now, turning back to your paper, you said in your review that large gaps remain in our understanding of the risk factors and etiologic mechanisms of ASD; isn't that right?

A Correct.

SPECIAL MASTER HASTINGS: Where were you reading from?

MR. MATANOSKI: This is page 132.

SPECIAL MASTER HASTINGS: 132 of the paper?

MR. MATANOSKI: The 2004 paper.

SPECIAL MASTER HASTINGS: Okay.

MR. MATANOSKI: These are all a series of questions that I will asking about. Parts of the paper, it didn't make their way into the expert

report, in this case.

SPECIAL MASTER VOWELL: Can you identify that by exhibit number for us, Mr. Matanoski?

MR. MATANOSKI: Certainly.

(Discussion held off the record.)

MR. MATANOSKI: I am sorry. We thought that this had been filed, but apparently it's not been filed by the Petitioner, this report. We will file this as a trial exhibit. I apologize. We assumed that a report by the expert --

SPECIAL MASTER VOWELL: That is something from my computer.

(Laughter.)

SPECIAL MASTER HASTINGS: No wonder we couldn't find it. All right.

MR. MATANOSKI: I apologize.

SPECIAL MASTER HASTINGS: That's okay. Why don't you file it as Respondent's Trial Exhibit 3.

MR. MATANOSKI: We will do so, sir.

SPECIAL MASTER HASTINGS: Okay.

(The document referred to was marked for identification as Respondent's Trial Exhibit No. 3 and received into evidence.)

THE WITNESS: Yes, I agree with that statement.

BY MR. MATANOSKI:

Q And you, also, state in your report that 'investigators have also failed to demonstrate causality between MMR vaccination and autism?'

A Yes.

Q Do you agree with that statement?

A Absolutely.

Q You stated in your report that 'conflicting data exists regarding the studies reported by Wakefield and colleagues' and 'purported to find persistent measles virus' --

A It's straight from the published literature.

Q And so, you agree with that statement?

A Oh, absolutely.

Q And you wrote 'there is little evidence to support persistent infection by the vaccine strain of measles virus except for individuals with a compromised immune system, where all immune dysfunctional.' You agree with that?

A Yes.

Q You, also, wrote 'the biological model linking MMR and autistic spectrum disorder remains incomplete;' correct?

A Correct.

Q And you agree --

A We don't have any good animal models that would allow us to approach these issues in a direct manner.

Q You agree with that -- and you agree with that statement?

A Oh, absolutely.

Q And you still agree with that statement?

A Yes. You guys didn't find my typing errors in there?

(Laughter.)

Q Just moving right along here.

A Getting back to Lubbock, my airlines is not easy, so I've got plenty of time.

Q In your 2004 report, you stated, you would need to find 'detection and isolation of the vaccine strain of measles virus in the gut of inflicted children' to be persuasive evidence of a biologic model linking MMR to ASD.

A Right. That was in reference to trying to develop an animal model that would closely mimic the human scenario and also was related to Koch's postulates.

Q In review today, is that what is necessary

to find persuasive evidence of a biologic model linking MMR to ASD?

A For something to say that this is a relevant model of the human situation and disease, yes. Without having that animal model, without being able to show that, then you would have some issues.

Q So, other animal models --

A Just don't do it.

Q And other models, we don't have other models; correct?

A You know, canine distemper, I don't know too many dog psychologists that figured out if they've got canine distemper, they're autistic dogs or not. So, that's why I kind of stick with neurologic manifestations, if it affects the brain, and it can cause a number of manifestations.

Q You referenced in your opinion in this case personal communications between you and Dr. O'Leary?

A Yes.

Q Were those the -- is that the meeting that you discussed earlier in your testimony?

A Yes. In fact, can I take this time to clarify? I called Dr. Sheils, the pathologist. She is actually not the pathologist. It's Dr. O'Leary. He's the pathologist. Thank you.

Q You said that that meeting took place in 2002 in the United Kingdom?

A Yes, to the best of my memory, 2000 -- early 2002, could be late 2001. I'm trying to remember my transition from Oklahoma to Texas Tech. It was in that time frame.

Q Where in the United Kingdom did that meeting take place?

A It was in London.

Q Do you recall when in 2002, 2001 that would have been?

A I would have to look back at my travel. I was doing a lot of travel in transition, so I can look back and report back to you on the specific dates. I tried to jog my memory. And I've been away from Lubbock for about two-and-a-half weeks now, so I haven't had a chance to go back and look and check to see specifically. But, it was in 2001, fall 2001, spring 2002, somewhere in that time frame.

Q Did you meet with him as part of the U.K. litigation of MMR?

A Actually, I was invited over there by a colleague to just evaluate the data. And as a result of my evaluation and criticism of the data presented by Dr. Sheils, the U.K. litigators decided that they

liked a guy from Texas, who wore a Texas shirt and wore cowboy boots, and they asked me at that time would I be interested.

Q So, the litigators were at this meeting?

A The litigators did not attend the meeting. They were not at that particular meeting. But after the meeting, that's when we had conversations.

Q Did they set the meeting up?

A To my knowledge, yes, but they did not offer the invitation.

Q I see. They set the meeting up, but the invitation was offered through an intermediary?

A Right.

Q And who was that intermediary?

A John Marchulonis. He is professor in chair at Microbiology-Immunology at the University of Arizona.

Q He was working with the litigators?

A Yes. I found out later on, yes. But, then, so did I, so --

Q When you were meeting with Dr. O'Leary, did he discuss with you any criticisms of the Unigenetics Lab?

A Actually, Dr. O'Leary was not physically present at the meeting. It was Dr. Sheils, who was

presenting the data, and Dr. O'Leary was available later on for teleconference. So, she presented the data in a scientific format. And over four-and-a-half to five hours, she was asked a number of questions relative to the technology, the standard operating procedures, the immunohistochemistry that was shown, how she detected what were her primers, what were the sensitivity, how was isolation done, what were controls, what were positive controls, how did she know that this was not contamination, what was the samples. She was essentially taken apart by, I would say, three or four extremely good microbiologists.

Q Okay. Yourself included?

A No. I took her apart, not that I'm an extremely good microbiologist, just from a standpoint of, you know, I wanted to know, you know, is this real or not.

Q And Dr. Sheils, she is a colleague of Dr. O'Leary?

A Yes.

Q And she worked in the Unigenetics Lab at Coombe Hospital?

A Yes. I never understood the connection between Trinity, because she was also at Trinity -- they were both at Trinity -- and Unigenetics until

later on.

Q Okay. And what was the connection?

A My understanding was that Unigenetics was set up as a for-profit laboratory to do testing for measles virus specifically for this situation to alleviate any legalities and issues and problems that may occur with Trinity College.

Q 'This situation' being litigation, correct?

A No, just from the standpoint of universities don't like to be involved with biotech companies or whatever, other than to own a little stock on the side. So, they like to set up foundations. That's how they do it in the States. So, if they're a state institution, you would set up a foundation, so you don't break state guidelines from the standpoint of ownership and things related to that. So, I thought it was kind of a standard situation that Trinity College being an old college in the U.K., this was the way that they were going to set up. Instead of setting up a foundation to do it first, they just set up a separate laboratory.

Q So, is it your understanding, then, that Trinity College set up Unigenetics or did Dr. --

A Dr. O'Leary set it up.

Q Because it was a for-profit --

A Because it was a for-profit and that is a non-for-profit entity.

Q So, he wasn't doing it for the University?

A He was -- so, I guess the best way to say it, this is a common practice that universities and institutions keep productive investigators around. So what they do is they allow them to be entrepreneurial, to set up, start up biotech companies, to have ownership in that. The university, also, figures out to have ownership by setting up a foundation. It's not really a university-sponsored company, but the university is aware of it.

Q Did the university have any stake in Unigenetics, Trinity College?

A I'm not aware of that. I'm not aware how Unigenetics was set up.

Q Where --

A Lawyers at Chadwick and I don't know how they set up corporations. I don't know if it was a Chapter S, an LLP or --

Q So, when you were at this meeting, you were provided with protocols, test results, all the information regarding how Unigenetics was operating and what their results were that were coming out of that lab?

A Correct.

Q Did they discuss with you the equipment used in the lab?

A Yes.

Q Did they discuss with you how the lab was laid out?

A Yes.

Q Did they describe how the samples were prepared?

A Yes.

Q Did they describe the primers that they used --

A Yes.

Q -- and their specificity?

A Yes.

Q Can you think of any information regarding how they were conducting their testing that they left out of that, at that meeting, that they left out of their presentation at that meeting?

A We didn't have -- there was one individual at the meeting, who had visited the Unigenetics Laboratory, but we didn't see, for instance, a visual on the layout. We had a description of how isolation was done in this laboratory, the PCR was done in this laboratory, our reverse transcriptase was bought from

here, here's how we bought the isolation kits for the RNA, here's how we prepared the samples. So, they actually had standard operating procedures, a list for a lot of the things that they were doing, because I think the anticipation was that this was going to be a long-term testing scenario.

Q Was there any information that you felt was lacking that you would need to understand whether or not their test results were reliable?

A Actually, we, a number of us asked for a lot of things that weren't presented initially, because they're standard stuff and why aren't they in there. And they were provided to us.

Q They were provided to you?

A Yes, not necessarily during the initial presentation, but we were able to see a number of things that were of concern about primer specificity, about things like cross contamination, about things like isolation, how you dealt with the tissues, the difference, the dyes used, the machine, the reproducibility of the machine, the standard curves used. So, we got a lot of information that is not necessarily available through the Uhlmann paper.

Q Were you satisfied you had everything you needed to determine whether or not it was reliable?

A We still had scientific discussions from a standpoint of in some of the assessments relative to -- a lot of us felt very uncomfortable from a standpoint that what they were doing was essentially using cycle number to determine faults -- I mean, to determine a positive versus a negative reaction. We were happy with they were doing quadruplicate samples. So, that was not an issue. But, where we had a problem is that they were pushing the sensitivity of this to the very low end and they were using cycle number, which was not really a standard at the time, which was not used at the time, to determine a positive versus negative. And some of the samples that were on the verge of the limit of sensitivity, there was a large amount of argument over whether these were truly positive or truly negative. And a number of suggestions were made on how do to improve that from a statistical standpoint.

Q Who else was at the meeting?

A There was Dr. Richard Tedder from the U.K. He's a professor of clinical virology. There were no lecturers present. So, it was all pretty senior people. There was a program chief from the National Institutes of Health, National Institutes of Neurologic Disorders and Stroke, Dr. Steven Jacobson,

myself, Dr. Marchulonis, Dr. Sheils. There was a pediatrician molecular biologist, whose name escapes me right now, who was real sharp -- I can come up with that name later -- and one or two others. It was a fairly small, small, intense session.

Q How much do you know about PCR?

A We do it in my laboratory.

Q Do you know enough about it to answer some questions as an expert?

A Well, it depends how technical you get.

Q Because you were offering an opinion earlier about the validity of the Unigenetics Lab result via Uhlmann.

A No. About the Uhlmann --

Q The Uhlmann paper.

A No. My opinion is based on the fact that it was detected. I relied --

Q And you've had --

A -- relied my opinion on the O'Leary and Sheils Lab, based on the information that I obtained at this and, also, through PubMed searches, to see that they are being productive in 2006 and 2007 with 12 joint co-authored papers in some excellent high-impact peer review journals, such as cancer research, the Journal of Micropathology, et cetera. So, the

laboratory is doing their thing.

Q So, well, I will ask you some questions about PCR. Before you conduct an experiment doing PCR, do you develop a standard operating procedure?

A Yes.

Q Would you follow that standard operating procedure throughout the experiment?

A There is -- if we're going to do a large amount of samples, we have to set up the conditions first. When we set up the conditions, that, then, allows us to determine what our standard operating procedure will be.

Q Okay. If you're going to operate the lab as a diagnostic, essentially a diagnostic lab, would you need a standard operating procedure?

A Absolutely.

Q Would you vary from that standard operating procedure, if you were doing it diagnostically?

A No. And if you did, there would be adequate notes taken as to why it occurred, why it happened, and what was the issue.

Q Would it make a difference to you, in terms of the reliability of a result that came out using a RT-PCR, if the quality of the probe varied from batch to batch?

A Absolutely.

Q And would you think the reliability of the results were less if the probe varied from batch to batch?

A So, the question was if the probe was messed up --

Q If the probe -- the quality of the probe varied from batch to batch, would you expect the results to be less reliable?

A So, your question is, if the probe is a problem, do you expect more variability?

Q No. My question is --

A Is that the question? I'm confused, sorry.

Q It's actually a lot simpler.

A Okay.

Q Would you call into question the reliability of a PCR test result, if the probe quality varied from batch to batch?

A So, if you ran out of probe during --

Q No.

A -- a large-scale testing, then -- and you substituted new probe from another place without going back and setting up your standard conditions, would I question that? Absolutely.

Q No. If you had the same -- if you were

using a probe and from batch to batch, the quality of your probe varied --

A The same probe?

Q Yes, the probe.

A The only way it would vary is if you had to have new probe synthesized.

Q Okay.

A So, that would mean you ran out. So, you would have to reset your conditions, have to order more. So, yes, that -- you don't do that, without resetting your conditions and --

Q Would the reliability of the PCR result -- would your assessment of the PCR result be less, if the amplification efficiency varied from batch to batch?

A So, batch to batch, what are you talking about? Your Taq polymerase? Your -- what is a batch-to-batch variation?

Q If you run one batch --

A Right.

Q -- and you have a certain amplification efficiency and then you run another batch and your amplification efficiency is different, does that call into question the reliability of the results overall?

A Are you running a standard curve at the same

time and do you have proper internal controls and have you standardized that --

Q So, these are the things that you would need to know?

A Right.

Q And could you go through, please, all the things that you would want to know, in order to assess whether it was reliable?

A I would want to know how it was run. All samples should be run at the same time with a positive and a negative control. It has to have a standard curve in there, has to have the number of internal controls. I know when I publish my PCR technology in peer review publications, I don't write down all of this stuff that's necessary for --

Q So, what is it that you would need --

A -- people learned in the art normally include, because it's a given.

Q If there was amplification variability, what is it that you would want to look at?

A I would say the Taq polymerase is bad. I would want to look at a new Taq polymerase. It could be primer is bad. It could be a number of different things.

Q These are all things that would go to the

reliability of the results you would get?

A Correct.

Q Would you want standardization, that you would use the same amount, extract the same amount of RNA each time for testing?

A It depends. So, a number of us use different techniques for quantitation. Some of use a standard housekeeping gene. So --

Q I'm talking about the amount that you extract for testing.

A So, you mean, the size of the tissue or the --

Q The amount of RNA that you're testing.

A Do you want -- well, it depends what you are trying to set up.

Q If there is variability from time to time -- now, you're using the procedure diagnostically and you vary the amount of RNA that you're extracting for testing.

A So, you're varying the amount that you add to each of the well?

Q Yes.

A Is that what you're asking?

Q Yes.

A That's how you generate the standard code,

okay, and that's how you, also, can quantitate, because you have to hit the linear portion of the standard curve to be able to tell how much is there. And that's a standard procedure. So to get the actual quantitation, you need to do --

Q I understand you, sir.

A -- by different lengths.

Q If you vary from your SOP in the amount that you extract, your SOP is set up to tell you how much to extract and you vary from that, does that call into question the result that you receive?

A So, the initial amount you extracted is different or the initial amount you had is different? I'm confused.

Q Never mind. It's fine, sir. Would you want to see that the test results for negative test consistently came back negative?

A It better, yeah.

Q And if they didn't, would that call into question the result?

A I would say, absolutely, and you would need to run them again and you would need to find out why that negative result became positive. It's part of the aspect of troubleshooting, which, you know, is with the very sensitive assays, is something that's

routinely done.

Q What is the gold standard PCR testing?

A As far as quantitative PCR, you mean?

Q What would be -- in terms of the accuracy of -- let's make it specific to this case. You want to get -- you are trying to identify measles virus RNA. What would be the best possible way of doing that, from PCR standpoint?

A So, you would have quantitative PCR and you would use multiple primer pairs.

Q That, you believe, is the best way to do that?

A Yeah, for PCR.

Q You wouldn't sequence?

A Oh, you're asking me after you detect it. You're saying for a PCR, in general?

Q Yes.

A All right. Once you've got a positive, yes, you want to sequence. In fact, Dr. Sheils was asked had they sequenced and verified that this was indeed vaccine strain and they had done sequencing of H and F.

Q Specific to vaccine strain?

A Specific -- an allelic discriminator from the F. They had actually sequenced the product that

came out.

Q Specific to vaccine strain?

A Specific to vaccine strain.

Q And they didn't publish this?

A This was in -- I had assumed that the Uhlmann paper had already been submitted. So -- and the Uhlmann paper came out --

Q And the Uhlmann paper was criticized --

A -- in 2002.

Q -- and they had this data and it was never published.

A I think it was criticized after the fact, that it was accepted for publication, that it was published. So, it went through peer review. Those criticisms were not levied. The criticisms came afterwards, after the paper was published, you should have done this, you should have done this. And I think if you take a look at anyone that was doing PCR at that time, that, you know, you knew that you needed to sequence to verify it. But, did you always do it? If the paper you submitted it to and those reviewers came back and said you need to sequence it, you did one of two things. You sequenced it to get it published or you submitted it to another journal and saw what the issues were then.

Q Okay. So, you could get it published without sequencing by just going to another journal?

A That's correct.

Q And after --

A Depending on the reviewers.

Q Okay. You have to sort of shop the paper around to find somebody, who didn't require sequencing?

A Actually, it's more the luck of the draw. You get a reviewer, who is out of town, who is in a hurry and is not, you know, as stringent as he might be.

Q Somebody --

A Or you get someone, who, you know, is not familiar with certain aspects --

Q And doesn't know to ask?

A Correct.

Q And in the five years since you saw this data, none of this has been published?

A To my knowledge, no.

Q Now, you said that Dr. O'Leary has been involved in this?

A Yes.

Q Okay. Does Dr. O'Leary believe that the data generated by his lab supports a causal

association between MMR vaccine and autism?

A He never made that statement to my knowledge.

Q So, he, presumably, has this data that you have seen, this data that no one else has seen and, yet, he doesn't believe --

A Well, the point is, it wasn't in every individual.

Q Which would mean that there could be a problem with the data, correct?

A No. It means that their positives were positives and their negatives were negative and not everybody was positive and there were negatives. So, not everybody had the same --

Q So, he didn't believe --

A -- situation --

Q -- there was enough there, though, to conclude that MMR was causing autism?

A I would say that anybody that would say that based on any sort of data, that would be very problematic in most scientific opinions and assessments.

Q We're talking about Dr. O'Leary.

A Oh, Dr. O'Leary, I can't speak for him, but, no, he never made that statement and I can't --

Q And he has this data?

A He has the data.

Q And he was the one that you said, I believe in your report, is well regarded and world- renowned.

A Yes. And he is, also, a pathologist by training, so he did a lot of the immunohistochemistry to prove that there was an N protein product present in these autistic enterocolitis children.

Q All right. You were asked a question, you were giving some testimony and you were asked a question by one of the Special Masters, I believe it was Special Master Campbell-Smith, to clarify your statement on -- she asked you a question on your statement on high copy number and what the significance of that was. Could you clarify what the significance of a high copy number in the measles virus RNA, what the significance is?

A Essentially to me, it suggests that the amount that's there is high compared to what you would anticipate with a natural measles virus infection. And if it's high and if it's there, then it had to amplify in some manner. And the only way it would amplify is to have it transcribed into the positive strand and have some evidence of replication. Does that make sense?

Q No.

A Okay.

Q It doesn't.

(Laughter.)

MR. MATANOSKI: Maybe it does to the Special Master, but it doesn't to me. I'm sorry.

BY MR. MATANOSKI:

Q I'm trying to figure out how you have higher copy numbers than you have in natural infection.

A So what happens is when you usually look at copy numbers for natural infection, you've got an effective immune response that is trying to clear things, okay. And as it clears things, the amount goes down. It's a natural process. So, the immune system is supposed to get rid of this. As it gets rid of this, the number, copy number would go down. As a result of the infection and there is no more measles virus there, then the copy number should be undetectable or zero.

Q Okay.

A In this situation, to get a higher copy number, I would state that in light, there's an IgG antibody response, that that is not effective at clearing, resolving the infection. So, you're getting an amplification of the measles virus RNA to higher

copy numbers than you would anticipate just through a natural infection, because it's amplifying and replicating. So, the measles virus enzyme is making more.

Q What if it was higher than in the peak times of an infection, what -- if the copy number is here or higher than in the peak times of an infection?

A I would anticipate that you've got a lot of inflammation then going on.

Q If the copy number is here exceed what you see at the peak of a natural infection, why would that be?

A Because you're pulling the copy numbers from a biopsy material where inflammation is ongoing and that high copy number, as the result of amplification, is probably resulting in that inflammation. When you look for copy numbers in a natural measles virus infection, you are not going to a specific site where it's inflamed. The individual would actually eventually resolve it.

Q So, it's your belief that there's more measles virus there, is that what you're saying?

A There is more measles RNA there.

Q Okay. And not necessarily more virus, is that what you're saying?

A There is virus there, because there's protein there. So, the process is going on. But, I can't argue that there is more measles virus there. There could be. There might not be.

Q So, are these copy numbers, in any way, related to the amount of measles virus that are there?

A No. As I mentioned before, it's not a one-to-one situation where you get one message producing one protein producing one virus particle. It's more stoichiometric than that. And you can get, you know, RNA without getting the protein. So, the RNA can accumulate and not necessarily get protein.

Q Okay. So, there's just this RNA, but without actually virus?

A Well, we know at least in the autistic enterocolitis kids, that there is nuclear protein by immunohistochemistry. So, there's protein. Did they isolate the virus?

Q I'm just trying to figure out how we get these high copy numbers.

A So, I would simply say, I would say that it's evidence that there is amplification in the infected cells that produces more RNA.

Q Amplification of RNA or amplification of virus?

A Of the negative strands and the positive strands.

Q In your report, in this case, you mentioned that you had been a reviewer for the IOM for the Immunization Safety Review Committee --

A Yes.

Q -- on MMR vaccine and on thimerosal containing vaccines?

A Yes.

Q What were your conclusions to that? What did you tell them about the issue?

A That essentially more studies needed to be done. My conclusions were not that different from theirs. But bottom line was, if this is such an important issue, we need to be putting more money into it from the federal government standpoint to resolve this issue and it needs to be a multi-front scenario. The limitations of what the report found from a standpoint of being able to say it causes or it doesn't cause, those limitations need to be examined from an experimental standpoint and need to be supported through funding agencies, like the National Institutes of Health, because it's an important issue and it needs to be resolved, including things like developing animal models, looking at sensitivity

issues from the standpoint of are there more and better assays that can be brought to bear, which are presently being used in cancer treatment and cancer diagnosis, which are very sensitive and work very well. Can we bring those expensive assays into the arena and address this issue?

Q Did you tell them that you believed that MMR vaccine and thimerosal containing vaccines acted together caused autism?

A Not to my knowledge.

Q Did you tell them that thimerosal vaccines caused immunosuppression in a vaccine recipient?

A I was not at that level of critique. So what I was, was essentially a reporter in there and I essentially evaluated what was going on and gave my opinion of where things need to go.

Q Did you agree with their conclusions?

A Yes, except for one.

Q What conclusion was that?

A The conclusion was that they didn't specifically come up with a recommendation for the National Institutes of Health to examine this as a priority issue.

Q Okay. So, you agreed with their conclusions favoring the rejection of a link between an MMR

vaccine and autism?

A It was -- they rejected the causal, but they didn't reject the possibility, if I'm correct.

Q And you agreed -- you agreed with their conclusions, but just you thought that there should be more research done; is that right?

A Absolutely. And let's address the question.

MR. MATANOSKI: I have no further questions at this time.

SPECIAL MASTER HASTINGS: All right. Will there be any redirect?

MS. CHIN-CAPLAN: Yes, Special Master, but could we take a break?

SPECIAL MASTER HASTINGS: I was going to say if there is going to be redirect, let's take a break. Take a 15-minute break.

(Whereupon, a short recess was taken.)

SPECIAL MASTER HASTINGS: Back from our break here. And I'm going to ask Ms. Chin-Caplan, if she has a redirect? Do the Special Masters have any questions?

SPECIAL MASTER VOWELL: Yes, we do.

SPECIAL MASTER HASTINGS: Special Master Vowell has some questions.

SPECIAL MASTER VOWELL: Okay. Doctor, would

you look at your slides. And I just have a couple of questions, one at least about the slide. And that is on page three of your slide, you refer to the products of the innate immune system and you refer to something called NO, but you didn't define that for us.

THE WITNESS: Yes. That's nitric oxide.

SPECIAL MASTER HASTINGS: Okay.

THE WITNESS: It's a mediator of inflammation.

SPECIAL MASTER VOWELL: Got it. And I may be misquoting you, so, please, correct me. I thought you testified, this was that long ago, that effector T cells provide immunity. Is that what you said?

THE WITNESS: Correct.

SPECIAL MASTER VOWELL: Could you explain that to me?

THE WITNESS: Okay. An effector T cell is one that has gone from being naive, has matured, has become activated, and is now armed and ready to destroy infected cells. There's also effector T cells that differentiate into the TH-1 and TH-2 types that secrete different cytokines to help arm, in the case of TH-2, B lymphocytes, activate them to produce antibodies. And in the case of CD8 cells, turn them into the cytotoxic T lymphocyte, which is then the

effector cell of the cell mediator immune.

SPECIAL MASTER VOWELL: So, do we have any particular term that we use for an effector T cell that is involved in the process of providing immunity, as opposed to --

THE WITNESS: So, examples of effector T cells involved in immunity would be the CD4 --

SPECIAL MASTER VOWELL: CD4, CD8?

THE WITNESS: CD8.

SPECIAL MASTER VOWELL: Okay. Then looking back to your slide, and this is your cell's picture at page 15, when the genes, the N, P, M, F, H, and L are produced, do you need all of those genes before a protein is made or will the N gene encode a particular protein?

THE WITNESS: The N gene will code a particular protein?

SPECIAL MASTER VOWELL: Yes.

THE WITNESS: Yes.

SPECIAL MASTER VOWELL: Okay. So, you can get proteins produced within the cell, if you only had the N gene replicated?

THE WITNESS: Correct, but you wouldn't get F protein off the N gene.

SPECIAL MASTER VOWELL: All right. And when

you are -- what you are measuring in the PCR is the genes, themselves, not the protein?

THE WITNESS: Correct.

SPECIAL MASTER VOWELL: Okay. And you testified on cross-examination about some knowledge about the interactions, both in the United Kingdom between universities or government entities and private firms, and within the United States; is that correct?

THE WITNESS: Yes.

SPECIAL MASTER VOWELL: Does the term 'CRADA' mean anything to you?

THE WITNESS: Yes.

SPECIAL MASTER VOWELL: Okay. Do they have in the United Kingdom CRADAs?

THE WITNESS: Not per se. What they do is instead of going -- the companies going outside and supporting -- so, they bypass their government, in their situation. So, the companies will specifically support laboratories.

SPECIAL MASTER VOWELL: So, that there is no -- there is not that linkage that we have within our system?

THE WITNESS: Correct.

SPECIAL MASTER VOWELL: Now, I hate to get

back into -- and CRADA would be -- it's an acronym --

THE WITNESS: Now, that was as of 2002, so it may --

SPECIAL MASTER VOWELL: And I noticed that my colleague just asked me to spell CRADA and it's C-R-A-D-A. It's an acronym. I hate to go back there in one sense, but in your report, when you talked about the high level in, and I'm leaving the object there blank, within Michelle, can you explain to me what specifically you are referring to?

THE WITNESS: It is the copy number I'm referring, the amount of RNA that's present.

SPECIAL MASTER VOWELL: Okay.

THE WITNESS: And it's measured as an amount per volume.

SPECIAL MASTER VOWELL: An amount per volume of what?

THE WITNESS: Of RNA that they pick off the standard curve.

SPECIAL MASTER VOWELL: Okay. And does that amount -- is that amount dependent on the amount of time in a replicator?

THE WITNESS: No.

SPECIAL MASTER VOWELL: Okay.

THE WITNESS: The amount is dependent. So,

everything is set at a threshold. The amount is dependent on the standard curve.

SPECIAL MASTER VOWELL: So, you plug something into the standard -- you plug the results into the standard curve and that tells you?

THE WITNESS: You have to find a straight line portion of the standard curve.

SPECIAL MASTER VOWELL: Okay.

THE WITNESS: So, that's why you do different dilutions or different amounts. You find a point on the straight line and that, then, tells you the amount that's present.

SPECIAL MASTER VOWELL: Okay. Let's suppose, and I am going to pick on folks here, Mr. Conway has an active measles viral infection and we take some tissue from his body and we measure the amount of measles virus RNA through a PCR method. Are you saying that the level in Michelle was higher than someone with an active -- than Mr. Conway -- with an active measles virus infection?

THE WITNESS: Yes.

SPECIAL MASTER VOWELL: Okay.

THE WITNESS: With the caveat, in, Mr. Conway, you are not measure his gut.

SPECIAL MASTER VOWELL: Okay. Well, let's

assume -- well, let's not even assume that we're measuring his gut. We won't go there, Mr. Conway.

(Laughter.)

SPECIAL MASTER VOWELL: Are you volunteering for a brain biopsy? I don't think so. All right. So, would it matter, then, what tissue we were measuring? You talked about --

THE WITNESS: No, it wouldn't. That would consider a high level --

SPECIAL MASTER VOWELL: Okay.

THE WITNESS: -- and the amplified level.

SPECIAL MASTER VOWELL: And it wouldn't matter how much tissue we started with, because you're using standard dilutions?

THE WITNESS: Correct.

SPECIAL MASTER VOWELL: So, it would mean that there was a high amount -- there was a high viral load; is that correct?

THE WITNESS: Yes.

SPECIAL MASTER VOWELL: Would that be a correct term?

THE WITNESS: Yes.

SPECIAL MASTER VOWELL: Okay. I think I have it now. Thank you.

SPECIAL MASTER CAMPBELL-SMITH: Further

related to Special Master Vowell's question, you indicated several times during direct examination that you were surprised, you would not have expected to have found replicating measles virus in the gut?

THE WITNESS: Correct.

SPECIAL MASTER CAMPBELL-SMITH: Is there someplace you might have expected to find replicating measles virus?

THE WITNESS: In this particular individual or in general?

SPECIAL MASTER CAMPBELL-SMITH: I'm asking you. It was your testimony.

THE WITNESS: Okay. So, no, I wouldn't expect to find it in the gut of this individual. I would have expected that the immune response would have taken care of that and you wouldn't see it. In an individual, who has an infection, then --

SPECIAL MASTER CAMPBELL-SMITH: A natural infection?

THE WITNESS: A natural infection, you can find it at various sites. And it depends on the cycle. So, the measles virus has a specific cycle, how it infects a host. It's got a viremia stage where it's found in the blood. It's got a droplet stage on how it can be spread through coughing and upper

respiratory. You can potentially isolate it from the legions that occur, as a result of an active measles virus infection. So, depending on the site, you can get amounts. But, I wouldn't anticipate on what's been reported to see the amount that you saw in Michelle at any one of those sites.

SPECIAL MASTER CAMPBELL-SMITH: Okay. Your answer there almost anticipates my next question, where you didn't expect to see it. You talked about the blood. And I was going to ask, based on what was discovered, what is alleged to have been discovered in the gut tissue here, would you expect to find the presence of measles virus in blood cells?

THE WITNESS: Not necessarily.

SPECIAL MASTER CAMPBELL-SMITH: That depends on the stage?

THE WITNESS: It depends on the stage. And viremia could have been cleared. The virus could be gone, but it's persisting somewhere else. And because it's there, that's another one of my arguments for persistence. If you found it in the blood, then it would be still trying to find its place, going to other places.

SPECIAL MASTER CAMPBELL-SMITH: Okay. One more, just to be certain that I understand this piece.

In a normal, meaning normally operating, with whatever range that means for an immune system, you would expect a natural virus to be cleared or at least to -- to be cleared entirely or to be cleared to the extent that it could not be detected, it either didn't exist or could no longer be detected even by PCR methods?

THE WITNESS: Cleared such that it couldn't be detected.

SPECIAL MASTER CAMPBELL-SMITH: So, it's possible that you could still have some remnant?

THE WITNESS: It's possible.

SPECIAL MASTER CAMPBELL-SMITH: Okay. Even in, I'm making sure, in a normally operated --

THE WITNESS: It's possible.

SPECIAL MASTER CAMPBELL-SMITH: Okay.

THE WITNESS: But the studies have not really looked at that to say if you can't detect it, the assumption is it's clear. But, if you can't detect it is it because you're not sensitive enough or is it because it's not there.

SPECIAL MASTER CAMPBELL-SMITH: Thank you.

THE WITNESS: And just let me clarify.

SPECIAL MASTER CAMPBELL-SMITH: Yes.

THE WITNESS: For the type of viruses that measles virus is grouped in, it is virologic dogma,

that once you amount an effective immune system -- an immune response and it's no longer there, then it is cleared, gone.

SPECIAL MASTER CAMPBELL-SMITH: Thank you.

THE WITNESS: Put the new sensitive techniques in, it may throw that dogma out.

SPECIAL MASTER CAMPBELL-SMITH: Got it.

SPECIAL MASTER HASTINGS: All right. Ms. Chin-Caplan, you had some redirect for this witness. Go ahead.

MS. CHIN-CAPLAN: Thank you, Special Master.

REDIRECT EXAMINATION

BY MS. CHIN-CAPLAN:

Q Dr. Kennedy, you were asked a series of questions about the meeting that took place in the United Kingdom; is that correct?

A Yes.

Q Did you identify the people, who were present at that meeting?

A Yes, I did, to the best of my knowledge.

Q And could you just remind the Court who they were?

A So, Dr. Richard Tedder, who is a professor of clinical virology from the U.K.; myself; Dr. John Marchulonis; Dr. Orla Sheils; Dr. Steven Jacobson, who

is a branch chief at National Institutes of Neurologic Disorders and Stroke; a pediatrician, who I can't remember; and a couple of other individuals from the U.K.-Ireland, who are molecular biology expertise that I would have to dig back and remember who they were.

Q And you, also, indicated that you grilled Dr. Sheils for about four hours; is that what you stated?

A Yes, four-and-a-half, five hours.

Q Okay.

A We made her cranky.

Q When you say you grilled her, did you question her about the procedure that subsequently constituted the Uhlmann paper?

A There were several of us that went in with the opinion with the null hypothesis. We don't believe you're data, prove it to us. So, we took a very strong ground and made her show and try to convince us that this was, indeed, what was going on.

Q And when you said that you tried to make you show that this is what was going on, did you ask her questions about whether cross contamination could occur?

A Yes.

Q And what was her response to that?

A That she deals with cross contamination the way that most laboratories do. She has a standard operating procedure. If she sees cross contamination, the run is invalid, nullified. She tries to figure out where the cross contamination occurred and the laboratory is set up, in that isolation takes place in one area, the actual PCR reaction takes place in another, and the analysis takes place in a third.

Q So the possibility of cross contamination after she explained that to you, how would you characterize the possibility of cross contamination?

A We didn't feel that cross contamination was an issue relative to the data that she was showing us in the final analysis.

Q Did you ask her about this issue of controls?

A Yes.

Q And what specifically did you ask her?

A Everything.

Q When you say, 'everything,' could you state to the Court what everything is?

A Why did you not run a no-template control.

Q And what was the response?

A We ran a no-template control. When you publish your PCR data, do you put that you ran a no-

template control in your materials and method section.

Q And what other questions did you ask her?

A Asked her things like what were your controls for your primers; how did you determine whether something was positive; why did you substitute in your consent to sequence a T for a C, because that would lower the potential melting curve and cause some issues. She explained that.

Q And what was her response to that?

A That she was looking for allelic discrimination, that this was an easy way to show the difference between wild type measles virus and the vaccine strain.

Q And were you satisfied with that explanation?

A Absolutely, because she showed us sequence data on the primer products that suggested that it was directly related to the vaccine strain.

Q And you indicated earlier that, and correct me if I'm wrong, that they had found vaccine strain in the F gene; was that it?

A Yes. That's where they look for the allelic discriminator.

Q And is that specific to vaccine strain?

A The primers that they used for that

particular thing, yes.

Q And Doctor, when you addressed the issues of control, were you satisfied that every positive that was supposed to be positive was positive and every negative that was supposed to be negative was negative?

A Yes.

Q And, Doctor --

A Was I positive with her interpretation of when something was positive versus negative from a standpoint of overall? We had a lot of discussion about that.

Q And did some of that discussion involve copy numbers?

A No. When we talked about copy numbers and she had high-level copy numbers, that was not a concern. It was when she was stretching the limits of sensitivity of the assay and trying to pick up these very low copy numbers, that there was a lot of discussion what she determined was a positive result versus a negative result.

Q And what did you consider a low copy number?

A Anything that was below 1,000.

Q Doctor, I am going to show you a lab result from Petitioners' Exhibit 28, page 179. Doctor, would

you take a look at this document and tell me what this document represents.

A Okay. It's a report on measles virus detection from Unigenetics Limited. It's from Michelle Cedillo.

Q And what is the date on this?

A The date of the test is 15/03/02, so that would be March 15, 2002.

Q And does this test indicate that measles virus was detected in this ileal biopsy?

A Yes.

Q What was the copy number that was obtained?

A 1.67 times 10 to the fifth copies per nanogram of total RNA.

Q Would that be considered -- how would you characterize that copy number?

A I would consider that high.

Q And, Doctor, you had previously testified that there was no dispute among the participants present at this meeting that high copy numbers were accurate?

A We had no issues when she had high copy numbers.

Q Now, Doctor, you, also, indicated that Dr. Sheils said she found some N protein; was that it?

A Yes. The group presented data on immunohistochemistry, where they identified N protein present in the biopsies and these were from children that had autistic enterocolitis. In addition, they provided some data from some individuals of the presence of measles virus RNA in cerebral spinal fluid.

Q And, Doctor, the presence of N protein, if we go to your chart on page 15, we've now gone through N gene, P gene, F gene, F gene, and H gene; correct? And we're now down in the green part here, correct?

A Right.

Q And does that mean that we have replicating virus here --

A Yes.

Q -- if you have protein present?

A In my assessment, yes.

Q And, Doctor, you've reviewed the report of Respondent's expert, Diane Griffin, have you not?

A Yes, I have.

Q And, Doctor, do you recall whether Dr. Griffin indicated that if protein was present, then it's an indication that it's replicating and spreading to other tissues?

A Not off the top of my head.

Q Okay. I am going to ask you to look -- I will bring you over Respondent's Exhibit B, page seven, number two on that page. Would you kindly read that into the record, please?

A Okay. This is from page seven. It says that 'viral protein production is also necessary for virus to replicate and for new virions to be made. Without this, there can be no virus replication and no spread of virus within the host to other cells or tissues.

Q When you read that statement, Doctor, is Dr. Griffin indicating that if there are proteins present, the virus is replicating?

A Yes, that's how I would read that statement. And since Dr. Griffin is the expert, I'm certainly not going to question anything that she says. I believe Dr. Griffin has over one hundred publications on measles virus, so --

Q Do, Doctor, at the end of this meeting, what conclusions did the group come to?

A We were satisfied with the results of the O'Leary and Sheils laboratory. We felt that they were confident, that they were capable. They did not find in every instance where they looked, measles virus. In some instances, in some individuals, they found

measles virus. So, that made us feel a lot better, in that it wasn't found in every instance. And in ones that they would run, they would have a positive individual and a negative individual. So, that argued that there was no cross contamination. There was no issue relative to that. And we felt fairly assured that this was a very competent laboratory, that they were skilled at what they did, that they were aware of the problems with PCR, and that their conclusions were supported for the presence of measles virus in gut biopsies and in the instance where it was found in cerebral spinal fluid.

MS. CHIN-CAPLAN: Thank you, Doctor.

SPECIAL MASTER HASTINGS: Mr. Matanoski, any re-cross?

MR. MATANOSKI: Yes, sir, briefly.

SPECIAL MASTER HASTINGS: Go ahead.

RE-CROSS EXAMINATION

BY MR. MATANOSKI:

Q I'm sorry, sir, I'm just taking a look at a document and unfortunately, it's very small print. Hello, again, Dr. Kennedy.

A How are you?

Q In the exhibit that you were asked to look at, the results from Unigenetics reported on Michelle

Cedillo, was there any positive result for protein, measles virus protein?

A In that document, no.

Q Your discussion about positive results for protein is based on the representations of Dr. Sheils at a meeting?

A Yes.

Q Did you see any of the actual lab reports?

A I actually saw some lab reports and data and we questioned her with regards to the use of the specific reagents that she was performing the immunohistochemistry with. One reagent, in particular, we felt as a group was very problematic. It was a polychronal antibody to the measles virus that was used and there was a concern that that polyclonal antibody had issues related to cross reactivity and may not be detecting measles virus protein. She addressed this by providing us with information that they used four different monochronal antibodies, in addition to the polyclonal, that were specific for epitopes on the measles virus in protein. And we felt confident with the monoclonal antibody that that dealt with the issue that we, as a group, had over the concern with them using a polyclonal antibody.

Q In terms of presenting this to you, she told you about it?

A No. We actually saw data.

Q Okay. Did you go to the lab?

A No, I did not.

Q Did you look at the lab notebooks?

A Yes, I did.

Q They had all of them?

A They had, I want to say four or five boxes. I didn't look at every individual one and every individual page, but there were certain ones that I was interested ones, certain ones that other in the group were interested in. And we looked at what our interest was and then discussed that later on in comparison what did we found, were we satisfied, was there a control, when was this run, what was the primer set. We were concerned about where were their primers, when they ran out of primers, what was the source of their Taq polymerase, where did they specific -- a lot of us not being from the U.K. didn't know where they got the specific reagents to isolate the RNA, where there issues related to that, sample preparation. So, it was a pretty rigorous go at it. I had a look at specific aspects that made me feel comfortable, that in the instances that I was looking

for, that the notebooks were a true representation of the data that she presented to us in general.

Q Okay. So, from your point of view, you had a good idea of what was going on in the lab?

A Yes.

Q And this data, though, this meeting wasn't to publish a scientific paper or anything like that; correct?

A You know, it was set up, in my mind, the way the presentation was, in that it was -- you know, eventually that this information was going to be published.

Q And it never was?

A I have never seen it in print.

Q That's five years ago that this took place?

A That's five years ago.

Q And this meeting was set up by lawyers, correct?

A Yeah, it was set up by the U.K. -- I forget what the firm was called -- what are they called -- they were representing --

Q The claimants?

A The claimants, thank you.

Q The claimants, who were alleging that MMR vaccine was causing autism?

A Correct.

Q And does Unigenetics exist anymore?

A No, it does not.

Q And do you know what happened to the litigation in the United Kingdom?

A To the best of my knowledge, what I heard was that the judge dismissed the claimants and that we were essentially no longer needed, so do not worry about it, and that anything that related to Unigenetics, I was not aware of until later on. I assumed that they no longer operate, because they no longer had a source of support for this activity.

Q Because the litigation was gone?

A The litigation was gone.

MR. MATANOSKI: Thank you.

SPECIAL MASTER HASTINGS: Anything further from this witness, Ms. Chin-Caplan?

MS. CHIN-CAPLAN: No, Special Master.

SPECIAL MASTER HASTINGS: Anything further?

(No response.)

SPECIAL MASTER HASTINGS: All right. Dr. Kennedy, we thank you, very much.

THE WITNESS: Thank you.

(Witness excused.)

SPECIAL MASTER HASTINGS: You are done for

this afternoon. Should we -- do we have anything else to talk about this afternoon? Is the plan to start with Dr. Byers in the morning?

MS. CHIN-CAPLAN: That's correct.

SPECIAL MASTER HASTINGS: Anything we should talk about before we adjourn for the day?

MR. MATANOSKI: No, sir.

SPECIAL MASTER HASTINGS: All right. Well, we are adjourned for the day, then. We will see you tomorrow morning at 9:00 for Dr. Byers' testimony.

(Whereupon, at 4:55 p.m., the hearing in the above-entitled matter was recessed, to reconvene on Thursday, June 14, 2007, at 9:00 a.m.)

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REPORTER'S CERTIFICATE

DOCKET NO.: 98-916V
CASE TITLE: Cedillo v. Sec., HHS
HEARING DATE: June 13, 2007
LOCATION: Washington, D.C.

I hereby certify that the proceedings and evidence are contained fully and accurately on the tapes and notes reported by me at the hearing in the above case before the United States Court of Federal Claims.

Date: June 13, 2007

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