

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.)

Pages: 576 through 858

Place: Washington, D.C.

Date: June 13, 2007

HERITAGE REPORTING CORPORATION

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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,)
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 Petitioners,)
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 v.)
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 SECRETARY OF HEALTH AND)
 HUMAN SERVICES,)
)
 Respondent.)

Docket No.: 98-916V

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

<u>WITNESSES:</u>	<u>DIRECT</u>	<u>CROSS</u>	<u>REDIRECT</u>	<u>RECROSS</u>	<u>VOIR DIRE</u>
<u>For the Petitioners:</u>					
Karen Hepner	582	636	--	--	--
	--	680	--	--	--
Ronald C. Kennedy	683	747	844	852	--

E X H I B I T S

<u>PETITIONERS '</u> <u>EXHIBITS:</u>	<u>IDENTIFIED</u>	<u>RECEIVED</u>	<u>DESCRIPTION</u>
7	585	585	Hepner presentation
8 presentation	690	690	Kennedy

E X H I B I T S

<u>RESPONDENT'S</u> <u>EXHIBITS:</u>	<u>IDENTIFIED</u>	<u>RECEIVED</u>	<u>DESCRIPTION</u>
3	805	805	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

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(9:02 a.m.)

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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.

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

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

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

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

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

12 Whereupon,

13 KAREN HEPNER

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

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

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

23 DIRECT EXAMINATION

24 BY MS. CHIN-CAPLAN:

25 Q Could you kindly state your name for the

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1 record, please?

2 A Karen Hepner.

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

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

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

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

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

1 Q Doctor, the techniques that were described
2 in the Uhlmann paper, are you familiar with them?

3 A Yes, I am.

4 Q Have you worked with those techniques?

5 A Yes, I have.

6 Q For how long a period of time did you work
7 with those techniques?

8 A Most of those PCR-based techniques I've
9 worked with for over 10 years. Some of them are
10 developed more recently, and I've worked on them more
11 recently.

12 Q Doctor, you're here to discuss the Uhlmann
13 paper with the Court, are you not?

14 A Correct.

15 Q And the Uhlmann paper utilized different
16 types of experimental techniques, did they not?

17 A Yes, they did.

18 Q Before we go into the Uhlmann paper, I'd
19 like you to run through the different types of
20 techniques that the Uhlmann paper cited and explain to
21 the Court how they are run.

22 A Okay. Do I have a laser pointer, or should
23 I get it?

24 SPECIAL MASTER HASTINGS: All right. Ms.
25 Chin-Caplan, let's mark this as Petitioner' Trial

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

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

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

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

16 MS. CHIN-CAPLAN: Certainly, Special Master.

17 SPECIAL MASTER HASTINGS: Please go ahead.

18 MS. CHIN-CAPLAN: Thank you.

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

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

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

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

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

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

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

1 also very critical information we'll discuss.

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

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

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

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

22 What scientists have been able to do is to
23 use this information as kind of a blueprint, as kind
24 of a fingerprint, like a detective uses a fingerprint,
25 so if someone wanted to determine the presence or

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

7 BY MS. CHIN-CAPLAN:

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

11 A Yes, it is.

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

14 A They're categorized by their host or by the
15 organism where they're found.

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

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

25 Q Okay.

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

5 Q And this is computerized?

6 A Yes, it is.

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

9 A Everywhere all over the world all the time.

10 Q And it's standard practice at this time?

11 A Yes, it is.

12 Q Okay.

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

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

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

25 A Yes. It's like a xerox machine. You're

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

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

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

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

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

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

1 to create the complementary strands.

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

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

11 Go ahead, Ms. Chin-Caplan.

12 BY MS. CHIN-CAPLAN:

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

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

17 Q There's no dispute about how this happens?

18 A No.

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

22 A Yes, they are.

23 Q So the body automatically manufactures it?

24 A Those are proteins that exist in our body.

25 Q And the body knows when it's supposed to

1 replicate?

2 A Correct.

3 Q All perfectly natural?

4 A Yes.

5 Q No dispute about the fact that it occurs?

6 A No.

7 Q Thank you.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

14 A Uh-huh.

15 Q And you look at the letters associated with
16 it?

17 A Right.

18 Q And you match up the letters?

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

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

1 develop the primers?

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

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

11 Q So this is commercially available?

12 A Commercially available.

13 Q Purchased from someplace else?

14 A Correct.

15 Q That's utilized by any laboratory who wishes
16 to seek this technical information?

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

19 Q Thank you.

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

25 That means that these primers need to be

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

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

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

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

16 A Correct.

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

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

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

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

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

6 Q So the computer does all this calculation
7 for you?

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

10 Q Okay.

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

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

23 This is theoretical because it doesn't
24 always work in that first cycle, but at least in
25 theory I want you to understand the concept, which is

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

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

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

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

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

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

1 cannot detect very, very small amounts of DNA.

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

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

9 A Absolutely.

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

12 A Well, this is the conventional PCR, the
13 solution-based PCR. This has been expanded, but this
14 is the classic solution-based PCR that people use.
15 We'll discuss some other techniques that are based on
16 this technique.

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

19 A Correct.

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

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

1 clear liquid. Now we need to see it somehow, so what
2 we do is there's this thing that we call an agarose
3 gel, and this feels kind of like very, very hard
4 jello.

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

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

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

22 Now, we still can't visualize it like that.
23 We apply a stain called ethidium bromide which
24 intercalates or basically inserts itself in between
25 the bases and under UV light it fluoresces, so what

1 we're looking at here are pieces of DNA that have been
2 amplified by PCR.

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

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

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

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

24 A Yes.

25 Q Is it accepted the way you described it as

1 standard practice within the field of molecular
2 biology?

3 A Yes.

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

6 A No.

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

9 A This is how you would look at DNA in PCR
10 products.

11 Q Thank you.

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

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

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

1 manipulate.

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

8 The next slide, please? Slide 8? Now, this
9 RNA undergoes a process called reverse transcription,
10 and what we're attempting to do here is to take an RNA
11 molecule and make it look like a DNA molecule.

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

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

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

22 A Yes.

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

25 A RNA is a labile molecule and therefore

1 unstable.

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

5 A Yes. One must do this process in order to
6 study RNA molecules.

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

10 A Correct.

11 Q Thank you.

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

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

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

25 It copies it along, along here, so we can

1 see that down here. It's copying along. Now we have
2 this complement to this RNA. It now copies the top
3 strand and displaces the original DNA.

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

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

11 A No.

12 Slide 9, please? We went back. Thank you.

13 Okay. So that was our little detour, okay, because I
14 needed to let you know that in some cases your
15 starting material is actually not DNA. It's RNA.

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

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

1 Now, what we're going to do here is the goal
2 of the Southern blot is to give us further indication
3 that what we amplified is what we think we've
4 amplified. The first thing we did was we ran it
5 through a gel, and we used our ruler and said I knew
6 it was going to be 150 base pairs. It is 150 base
7 pairs.

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

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

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

1 of filter.

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

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

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

25 If you design the probe to be complementary

1 to the strand and you know what your target gene is,
2 you will now know if this binds that you have
3 amplified what you think that you have amplified, what
4 you plan to amplify.

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

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

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

12 A Yes.

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

15 A Yes, it is.

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

18 A Yes.

19 Q Thank you.

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

1 Now, you should be familiar already with the
2 concept of a primer, so you have a primer going in
3 this direction, a primer going in this direction.
4 These are designed specifically for your target genes,
5 and they will amplify from the ends of that target
6 towards the middle, making another copy of that
7 target.

8 What TaqMan PCR does, it does two more
9 layers that are important. In TaqMan PCR you design a
10 probe which is complementary to one of the strands.
11 Now you have a primer complementary to the strand, a
12 primer complementary to the strand, and now we have a
13 probe that's complementary to the strand.

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

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

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

1 specific way of finding your gene?

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

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

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

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

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

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

1 A Yes.

2 Q And is it an accepted technique nationally?

3 A Yes.

4 Q Internationally?

5 A Yes.

6 Q Thank you.

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

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

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

22 The more you start with, the more starting
23 material you have, the faster this reaction will enter
24 that logarithmic phase, so if we go back to our penny
25 example, if I give you a penny and then I give you a

1 penny for every penny, it will take you a long time to
2 get to \$1 million.

3 If I give you a dime and then give you a
4 dime for every dime, it will take you a shorter amount
5 of time to get to \$1 million. If I give you \$1 and
6 give you \$1 for every \$1, it will take you less time,
7 so the faster that it gets to logarithmic phase, the
8 faster that it gets to exponential phase, the more
9 starting material you must have had.

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

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

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

22 Q The cycle number is just standard? Is that
23 it?

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

1 Q So you now know that if you have five copies
2 let's say, you will enter logarithmic phase at Cycle
3 30. If you have 10 copies, you will enter it earlier.
4 If you have one copy, you will enter it later.

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

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

17 Next slide, please?

18 SPECIAL MASTER HASTINGS: Now we're on Slide
19 12.

20 THE WITNESS: Correct. Yes.

21 SPECIAL MASTER HASTINGS: Go ahead.

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

25 BY MS. CHIN-CAPLAN:

1 Q Dr. Hepner, in-situ PCR. Is that an
2 accepted technique within the field of molecular
3 biology?

4 A Yes, it is.

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

7 A Yes.

8 Q And internationally?

9 A Yes.

10 Q Thank you.

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

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

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

25 Q Immunohistochemistry is an accepted

1 technique within the field of molecular biology?

2 A Yes.

3 Q And is it practiced on a national level?

4 A Yes.

5 Q And is it practiced on an international
6 level?

7 A It is.

8 Q And when somebody says that this was
9 obtained by immunohistochemistry, is there any
10 question about the technique that's utilized?

11 A Not in general. In detail perhaps.

12 Next slide? Last slide, please? This is
13 Slide 13. Just to show you how it is that all of this
14 got started, you probably heard about the ileum
15 yesterday and the small intestine.

16 Tissue is extracted from the ileum, and it
17 can either be put on a slide to be subjected to things
18 like the immunohistochemistry and in-situ PCR, or it
19 can be stored in a tube that preserves the RNA for
20 later extraction.

21 The RNA is purified and tested for RNA
22 integrity. Because it's a highly labile molecule, you
23 need to test the integrity of the RNA, and then it can
24 be subjected to the experiments that we described.

25 That would be it.

1 Q Doctor, this is a description of the
2 techniques, the experimental techniques that are
3 detailed in the Uhlmann paper? Is that correct?

4 A Correct.

5 Q Doctor, when you reviewed the Uhlmann paper
6 how did you know that the results of that paper were
7 valid?

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

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

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

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

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

25 A Yes.

1 Q What was the next technique that they
2 utilized?

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

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

7 A Yes, it is.

8 Q Did they utilize any other experimental
9 techniques?

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

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

15 A Yes, it is. Yes.

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

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

1 about controls.

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

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

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

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

1 reaction or your experiment is specific for measles
2 virus RNA. If it's negative, then there is a flaw in
3 your experimental design. You cannot get any
4 information from an experiment where the positive
5 control is negative.

6 The next thing you have to do is look at the
7 negative control. The negative control is a sample
8 where it is necessarily -- where measles virus RNA or
9 cDNA is not present. If the negative control is
10 positive, there is either a flaw in your experimental
11 design -- one example would be where your primers are
12 not specific to your target -- or you have some level
13 cross-contamination where measles virus RNA or cDNA
14 contaminated your samples.

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

19 A Correct.

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

22 A So the second level are the experimental
23 controls. So you have your experimental group. Your
24 experimental group in this case was ASD patients with
25 GI bowel -- with this idiopathic bowel disease, and

1 it's the GI biopsies from this cohort of patients.
2 That was your experimental group because that's the
3 group about which you are asking your question.

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

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

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

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

1 relative to its controls.

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

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

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

8 A That is standard laboratory practice.

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

13 A Yes.

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

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

19 Q Okay.

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

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

1 A Let's start with the solution phase PCR,
2 which is in Figure 2, for those who have it.

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

7 MS. CHIN-CAPLAN: That's correct.

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

12 THE WITNESS: Okay, so if we look at Figure
13 2 --

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

16 THE WITNESS: Do you have the --

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

20 THE WITNESS: Figure 2 is the figure for the
21 solution phase PCR reaction. And what they show here
22 is that they had a positive control that acted true to
23 designation, which means the positive control RNA
24 consistently performed -- was positive. They also had
25 a no-template control, which is a control that does

1 not contain any nucleic acid, and therefore, is
2 necessarily negative for measles virus RNA. And that
3 acted true to designation.

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

10 BY MS. CHIN-CAPLAN:

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

13 A It appears to have worked.

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

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

25 Now, in panels B and C, what they did was --

1 oh, we should go back up to panel A. The left side,
2 the first seven lanes of this panel are using gene
3 primers that are specific for a particular gene called
4 the F gene in the measles virus. The right half of
5 this panel, 8 through 14, used primers that are
6 specific for the F gene of measles virus, as this
7 actually adds another layer of confidence because we
8 are actually seeing two genes amplified, not just one.

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

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

18 A Oh, my handout, yes.

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

21 THE WITNESS: Okay.

22 BY MS. CHIN-CAPLAN:

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

25 A Right.

1 Q Is that true?

2 A Yes.

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

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

13 Q And they are both present on the schematic
14 here?

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

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

20 A Yes.

21 Q Uhlmann ran one last experiment?

22 A Well, they did a TaqMan PCR, which is the
23 summary of the TaqMan PCR as found in Table 2. And
24 they designed multiple primers and multiple probes to
25 determine the presence of measles virus RNA and to get

1 some information about the amount. This could be --
2 because remember, this is a quantitative experiment as
3 well. So they get some information about the quantity
4 of the starting material. Yes?

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

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

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

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

1 A The TaqMan and the solution phase PCR?

2 Q Yes.

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

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

8 A Yes.

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

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

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

23 A You have to run the controls every single
24 time and if your negative control is consistently
25 negative, and it is negative every time, then you

1 simply can -- it is illogical to assume that you would
2 have contamination in all your samples but your
3 negative control.

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

6 A Yes.

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

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

12 Q And are you referring to specific studies?

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

16 (Pause.)

17 THE WITNESS: What do you want?

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

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

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

25 SPECIAL MASTER HASTINGS: F as in Frank?

1 MS. CHIN-CAPLAN: L as in Larry.

2 SPECIAL MASTER HASTINGS: Okay, thank you.

3 BY MS. CHIN-CAPLAN:

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

6 A Sure. Again, when you attempt to reproduce
7 a study, you need to reproduce all the variables of
8 that study. Now, the study in question, the study
9 that is being reproduced, if the study is attempting
10 to show that measles virus is present in the bowel
11 biopsies of this specific cohort of patients, all
12 reproductions of the study must use the same starting
13 material and must use all biopsies from this cohort of
14 patients.

15 In Afzal et al., they attempted to reproduce
16 these results, however, they neglected to use the same
17 starting material. Instead of using GI biopsies from
18 this cohort of patients, they used peripheral blood
19 mononuclear cells from ASD patients. It actually was
20 not even restricted to ASD patients with GI disease,
21 so it was just ASD patients looking at the peripheral
22 blood.

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

1 A Yes, so, what I believe that they managed to
2 do was --

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

5 A Correct.

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

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

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

12 A Yes.

13 Q Go on Doctor, I'm sorry.

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

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

1 Q Now, Doctor, you also mentioned the D'Souza
2 paper?

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

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

10 A Correct. May I add something?

11 Q Sure.

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

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

22 A Correct.

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

1 A That's right.

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

5 A Can you ask that question differently?

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

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

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

20 Q So Doctor, just to be clear on that, this
21 one laboratory took this big batch of solution, is
22 that it, and they divided it up into seven parts, and
23 they sent it out to seven different laboratories to
24 test, and when those seven different laboratories
25 tested it, the difference between the seven

1 laboratories is a thousand times different?

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

14 In one lab, that's X. In another lab, I can't
15 detect X. In fact, I can't even detect it until it's
16 1000 times X. And that would be the difference in
17 those detection thresholds between the labs. And that
18 reflects, most likely, it could reflect differences in
19 operators, the people who are conducting the study,
20 and it also reflects a difference in reagents and
21 technical design.

22 SPECIAL MASTER HASTINGS: And just for the
23 record, looking at your paper, Dr. Hepner, I think
24 that study you were just talking about was the Afzal
25 2003 study?

1 THE WITNESS: Yes, my apologies. That was a
2 different study. This is 2003.

3 SPECIAL MASTER HASTINGS: Okay. Thank you.
4 Go ahead.

5 BY MS. CHIN-CAPLAN:

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

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

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

18 A Correct.

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

21 A Sure. This was an IMFAR abstract.

22 Q Yes.

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

1 design, to detect MV RNA in the bowel biopsies of ASD
2 patients. And what we did was we generated multiple
3 primer sets and tested them for their specificity and
4 their sensitivity.

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

13 Q And what were the preliminary results of
14 your study?

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

23 MS. CHIN-CAPLAN: Thank you.

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

1 MS. CHIN-CAPLAN: Yes, thank you.

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

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

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

9 MR. MATANOSKI: Five minutes.

10 SPECIAL MASTER HASTINGS: All right. Go
11 ahead.

12 (Whereupon, a short recess was taken.)

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

18 MR. MATANOSKI: Thank you, sir.

19 CROSS EXAMINATION

20 BY MR. MATANOSKI:

21 Q Dr. Hepner, good morning.

22 A Good morning.

23 Q How long ago did you receive your PhD?

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

1 Q And you are the first author on one
2 published article and a coauthor on three?

3 A Correct.

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

5 A Correct.

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

8 A They do not.

9 Q Those articles generally concern cancer,
10 correct?

11 A Correct.

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

14 A They do not.

15 Q What types of PCR have you worked with?

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

18 Q About how many times have you personally
19 used PCR?

20 A It's hard to really answer that question.

21 I've done it so many times, it's hard to say.

22 Hundreds.

23 Q Hundreds?

24 A Yes, well, my most recent study, which is in
25 preparation, I generated over 20 mutant clones that

1 required an inordinate number of PCR reactions.

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

4 A I design primers for all of my PCR
5 reactions.

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

7 A Oh, for viruses?

8 Q Yes.

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

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

13 A I designed some of them.

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

16 A No, it did not.

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

20 A I did not.

21 Q Do you believe that is proven?

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

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

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

5 Q It's a hypothesis?

6 A A plausible hypothesis.

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

9 A I believe that they are reliable.

10 Q So --

11 A And not subjective.

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

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

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

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

25 Q You are basing that on published work?

1 A The published Uhlmann study?

2 Q Yes.

3 A Yes.

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

10 A Correct.

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

15 A That's right.

16 Q And why would that not be appropriate?

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

24 Q Is there a reason to believe from a
25 biological standpoint that if the measles virus was

1 present in the gut tissue that it also would not be
2 present in the blood?

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

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

12 A That is what the data is showing currently.

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

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

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

1 A They reported a range.

2 Q And weren't some of those very high?

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

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

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

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

19 A It's possible.

20 Q Isn't that likely?

21 A It's not what the data is showing.

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

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

1 Q And it would not be detectable because it
2 was a low copy number?

3 A Correct.

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

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

14 Q They didn't use the proper reagents?

15 A The starting material, the RNA source.

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

19 A That's right.

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

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

1 the Uhlmann study also used. Besides that, there is
2 nothing specifically that I can say is flawed.

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

5 A D'Souza.

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

8 A They addressed that question.

9 Q Again, they used peripheral blood --

10 A That's right.

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

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

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

25 A That was not their conclusion because --

1 actually, it would be helpful if I could refer to that

2 --

3 Q Sure.

4 A Can you just point me in the right
5 direction?

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

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

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

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

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

23 A That's right. In peripheral blood.

24 Q You've criticized peripheral blood. Didn't
25 -- in the Uhlmann paper, take a look at it. Didn't

1 they use peripheral blood as one of their controls?

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

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

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

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

16 A Right, and this was normal.

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

20 A Yes.

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

25 A If there was contamination in the lab, yes,

1 it could be.

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

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

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

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

13 Q So that was incorrect in your report?

14 A That was incorrect.

15 Q You have read the paper though?

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

18 Q They are not admissible here?

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

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

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

6 Q In the Uhlmann paper?

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

9 BY MR. MATANOSKI:

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

13 A I mean that the experimental samples were
14 positive at a frequency greater than controlled.

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

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

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

25 A I would say in an individual sample it would

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

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

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

6 Q -- analyze by both methods?

7 A Actually in the TaqMan PCR, according to the
8 Table 2 summary of the TaqMan and in-situ PCR results,
9 70 out of 91 were positive for TaqMan, and 42 out of
10 57 were positive for in-situ PCR.

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

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

17 Q The information presented in the paper?

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

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

1 THE WITNESS: May I add something?

2 MR. MATANOSKI: Yes.

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

8 BY MR. MATANOSKI:

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

11 A Yes. Yes.

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

13 A You won't always get the same results.

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

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

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

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

25 A This was actually referred to. Yes. This

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

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

6 Q So they weren't actually --

7 A No. In fact --

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

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

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

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

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

21 SPECIAL MASTER HASTINGS: Okay.

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

25 The immunohistochemistry in this paper was

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

4 BY MR. MATANOSKI:

5 Q So that was an error?

6 A That was an error.

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

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

16 Q Uhlmann doesn't do that?

17 A Uhlmann does not.

18 Q So it's not supported there in the Uhlmann
19 paper?

20 A That particular level of support is not
21 here.

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

25 Wouldn't that variability of detection limit

1 make reproducibility of results that much more
2 important?

3 A It certainly does.

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

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

12 A No, they did not.

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

15 A Yes, I believe it is.

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

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

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

24 A It's a criticism, yes.

25 Q -- paper lacks essential data for evaluating

1 it?

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

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

7 A Correct.

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

11 A Yes.

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

14 A Yes.

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

17 A Yes, it would.

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

20 A Uh-huh.

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

24 A Sure.

25 Q I have a number of questions to ask you

1 about that work.

2 A Sure.

3 Q Do you have the poster available?

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

6 Q It's probably Exhibit 3. It came in
7 yesterday.

8 A I have it now.

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

12 A Sure.

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

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

20 Q How was it stored?

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

23 Q Dr. Walker did the RNA extraction?

24 A Yes, he did.

25 Q How did he do that? What method?

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

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

7 A I'm sorry?

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

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

12 Q Actually from start to finish.

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

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

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

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

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

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

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

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

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

14 A That's standard practice, yes.

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

18 A Yes.

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

21 A In the Walker study?

22 Q In the Walker study, yes.

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

1 samples.

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

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

9 A There was some slight degradation.

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

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

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

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

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

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

25 A Irrelevant tissue, which would mean tissue,

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

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

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

7 Q Do you have the data?

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

9 Q But there is data?

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

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

19 A It is very preliminary.

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

22 A Yes.

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

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

6 Q Okay. How much RNA was used?

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

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

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

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

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

21 Q How many cycles of PCRs do you conduct?

22 A Thirty-five to 40.

23 Q And how many cycles of nested PCR?

24 A Thirty-five to 40.

25 Q It's all nested?

1 SPECIAL MASTER HASTINGS: It's all what?

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

3 SPECIAL MASTER HASTINGS: Nested?

4 THE WITNESS: Nested.

5 MR. MATANOSKI: Nested.

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

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

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

13 BY MR. MATANOSKI:

14 Q Could you describe what your technical
15 controls were?

16 A For the nested PCR? For this PCR reaction?

17 Q For the PCR reaction.

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

23 Q Did you include negative environmental
24 controls?

25 A What do you mean by environmental controls?

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

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

5 Q What were your positive controls?

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

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

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

23 A We don't have that at present.

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

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

3 Q The biopsies.

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

7 Q When did you run the positive control?

8 A When?

9 Q How long ago did you run the positive
10 control?

11 A Positive controls are run with each
12 experimental run.

13 Q And where do you run them?

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

16 Q Do you do it in the same lab?

17 A Everything is run in the same lab.

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

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

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

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

1 Q Yes. I'm sorry.

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

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

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

14 A Twelve positive samples or 12 --

15 Q You talked about 12 in the poster.

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

19 Q Can you turn to the chart that you have?

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

22 THE WITNESS: Yes.

23 MR. MATANOSKI: We're still on the poster.

24 Yes, sir.

25 SPECIAL MASTER HASTINGS: Would you bear

1 with me about 20 seconds while I get my copy?

2 Go ahead.

3 MR. MATANOSKI: Thank you.

4 BY MR. MATANOSKI:

5 Q Why did you use five different strategies?

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

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

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

23 Q You said F1-R and F2-R were the Uhlmann
24 primers?

25 A Yes. Anything with an F1 or F2 designation

1 were Uhlmann, so F1-R is actually the F1 reverse
2 primer. F2-F is the forward primer.

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

7 Q He redesigned some of the Uhlmann primers?

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

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

13 A That's right.

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

16 A No. They were different.

17 Q They were different?

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

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

1 A He had not, as far as I know.

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

4 A He did use some plasma DNA.

5 Q He'd need that for the controls?

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

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

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

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

23 Q What were you using as your positive
24 control?

25 A It was a wild type plasmid.

1 Q I'm sorry?

2 A A wild type plasmid.

3 Q Okay.

4 A A wild type piece of DNA.

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

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

10 Q Okay. So it's an artificial construct?

11 A Yes.

12 Q That's your control?

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

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

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

23 A Yes.

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

1 conducted to determine whether those results are
2 reliable, correct?

3 A Yes.

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

6 A Changes in what?

7 Q Study design.

8 A Changes in the design of the experiment?

9 Q Yes.

10 A Yes.

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

13 A By calibration you mean what?

14 Q Is the PCR actually working properly.

15 A The PCR machine?

16 Q Yes.

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

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

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

25 However, if you treat the sample, the

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

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

6 A Yes. In some of the experiments, yes.

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

10 A Yes.

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

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

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

18 A Certainly.

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

22 A Yes.

23 Q And whether your negative controls
24 consistently came up negative?

25 A Yes.

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

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

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

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

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

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

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

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

11 Q The same?

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

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

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

25 Q It might make you want to do it again?

1 A It would make you want to do it again.

2 Q Or maybe check how you were doing your
3 experiment?

4 A Yes.

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

7 A We want to optimize conditions so that
8 happens less.

9 Q What's the gold standard for PCR
10 specificity?

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

14 Q And Uhlmann didn't do sequencing, did they?

15 A No, they did not.

16 MR. MATANOSKI: I have no further questions.

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

19 Go ahead.

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

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

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

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

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

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

9 THE WITNESS: Okay.

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

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

18 SPECIAL MASTER CAMPBELL-SMITH: Are only
19 wild virus?

20 THE WITNESS: Uh-huh.

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

25 THE WITNESS: Okay. Let me go back.

1 SPECIAL MASTER CAMPBELL-SMITH: Okay.

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

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

9 SPECIAL MASTER CAMPBELL-SMITH: Okay.

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

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

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

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

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

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

7 SPECIAL MASTER CAMPBELL-SMITH: Okay.

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

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

15 THE WITNESS: Yes.

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

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

25 SPECIAL MASTER CAMPBELL-SMITH: Okay. Thank

1 you.

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

7 THE WITNESS: Uh-huh.

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

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

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

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

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

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

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

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

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

16 SPECIAL MASTER CAMPBELL-SMITH: Thank you
17 very much.

18 THE WITNESS: Okay.

19 SPECIAL MASTER HASTINGS: Any questions?

20 (No response.)

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

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

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

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

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

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

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

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

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

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

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

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

5 THE WITNESS: Yes.

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

8 THE WITNESS: That's right.

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

11 Any redirect for this witness?

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

14 SPECIAL MASTER HASTINGS: All right.

15 MR. MATANOSKI: Excuse me, Your Honor.

16 Based on the questions, I have just three brief
17 questions on the Walker study.

18 SPECIAL MASTER HASTINGS: Go ahead.

19 FURTHER CROSS-EXAMINATION

20 BY MR. MATANOSKI:

21 Q On the Walker study --

22 A Yes. I'm opening it up again.

23 Q Actually, I think it's pretty easy.

24 A Okay.

25 Q You may have answered it. I just want to

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

3 A Yes.

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

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

8 Q For the experimental samples themselves.

9 A Uh-huh.

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

12 A In this study that was done here?

13 Q Yes.

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

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

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

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

3 A Absolutely.

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

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

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

16 MR. MATANOSKI: Thank you.

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

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

20 SPECIAL MASTER HASTINGS: All right.

21 (Witness excused.)

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

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

1 SPECIAL MASTER HASTINGS: Okay.

2 MS. CHIN-CAPLAN: A five minute break.

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

6 (Whereupon, a short recess was taken.)

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

10 We have Dr. Kennedy in the witness chair.

11 Ms. Chin-Caplan, when you're ready go ahead.

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

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

16 Whereupon,

17 RONALD C. KENNEDY

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

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

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

23 DIRECT EXAMINATION

24 BY MS. CHIN-CAPLAN:

25 Q Dr. Kennedy, could you kindly give the Court

1 a brief description of your educational background
2 from college?

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

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

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

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

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

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

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

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

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

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

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

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

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

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

23 A Yes.

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

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

5 Q Doctor, have you authored any peer reviewed
6 articles?

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

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

11 A Yes, they do.

12 Q Doctor, have you done vaccine work at all?

13 A Yes.

14 Q Could you describe for the Court your
15 vaccine work?

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

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

1 developing vaccines.

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

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

11 Q And are you currently researching actively
12 in HIV still?

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

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

21 Q Doctor, do you hold any patents?

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

1 Q Have you done any work with MMR or measles?

2 A I've done some work with MMR and measles.

3 Q And have you authored any articles on
4 measles?

5 A Yes, I have.

6 Q Your current work with the primates. Do
7 they involve any sort of measles-related activity?

8 A Yes, they do. We're trying to develop
9 what's called a specific pathogen free colony of
10 baboons.

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

19 Q Now, Doctor, we're going to turn to your
20 slides here. Could you kindly describe to the Court
21 what the immune system is?

22 A Okay. The immune system is essentially a
23 two component system. The first component is called
24 the innate immune system. The second component is the
25 adaptive immune system. They both function together,

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

24 SPECIAL MASTER HASTINGS: Go ahead.

25 BY MS. CHIN-CAPLAN:

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

4 A Correct.

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

8 A Correct.

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

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

14 SPECIAL MASTER HASTINGS: Can I interrupt?

15 THE WITNESS: Yes.

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

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

20 SPECIAL MASTER HASTINGS: Okay. Yes.

21 Certainly.

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

25 SPECIAL MASTER HASTINGS: Yes, that's what

1 we have as No. 4.

2 (Pause.)

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

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

7 THE WITNESS: The other two slides weren't
8 identical, but they were close enough. I believe No.
9 2 was identical. The third one, the characteristics
10 are very similar to what I had. I just put in a more
11 specific situation where the products I specifically
12 give products like tumor necrosis factor alpha,
13 interleukin-1 B, IL6, as being part of the innate
14 immune system.

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

18 BY MS. CHIN-CAPLAN:

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

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

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

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

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

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

1 resolve the infection. It's the affective *T* cell in
2 this case that is responsible for providing immunity.

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

5 A Yes.

6 Q So to summarize on page 5 innate immunity?

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

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

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

1 which is interleukin-1 *B*.

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

5 A Correct.

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

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

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

25 And this differs from the next type I'll

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

3 Q So the *B* lymphocytes, Doctor, are they the
4 immunoglobulins?

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

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

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

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

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

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

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

1 functioning normally.

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

5 A Absolutely. Within the IgGs there are
6 actually four subclasses in humans. There's IgG1, 2,
7 3 and 4. Each one has specific components. There are
8 a number of examples in the literature where
9 individuals are deficient in one particular subclass,
10 and they are more prone to infection because they are
11 lacking that subclass.

12 Q Now, Doctor, the next slide, which is page
13 7, is this a depiction of what happens with the
14 adaptive immune system and *T* lymphocytes?

15 A Yes.

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

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

25 Essentially what happens is that exposure to

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

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

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

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

24 A Absolutely.

25 Q Now, Doctor, do these systems talk to one

1 another?

2 A They absolutely do.

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

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

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

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

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

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

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

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

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

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

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

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

1 about leprosy.

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

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

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

17 A Absolutely.

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

20 A It could, yes.

21 Q Now, Doctor, are there known
22 immunodysfunctional conditions?

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

4 There are agents that cause
5 immunosuppression. The classic acquired
6 immunodeficiency syndrome is AIDS, so the human
7 immunodeficiency virus is an acquired immunodeficiency
8 syndrome. Human *T* cell leukemia virus 1 is also
9 immunosuppressive. Measles virus surprisingly is an
10 entity or a virus that can cause immune suppression or
11 immunodeficiency.

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

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

25 The one that's bothering me right now is

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

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

11 BY MS. CHIN-CAPLAN:

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

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

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

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

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

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

15 A Yeah. This is a cartoon of what the measles
16 virus looks like, and I thought this might be
17 important because we keep hearing about *F* gene, *H*
18 gene, *N* gene. What does that mean? From a pictorial
19 standpoint of the measles virus if you look at measles
20 virus by electron microscopy its size is approximately
21 150 to 300 nanometers. You need an electron
22 microscope to look at it, so it's small.

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

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

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

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

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

22 A Yes. It's been attenuated.

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

25 A Yes. So if you take a wild type measles

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

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

12 Q Doctor, does the vaccine strain also contain
13 phosphoprotein?

14 A To a degree, yes.

15 Q And does it contain nucleoprotein?

16 A Yes.

17 Q Matrix protein?

18 A Yes.

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

21 A Yes. And it contains the negative strand
22 RNA.

23 Q And the structure is essentially the same?

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

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

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

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

9 SPECIAL MASTER HASTINGS: Now we're on Slide
10 11.

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

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

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

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

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

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

1 BY MS. CHIN-CAPLAN:

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

4 A From the standpoint of?

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

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

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

15 A Correct.

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

19 A Yes.

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

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

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

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

3 A Three to six months.

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

6 A Yes.

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

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

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

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

1 memory.

2 Q Once that population regenerates is it fully
3 functional?

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

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

11 A Yes.

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

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

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

18 A It could be. Yes.

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

22 A Yes.

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

25 A It affects the professional antigen

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

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

8 A Correct. However, to a new infection.

9 Q To a new infection.

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

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

14 A Yes, or to invoke.

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

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

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

21 A From what perspective?

22 Q From immunosuppression standpoint.

23 A If the measles vaccine is immunosuppressive,
24 yes.

25 Q So is the vaccine strain

1 immunosuppressive --

2 A It can be. Yes.

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

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

7 Q Now, Doctor, have you read the Uhlmann
8 paper?

9 A Yes.

10 Q Just very generally since we had a
11 discussion about the Uhlmann paper today can you tell
12 the Court what that study was designed to do?

13 A To show the presence of measles virus in
14 biopsy samples from patients that had an intestinal
15 disorder, which was called IBH.

16 Q IgG?

17 A Whatever. Some hyperplastic condition that
18 normally you don't find in the ileum.

19 Q Now, Doctor, from that study do you know
20 were they studying measles specifically?

21 A Yes, they were looking for measles.

22 Q In their results did they recover some
23 portions of measles virus?

24 A They were using quantitative PCR technology,
25 and they reported recovering products that were the H

1 gene and the *F* gene.

2 Q And the *H* gene and the *F* gene, is that
3 contained on the envelope of the measles virus?

4 A Yes.

5 Q And that you had shown earlier on the slide?

6 A Yes.

7 Q Doctor, what is the likelihood that the *F*
8 gene and the *H* gene are false positives?

9 A If you'd let me get through my slides I'll
10 show you the possibility that it's probably not.

11 Q Okay. All right. Let's talk about
12 replication, Doctor.

13 A Okay. Good. Because it's important to
14 understand replication before you look to see why look
15 for *H* gene and why look for *F* gene products?

16 SPECIAL MASTER HASTINGS: So we're going to
17 Slide 12, right?

18 THE WITNESS: Yes.

19 SPECIAL MASTER HASTINGS: Okay. Go ahead,
20 Doctor.

21 THE WITNESS: So essentially for replication
22 the *H* protein on the envelope attaches to the host
23 cellular receptor for measles virus, which is referred
24 to as CD46 for cluster of differentiation antigen 46,
25 so the receptor for measles virus has been identified.

1 Replication takes place in the cytoplasm. The
2 negative strand RNA serves as a template for messenger
3 RNA synthesis.

4 Messenger RNA synthesis is a positive strand
5 RNA, and this is the part that will be translated into
6 viral protein. Also, the negative strand serves as a
7 template so one can synthesize additional positive
8 strand RNA to then make new negative strand RNA to
9 produce new virus. The virus contains an RNA
10 transcriptase used to generate the messenger RNA, the
11 positive strand.

12 SPECIAL MASTER HASTINGS: Now we're going to
13 Slide 13?

14 THE WITNESS: Slide 13.

15 SPECIAL MASTER HASTINGS: Go ahead.

16 THE WITNESS: The RNA transcriptase is
17 packaged into the infectious virus particle, and again
18 you need to generate more positive strand RNA to
19 produce the negative strand that is actually the
20 entity that is packaged into the measles virus
21 particle. You've got *M* protein involved, and that's
22 important for assembly and for the virus to release
23 itself from the cell it's infecting.

24 The *F* protein is responsible for fusion, and
25 it's important in the entry process of the virus into

1 the infected cell following the attachment of the *H*
2 protein. The *F* protein allows fusion with the host
3 cell membrane and entry of the virus particle in
4 encoding. *N* protein, which is a nucleoprotein,
5 functions in viral replication and is further involved
6 in allowing the genome to be packaged and processed.

7 SPECIAL MASTER HASTINGS: His last testimony
8 was about Slide 14.

9 MS. CHIN-CAPLAN: That's correct, Special
10 Master.

11 THE WITNESS: Correct. Slide 14.

12 BY MS. CHIN-CAPLAN:

13 Q So, Dr. Kennedy, you've just described how a
14 measles virus multiplies. Is that true?

15 A Yes. How it replicates. How it infects,
16 and how it replicates, and how it can potentially
17 persist and how it can produce new virus particles.

18 Q Now, Doctor, why is this important?

19 A Well, it's important for a number of
20 aspects. So the next slide will be 15, and I don't
21 think we can do -- back one. Sorry. So this is Slide
22 15 entitled Replication. It's a cartoon, and
23 unfortunately I don't think the Power Point
24 presentation is working because it's animated, but
25 I'll take you through it anyway.

1 What I want to show from this is the process
2 of what it takes for a measles virus to not only
3 produce the transcriptase that are picked up by
4 quantitative RT-PCR, but also what it takes to produce
5 protein from those transcriptase and have that protein
6 been assembled with additional negative strand RNA to
7 form new virus particles to go on and infect other
8 cells.

9 So if you take a look at the red line up
10 there, that is the initial negative strand RNA. We
11 use the term nonsegmented. Now, how does that become
12 separated from the rest of the virus? When the virus
13 attaches via the hemagglutinin to the CD46, which you
14 see up top, the *F* gene product then comes into play
15 and allows the entire encapsidated measles virus to
16 enter the cell.

17 So it goes through a phase of encoding.
18 When it encodes two things happen. That negative RNA
19 becomes free and the viral polymerase, the RNA
20 transcriptase, the enzyme that the measles virus
21 carries, becomes free and active and can go and do its
22 work. The first thing it does is from that negative
23 strand RNA it produces a positive strand RNA.

24 That positive strand RNA becomes the
25 messenger RNA. You see the messenger RNA contains

1 specific gene products in a particular order.

2 SPECIAL MASTER HASTINGS: Let me stop you,
3 Doctor, because maybe you're going a little too fast
4 for me or whatever, but is this an important --

5 THE WITNESS: Yes.

6 SPECIAL MASTER HASTINGS: I sense this is an
7 important part of your presentation.

8 THE WITNESS: Yes.

9 SPECIAL MASTER HASTINGS: Why don't you
10 start with this slide over again, and I think I'm
11 following the segment, but let's see. Let's start
12 with this slide over again and watch me. If I put my
13 hand up and stop you --

14 THE WITNESS: Hold up your hand and then --
15 okay.

16 SPECIAL MASTER HASTINGS: Go ahead.

17 THE WITNESS: The important thing is that
18 the measles virus when it causes infection in a cell
19 it is an orderly process that occurs to ultimately
20 produce more measles virus to then go on and infect
21 additional cells, so it's a self-regenerating, self-
22 procuring process with the ultimate goal of producing
23 more measles virus particles.

24 SPECIAL MASTER HASTINGS: Got that.

25 THE WITNESS: The process is defined, it's

1 characterized and it's very orderly, and it occurs in
2 a very orderly fashion using not only a specific set
3 of enzymes that the measles virus carries with it,
4 those enzymes then allow the ability to produce the
5 necessary components to then produce new measles virus
6 that can then go on to infect another cell and do the
7 same process over.

8 So what I'd like to point out as the
9 important components, the red strand. That is the
10 nonsegmented negative strand of measles virus.

11 SPECIAL MASTER HASTINGS: The red strand at
12 the top?

13 THE WITNESS: The red strand at the top.
14 That will serve as a template for the virus enzyme to
15 produce the blue strand. That blue strand is
16 positive, so negative goes to positive. The positive
17 RNA is what's referred to as messenger RNA, and that
18 messenger RNA contains defined genes in a specific
19 order. Those genes will then go through a cell
20 machinery to produce proteins, and those proteins are
21 the green and different green colored things down
22 below.

23 So at No. 2, that is the cell machinery.
24 Things called ribosomes, things called ribosomal RNA,
25 transfer RNA, bring amino acids that allow the protein

1 to be produced.

2 SPECIAL MASTER HASTINGS: What do you mean
3 by the cell machinery? I'm not following that. Which
4 cell machinery?

5 THE WITNESS: The host cell. So measles
6 virus takes over the host cell and uses its synthesis
7 machinery to assist it in producing its protein. So
8 it just says your protein is not being made, I'm
9 making my protein.

10 SPECIAL MASTER HASTINGS: One minute.

11 SPECIAL MASTER VOWELL: I have a question,
12 Dr. Kennedy.

13 THE WITNESS: Yes?

14 SPECIAL MASTER VOWELL: This cartoon is
15 essentially a cell.

16 THE WITNESS: A cell. Yes.

17 SPECIAL MASTER VOWELL: And so at the upper
18 right-hand corner when you have the schematic cartoon
19 you've been using to show the measles virus you show
20 the measles virus entering the cell.

21 THE WITNESS: Right.

22 SPECIAL MASTER VOWELL: And then at the
23 bottom you show the measles virus exiting the cell.

24 THE WITNESS: Correct.

25 SPECIAL MASTER VOWELL: So I understand your

1 cartoon.

2 THE WITNESS: Okay.

3 SPECIAL MASTER VOWELL: Thank you.

4 THE WITNESS: Yes.

5 SPECIAL MASTER HASTINGS: Okay. That is
6 helpful to me.

7 THE WITNESS: Okay. The important take away
8 point is if you look at the blue, the blue has *N*, *P*,
9 *M*, *F*, *H*, *L* from left to right. Those correspond to
10 genes that will be involved in making those proteins
11 and from a standpoint of the quantitative PCR that's
12 what is being detected, those products.

13 You have to make *N* before you can make *P*,
14 you have to make *M* before you make *F*, you have to make
15 *F* before you make *H*, and you have to make the whole
16 thing to get all the proteins necessary for what's
17 called a productive infection. A productive infection
18 means that new measles virus is produced and released.
19 Does that make sense? So it's an orderly fashion.

20 You can't find *H* message sequences without
21 being able to find *F*, you can't find *F* without *M*, you
22 can't find *P* without *N*. So it's a process. *N* is made
23 first, *P* follows, *M*, *F* and *H*. This is going to become
24 very important later on. The other thing to note is
25 going from the negative, the red, to the blue there is

1 an amplification process.

2 When that occurs replication has occurred.

3 So infection starts once that negative red starts
4 becoming blue.

5 SPECIAL MASTER HASTINGS: And that occurs
6 naturally in the infection process?

7 THE WITNESS: Absolutely. Now, protein. We
8 heard issues related to protein. Can you detect
9 protein? Protein is a later process. So you detect
10 the blue first, and then you detect the green later,
11 and then once everything is together it's organized
12 and put together in a particle and that particle is
13 released from the cell. Now, that is a productive
14 infection.

15 Persistence doesn't necessarily mean that
16 you have to produce more virus. Persistent means
17 you're at some stage of this process. So you can be
18 blue, you can be green, but you don't have to produce
19 new virus particles. Does that make sense?

20 SPECIAL MASTER HASTINGS: I'm not sure
21 exactly what that means. You're saying for
22 persistence that the virus doesn't have to keep
23 reproducing itself?

24 THE WITNESS: Correct. You don't have to
25 have that.

1 SPECIAL MASTER HASTINGS: Those virus
2 particles, or is it the whole virus that just remains
3 living indefinitely?

4 THE WITNESS: It's pieces of the virus.
5 Depending on the situation at some point it may be
6 able to reactivate and produce new viruses, at other
7 points it may not. It really depends on the host cell
8 that is persisting it.

9 SPECIAL MASTER HASTINGS: So you can have
10 persistence of portions of the virus?

11 THE WITNESS: Correct.

12 SPECIAL MASTER HASTINGS: For an indefinite
13 period?

14 THE WITNESS: For as long as that cell stays
15 around. Or you can have persistence where you're
16 getting new infections occurring, but it's at a very
17 low level.

18 SPECIAL MASTER VOWELL: Let me ask you, Dr.
19 Kennedy, that calls a question into my mind. You made
20 a point about that in this sequencing they can only
21 occur in a particular sequence.

22 THE WITNESS: Correct.

23 SPECIAL MASTER VOWELL: But when you refer
24 to persistence is it possible that you can have
25 portions of that sequence that remain and others are

1 not there?

2 THE WITNESS: No. Actually, you could have
3 a situation where you could get *N* but not necessarily
4 *P*, *M*, *F* and *H*, but it would stop there. So it would
5 tend to stop at a particular stop point.

6 SPECIAL MASTER VOWELL: So what had been
7 generated would be all that would persist?

8 THE WITNESS: Right.

9 SPECIAL MASTER VOWELL: Thank you.

10 THE WITNESS: So is that fairly clear?

11 SPECIAL MASTER HASTINGS: I think up to the
12 point you're at. Right.

13 THE WITNESS: Okay.

14 BY MS. CHIN-CAPLAN:

15 Q Okay. Now, so, Doctor, just to be clear if
16 one finds *F* gene then *N*, *P* and *M* must have existed
17 before *F* gene to appear?

18 A Yes.

19 Q And if one finds *H* gene then *N*, *P* and *F* has
20 appeared before it?

21 A Yes.

22 Q So is that an indication of replication?

23 A Yes.

24 Q Now, Doctor, you also just generally talked
25 about persistence, that the presence of these genes

1 indicate the viral persistence. Would the presence of
2 these genes cause harm to the cell?

3 A They could, they may not. Persistence
4 usually means you don't harm the cell. If you harm
5 the cell then you no longer persist.

6 Q So if it persists it's potentially harmful,
7 and it depends on the host's immune system?

8 A A persistent infection to the general host
9 can be harmful, to that cell probably not.

10 Q Thank you.

11 A Does that make sense? So in other words to
12 persist it has to stay in that cell. If it harms the
13 cell and the cell is lived then it's not persisting in
14 that cell. Now, its persistence in that cell, that's
15 now an infected cell so the host wants to try to get
16 rid of it.

17 SPECIAL MASTER VOWELL: Let me ask two
18 question, Dr. Kennedy. Do we know anything at all
19 about the length of time these genes can persist
20 within a cell? In other words, the cell makes *N*, and
21 then stops for a while, and then goes back and
22 something triggers it and it makes *P*. Do we know
23 anything about the length of that process?

24 THE WITNESS: About the individual ones?
25 No.

1 SPECIAL MASTER VOWELL: Okay. Another
2 question. If that cell is infected with a persistent
3 but not actively replicating virus so the cell doesn't
4 die can that cell's particular function in the body be
5 impaired by the presence of the virus?

6 THE WITNESS: Yes.

7 SPECIAL MASTER VOWELL: And in what way?

8 THE WITNESS: Metabolic. It can be a target
9 for neighboring cells to be damaged or destroyed as a
10 result of a developing immune response.

11 SPECIAL MASTER VOWELL: So the body is
12 responding to the infection in that particular cell
13 but other cells are --

14 THE WITNESS: Are hurt. Yeah. A bystander.

15 SPECIAL MASTER VOWELL: Okay.

16 SPECIAL MASTER HASTINGS: Go ahead.

17 MS. CHIN-CAPLAN:

18 Q Doctor, to summarize your report here?

19 A Okay. So this is the next slide.

20 SPECIAL MASTER HASTINGS: Number 16. Go
21 ahead.

22 THE WITNESS: So measles virus and close-
23 related viruses in the same subfamily and genus are
24 neurotropic and can cause neurologic disorders and
25 sequelae in humans and other species. Measles virus

1 and other morbilliviruses can persist and cause
2 persistent infection and have been detected in the CSF
3 and the brain of individuals that exhibit neurologic
4 disorders. Measles virus infection and replication
5 requires the presence of measles virus RNA.

6 Measles virus is produced from measles virus
7 protein that is produced from measles virus RNA and is
8 required to produce new measles virus progeny.

9 "Immune dysfunction" is a term that
10 encompasses problems associated with the normal
11 function of the immune response.

12 SPECIAL MASTER HASTINGS: Now you're
13 shifting to --

14 THE WITNESS: -- Slide 17.

15 SPECIAL MASTER HASTINGS: -- Slide 17. If
16 you could, let us know when you're shifting, for the
17 record, and it will make it easier for us to follow
18 this testimony later on when we reread this, which we
19 will be doing.

20 THE WITNESS: Okay. I apologize. "Immune
21 dysfunction" is a term that encompasses problems
22 associated with the normal function of the immune
23 response.

24 Viruses, in general, do not persist in a
25 host because the host has an effective immune

1 response. Viruses, in general, persist in a host
2 because of an ineffective immune response, and this
3 would be Slide 18.

4 A number of contraindications for
5 administering the measles/mumps/rubella vaccine are
6 described in the Physician's Desk Reference. Adverse
7 events involving the measles/mumps/rubella vaccine
8 have included neurologic disorders. Next slide.

9 BY MS. CHIN-CAPLAN:

10 Q Doctor, can we just stop here for one
11 minute?

12 A Yes.

13 Q You're moving on into the Uhlmann paper
14 here, are you not?

15 A Yes.

16 Q Doctor, we've heard a lot about the Uhlmann
17 paper today. You've reviewed the Uhlmann paper?

18 A I have.

19 MS. CHIN-CAPLAN: Could we just go back to
20 Slide 15, please?

21 BY MS. CHIN-CAPLAN:

22 Q Doctor, the Uhlmann paper detected measles
23 genes in the gut tissue. Is that correct?

24 A Correct.

25 Q Could you tell the Court which measles genes

1 the Uhlmann paper detected?

2 A The H and the F.

3 Q Doctor, from your prior testimony, before
4 one can reach F, it must have gone through N, P, and
5 M. Is that correct?

6 A Correct.

7 Q So, for F to exist, does that mean that
8 there has to be replication prior to F?

9 A Yes.

10 Q And you indicated also that they had
11 recovered H gene. Is that correct?

12 A Yes.

13 Q So to recover H gene, they would have to
14 have gone through N, P, M, and F gene.

15 A Yes.

16 Q And is that an distinction of replication as
17 well?

18 A Yes.

19 (Discussion held off the record.)

20 BY MS. CHIN-CAPLAN:

21 Q So, Doctor, if there is recovery of F gene
22 and H gene, this is an indication that the virus is
23 replicating, you said.

24 A Yes.

25 Q Is that an indication also that the virus is

1 persistent?

2 A Not necessarily.

3 Q Could you kindly explain that?

4 A Okay. The fact that it's replicating means
5 that it's there. How long is it there? That's what
6 determines persistence. If it's there for a period of
7 time, three to six months, in that site where it
8 causes bi-standard effects to occur in nearby cells as
9 a result of an ineffective immune response, then it
10 persists. It's there.

11 Q Doctor, if we could go to Slide 19, you were
12 continuing your summary of your report here. Are you
13 familiar with the lab of John O'Leary and Dr. Orla
14 Sheils at Trinity College in Dublin?

15 A Yes, I am.

16 Q Are you familiar with their reputation in
17 the community as molecular biologists?

18 A Yes, I am.

19 Q Could you tell the Court what that
20 reputation is within the community?

21 A Well, if you look at it just in general
22 terms, in the past two years, the laboratory has
23 published 12 peer-review publications in excellent
24 scientific journals in the area of PCR and molecular-
25 based diagnosis. It's primarily been in the area of

1 cancer, cancer diagnostics and cancer detection, but
2 they are highly productive and highly qualified to
3 perform these types of analyses.

4 Q Doctor, they used PCR technique. Is that
5 true?

6 A Correct.

7 Q Is PCR a generally accepted standard test to
8 detect measles RNA?

9 A Yes. In this decade, yes.

10 Q In previous decades, no? Doctor, if we
11 could go on to the next slide, please.

12 A Okay. So this is the --

13 THE WITNESS: Next slide.

14 SPECIAL MASTER HASTINGS: Number --

15 MS. CHIN-CAPLAN: -- 20.

16 SPECIAL MASTER HASTINGS: -- 20. Correct?

17 THE WITNESS: -- 20. Okay. So the
18 detection of measles virus RNA from several measles
19 virus structural gene products; we didn't talk about
20 it, but the high levels of measles virus RNA detected
21 in the intestinal samples of Michelle Cedillo at a
22 time point when an effective MMR vaccine-induced
23 immune response should have cleared the measles virus
24 indicates that measles virus RNA has amplified as a
25 result of replication and persists in part of her body

1 where it is not expected to be found.

2 The detection of measles virus protein in
3 intestinal tissue samples from autistic enterocolitis
4 children further supports measles virus replication
5 and persistence.

6 BY MS. CHIN-CAPLAN:

7 Q Doctor, there was previous testimony about
8 the copy number that was found in the gut tissue of
9 Michelle Cedillo. Are you familiar with the copy
10 number?

11 A Yes.

12 Q And the copy number that was found; would
13 characterize it as high, medium, low?

14 A Higher than one would anticipate.

15 Q Higher than one would anticipate --

16 A -- with a natural measles virus infection.

17 Q And is there a reason for that?

18 A Yes. In my opinion, this is evidence that
19 replication occurred. There was amplification. The
20 only way you get higher amounts is if that process
21 that I showed you in that cartoon slide occurred.
22 There was amplification of that red strand into blue.

23 SPECIAL MASTER CAMPBELL-SMITH: Can I just
24 interject? Dr. Kennedy, you said higher than you
25 would expect to find with a natural measles virus.

1 THE WITNESS: With a natural measles virus.

2 SPECIAL MASTER CAMPBELL-SMITH: So even
3 beyond what you would expect to find for someone who
4 had been vaccinated, you're saying --

5 THE WITNESS: Correct, correct.

6 SPECIAL MASTER CAMPBELL-SMITH: Thank you.

7 BY MS. CHIN-CAPLAN:

8 Q Doctor, did you also look at an immune
9 workup that was done on Michelle Cedillo?

10 A Yes.

11 Q Was that workup performed by Dr. Gupta?

12 A Yes.

13 Q Do you recall what the values of that immune
14 workup was?

15 A Not specifically, but I remember certain
16 things that I thought were relevant.

17 Q And what were those things that you thought
18 were relevant?

19 A An elevated IgG-2 response.

20 Q What's the significance of an elevated IgG-2
21 response?

22 A It would be indicative of some sort of
23 immune dysfunction. It would also suggest that there
24 was a skewing toward TH Type 2 response because, if
25 you look in humans, IgG-1 and IgG-3 are evidence of a

1 TH Type 1 response. IgG-2 and 4 are evidence of a TH
2 2-type response. The fact that IgG-2 was elevated
3 would suggest to me that, based on subclass
4 characterization, that there was a skewing of the
5 response.

6 Additionally, I noted that Dr. Gupta did not
7 perform any sort of serology for functional activity
8 of measles virus antibodies. The IgG that he looked
9 at; he did not look to see if there was any
10 neutralizing activity or any functional activity that
11 would tell me that that is evidence of a protective
12 immune response.

13 So, based on those two things, the fact that
14 there appears to be an ineffective immune response
15 that lacks any sort of viral clearing activity and
16 elevation of a particular subclass that is indicative
17 of a specific type of response, a TH Type 2, that
18 there is some sort of immune dysfunction.

19 Q What is the significance of the TH Type 2
20 elevation?

21 A TH Type 2, as I showed in previous slides,
22 can be very problematic, from a standpoint of
23 resolving an infection. If you skew the response in
24 the wrong direction, you can make a situation worse
25 instead of better.

1 Q And in this situation, if you had a TH-2
2 response, would she have been able to clear the
3 measles infection?

4 A I wouldn't be able to speak to that unless
5 additional assays were performed, but I would suspect
6 that since those assays were not available, that she
7 had a difficulty of clearing it. That elevated IgG-2
8 didn't help her and may have hurt her.

9 Q Doctor, do you have an opinion as to whether
10 he measles virus results that were found in Michelle
11 Cedillo's chart is indicative of replication in the
12 gut?

13 A I would say yes.

14 Q Do you have an opinion whether the presence
15 of the F gene and the H gene, as reported in the
16 Uhlmann paper, would indicate persistence of that
17 virus?

18 A Yes, with all of the other caveats that I
19 mentioned.

20 Q Doctor, what is the basis of your opinion?

21 A The immunology and virology workup and the
22 only plausible explanation, in my mind.

23 MS. CHIN-CAPLAN: Thank you, Doctor.

24 SPECIAL MASTER HASTINGS: Doctor, before we
25 break here, could we back up to the previous slide,

1 Number 20?

2 THE WITNESS: Sure.

3 SPECIAL MASTER HASTINGS: Now, there's two
4 points here. The first one talks about the detection
5 of the MV RNA, and in that, you're talking about the
6 Uhlmann paper.

7 THE WITNESS: Correct.

8 SPECIAL MASTER HASTINGS: Then the second
9 point there, the detection of MV protein; now there
10 are you talking about the research described earlier
11 today by Dr. Hepner that was contained in the poster?

12 THE WITNESS: No.

13 SPECIAL MASTER HASTINGS: No.

14 THE WITNESS: I'm talking about additional
15 information that I have from attending a meeting where
16 Dr. Sheils presented work from the O'Leary and Sheils
17 Laboratory.

18 SPECIAL MASTER HASTINGS: Is this published
19 anywhere?

20 THE WITNESS: To my knowledge, no. This
21 would be a personal communication, actually, a
22 personal observation.

23 SPECIAL MASTER HASTINGS: Of who, Dr.
24 Sheils?

25 THE WITNESS: The O'Leary-Sheils group. Dr.

1 Sheils is a pathologist, so he does a lot of
2 immunohistochemistry, and I'm aware of the types of
3 specific antibodies that they used. I attended a
4 meeting where the data was presented from a standpoint
5 of both detection of the F gene and N gene products by
6 PCR and also the detection of the nuclear protein by
7 immunohistochemistry in gut tissues from autistic
8 enterocolitis children that was positive for
9 immunohistochemistry.

10 Part of this came up as a result of a report
11 by a former student in -- I don't remember the
12 specific report -- that was criticizing the
13 immunohistochemistry that was being used using a
14 polyclonal antibody, the measles virus. Do you
15 remember which report that is, Counselor?

16 It was a graduate student of Dr. Wakefield
17 who submitted a rebuttal letter. If you'll give me a
18 second to look --

19 SPECIAL MASTER HASTINGS: Done by Dr.
20 Chadwick?

21 THE WITNESS: Dr. Chadwick. Thank you. So
22 Dr. Chadwick talks about immunohistochemistry studies
23 in there, and I attended a meeting that Dr. Chadwick
24 was not present at that Dr. Wakefield was not present
25 at, but had the O'Leary-Sheils group there, and they

1 presented this data.

2 SPECIAL MASTER HASTINGS: Is there any
3 record of this meeting that you're aware of?

4 THE WITNESS: Just the individuals that were
5 there would be the record.

6 SPECIAL MASTER HASTINGS: Where and when did
7 this meeting take place?

8 THE WITNESS: It took place in the U.K. in
9 2002.

10 SPECIAL MASTER HASTINGS: Why don't we take
11 a lunch break right now? You're done with your
12 direct, I assume.

13 MS. CHIN-CAPLAN: I am, Special Master.

14 SPECIAL MASTER HASTINGS: Let's take a lunch
15 break of about one hour, and we'll be back here, and
16 there probably will be some cross-examination.

17 (Whereupon, at 1:00 p.m., a luncheon recess
18 was taken.)

19 //

20 //

21 //

22 //

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25 //

1 Q You said you published on MMR.

2 A I stated that, in that publication, it
3 discusses MMR.

4 Q So the same publication that talks about
5 measles virus is the one that talks about MMR.

6 A Correct.

7 Q Have you ever testified in court before?

8 A No. This is my first time.

9 Q Welcome.

10 A It's loads of fun.

11 Q Have you ever participated as an expert
12 witness or a consultant for litigation before?

13 A Yes, I have.

14 Q How many times?

15 A Patent, probably three or four over patent
16 issues, from a standpoint of I'm doing one relative to
17 an environmental toxin. How about less than five,
18 other than patent?

19 Q Other than patents.

20 A Other than patent.

21 Q Of these five, one, you said, was on an
22 environmental toxin.

23 A Yeah. It involves an environmental toxin,
24 something called acrylamide.

25 Q Okay.

1 A The other was an HIV needle-stick situation.
2 There was the U.K. report on the
3 measles/mumps/rubella issue and another one relative
4 to these annuity companies wanting to cash in on AIDS
5 patients' life insurance policies early and to
6 essentially give them a lump sum at a discount to kind
7 of -- what was the status of their HIV/AIDS infection.
8 So I believe that's it.

9 Q That's four.

10 A Four? And this is five.

11 Q Okay. You didn't participate in litigation
12 involving a claim that MMR, other than this
13 litigation.

14 A The U.K. one; I was an expert witness.

15 Q Other than those two, you don't recall any
16 others.

17 A No. I honestly don't like being in court,
18 so I try to avoid it.

19 Q Well, neither do I.

20 In 1999, you were invited by Representative
21 Burton, Dan Burton, to speak before the House
22 Committee on Government Reform. Do you recall that?

23 A Yes.

24 Q In your report, you mentioned that
25 particular instance, and you said that when you were

1 preparing for your testimony there, you were surprised
2 to find that there were some contraindications for MMR
3 vaccine.

4 A Yeah. Yes.

5 Q You don't prescribe medications.

6 A No, I don't, but I can read the Physician's
7 Desk Reference.

8 Q Now, aren't there contraindications, though,
9 if you read the Physician's Desk Reference, to just
10 about every single medication?

11 A From the standpoint of vaccines, I would say
12 it varies on the type of vaccines, that the live,
13 attenuated group, of which MMR is, has more
14 complications than, let's say, a subunit or a
15 recombinant derived or a glycoconjugate type of
16 vaccine, such as the HiB, and with the HiB vaccines,
17 there are different conjugate forms, depending on
18 which carrier protein you use, or whether you use a
19 synthetic oligopolysaccharide. So it's really vaccine
20 dependent.

21 Q Okay. If I were to take a look at, say,
22 Tylenol, would I find contraindications to that?

23 A Yes.

24 Q Do you think there may be more for a live
25 vaccine? Is that what you're implying?

1 A I would say a live vaccine has special
2 issues and contraindications that you wouldn't find
3 for other vaccination forms.

4 Q Now, you were in front of Representative
5 Burton to talk about Hepatitis B vaccine. Correct?

6 A Actually, I was there to talk about issues
7 related to safety considerations and informed consent
8 and essentially educating physicians on what they
9 should tell parents relate to providing vaccines.

10 Q For Hepatitis B vaccine. Correct?

11 A For Hepatitis B, yes.

12 Q Hepatitis B vaccine is not a live vaccine,
13 is it?

14 A No. It's a subunit: early subunit, later
15 recombinant form.

16 Q Now, I know this is 1999 when you were
17 testifying, but in your report you profess surprise at
18 these numbers of contraindications. Does that mean,
19 up to 1999, you really didn't know much about MMR
20 vaccine?

21 A I would say I knew more about Hepatitis B
22 vaccines, and I knew the most about failed HIV
23 vaccines, but certainly I looked at live attenuated
24 approaches as one of the approaches to develop new
25 types of viral vaccines in my laboratory. So I was

1 certainly aware of anything that was a successful
2 vaccine and certainly anything that was licensed. I
3 believe I used the MMR vaccine in my first medical
4 school lecture in 1989.

5 Q In 1999, you didn't know what the
6 contraindications were before you prepared --

7 A I was surprised at the level.

8 Q And the contraindications are that you
9 shouldn't give it to someone who has a neomycin
10 sensitivity or allergy.

11 A That's one. There's a number, and it's
12 developed with time. So if you look at the various
13 Physician's Desk References, the various volumes over
14 the years, so Volume 51 differs from Volume 54. So
15 it's developed as things have become clearer.

16 Q Is there any contraindication there to
17 administering MMR vaccine in someone who has autism?

18 A To my knowledge, no.

19 Q You mentioned that you thought that, ideally
20 -- to be fair, you said it wouldn't be practical, and
21 the cost-benefit wouldn't justify this, but you
22 thought that everybody, ideally, should have an
23 immune workup before they got any vaccinations.

24 A I think that they should not only have an
25 immune workup; they should be tested prior to and post

1 because there's issues, from a standpoint of
2 transplacental transfer of immunity. So if mother has
3 been exposed and transfers IgG antibodies to the
4 infant, that can affect the vaccine's efficiency, its
5 ability to induce an immune response. There are a
6 number of conditions that can be measured prior to
7 decision when is the optimal time to give the vaccine
8 or when the vaccine should not be given.

9 Q Has any public, scientific body advocated
10 such an approach?

11 A Not to my knowledge.

12 Q Now, when you were giving your testimony to
13 Congressman Burton, did you state, at any time during
14 that testimony, that you believed that MMR vaccine was
15 causing problems?

16 A To the best of my recollection, I was there
17 to talk about the Hepatitis B vaccine, and I talked
18 about, in general terms, vaccines and adverse events
19 and reporting systems and VAERS in very general terms.

20 Q And did you mention any problems with MMR
21 vaccine?

22 A At that time, I didn't specifically mention,
23 to my knowledge, anything relative to MMR or DTaP or
24 any other vaccines. I was more present to discuss the
25 immaturity of the neonatal immune system.

1 Q You didn't advocate, at that point, that
2 there should be a choice with regard to whether to
3 vaccinate with MMR vaccine. Is that right?

4 A I'm not a health policy person, so, no, I
5 didn't.

6 Q But you were advocating that there be a
7 choice for Hepatitis B vaccine.

8 A Hepatitis B is a certain situation. For
9 instance, Hepatitis B is transmitted by infected blood
10 products, via sex, and, in utero, from a chronic
11 carrier mother to its infected infant. I don't know
12 too many newborns or one- or two-year-olds that are IV
13 drug users, that are sexually promiscuous, or if
14 mother is chronically infected, that the physician or
15 pediatrician or OB/GYN is not aware of that prior to.

16 Q So you were willing to comment on health
17 policy, as far as that vaccine.

18 A I believe I didn't comment to those direct
19 terms -- that was 1999 -- but I would be happy to
20 comment on it now.

21 Q I think you just have.

22 A Yes.

23 Q Now, measles vaccine, or measles virus, I
24 should say, has never been the focus of your research,
25 has it?

- 1 A Actually, it is.
- 2 Q It is the focus of your research.
- 3 A One aspect of a lot of my research
- 4 activities.
- 5 Q And you have yet to publish anything --
- 6 A Correct.
- 7 Q -- other than your one paper.
- 8 A The one paper.
- 9 Q Anything in press?
- 10 A No.
- 11 Q Any studies that any data has been generated
- 12 from?
- 13 A Yeah. Actually, we're trying to develop a
- 14 specific, pathogen-free baboon colony that is free of
- 15 all pathogens that potentially cause
- 16 immunosuppression. So what we're doing is trying to
- 17 select females and males that are characteristically
- 18 free, using qualitative PCR and some serologic
- 19 diagnosis of a number of infections, of which we're
- 20 examining measles virus, or measles virus-like
- 21 entities, along with Simian Virus 40, the retroviruses
- 22 that are present in human and Simian species; Herpes B
- 23 virus, so a number.
- 24 Q You're using measles virus to help develop a
- 25 certain subspecies or subset of primate for use in

1 research. Correct?

2 A Correct.

3 Q So the focus of your research is developing
4 that primate, is it not?

5 A Correct. But I use measles virus, among
6 others, to develop that primate model.

7 Q And that's your research.

8 A One of them. I do other things.

9 Q None of which that have been published.

10 A No. Actually, I have eight publications
11 this year.

12 Q On measles virus.

13 A No.

14 Q I'm sorry.

15 A I was talking about the other things.

16 Q Okay. So you have other research going on.

17 A Yes.

18 Q But that's not involving measles virus.

19 A Correct.

20 Q And you're not researching mumps or rubella
21 either. Correct?

22 A No.

23 Q And neither at Texas Tech, nor at any of
24 your previous academic positions, were you researching
25 measles virus or MMR. Correct?

1 A Could you restate the question?

2 Q Neither at Texas Tech, nor at any of your
3 previous academic positions were you researching
4 measles virus or MMR, and let me qualify that, other
5 than to the extent you've talked about, with this
6 study to try to develop the primate --

7 A "Research," do you mean physical, hands-on,
8 my laboratory is working on it, or "research," do you
9 mean that I am competent in reading the literature and
10 being aware of --

11 Q I mean research that you're carrying out
12 with your laboratory --

13 A Laboratory. Okay.

14 Q -- as part of your academic research.

15 A No.

16 Q This is just a point of clarification. You
17 mentioned that you've been working with HIV, what
18 turned out to be HIV, an unidentified virus at the
19 time, and don't take this question the wrong way; I
20 just want to make sure that it's clarified. You
21 weren't suggesting that you had discovered HIV.

22 A Oh, no, not at all.

23 Q I want to make sure I understand your theory
24 of causation here. Can you please state it as simply
25 as possible, step by step, what your theory of

1 causation is?

2 A With regards to?

3 Q Why are you here to testify, in a sense?

4 How do you think the MMR vaccine led to Michelle
5 Cedillo having an autistic spectrum disorder?

6 A Well, if you look at my Slide 20 and 21, I
7 think it's pretty clear there, if we could have that,
8 my last two slides. I didn't get a chance to memorize
9 my slides, just my lectures. I don't have a copy.

10 Q I guess I could start by saying, does this
11 encapsulate your theory?

12 A Yes. Do you need me to read it again?

13 Q No. I don't need you to read it. I need to
14 go through it to make sure that I understand.

15 A Okay.

16 SPECIAL MASTER HASTINGS: Wait. I'm not
17 sure what the question was. He said "this." Are you
18 referring to the summary of the expert report?

19 THE WITNESS: Yes.

20 SPECIAL MASTER HASTINGS: When you answered
21 yes, you were talking about the entire summary of
22 expert report, which goes from 16 through 21?

23 THE WITNESS: No. I would just say the
24 summary, which are the last two pages, 20 and 21,
25 relative to the last question.

1 SPECIAL MASTER HASTINGS: Twenty and 21.

2 THE WITNESS: Right.

3 SPECIAL MASTER HASTINGS: Okay. Go ahead,
4 Mr. Matanoski.

5 MR. MATANOSKI: Thank you.

6 BY MR. MATANOSKI:

7 Q This would be Petitioners' Trial Exhibit No.
8 8, I believe, on page 20.

9 A Yeah.

10 Q And you have that before you, don't you,
11 Doctor?

12 A Yes.

13 Q Now, it starts out, and it says: "The
14 detection of MV --" measles virus "-- RNA from several
15 measles virus structural-gene products," and I won't
16 go further. Where is the immunosuppression? How does
17 immunosuppression play a role in this? I heard a lot
18 of testimony about immunosuppression.

19 A I gave essentially basic aspects related to
20 immune dysfunction, immune dysregulation, and immune
21 deficiency, and one of the contraindications for MMR
22 is an individual that is immunosuppressed. So I
23 really didn't discuss aspects relate to
24 immunosuppression.

25 I think it's best addressed by the clinical

1 immunologist, but certainly I provided aspects that
2 this is how immunosuppression, immunodysfunction, and
3 immunodisregulation can occur; what are the
4 environmental triggers; what are factors; and what is
5 the difference between a congenital or primary versus
6 a secondary or acquired?

7 Q In your hypothesis, do you need immune
8 suppression?

9 A I would say it definitely would help, yes.

10 Q So you do need it. Is that a "yes," you do
11 need it, or, in some circumstances, you don't need
12 immune suppression?

13 A I would say that immune suppression is one
14 of the environmental requirements or factors that are
15 necessary to allow persistence of the virus, and the
16 virus must persist for the scenario to occur.

17 Q So, in the absence of immune suppression, in
18 your postulate, or your hypothesis, you wouldn't hold
19 to that.

20 A I would say it's less likely, yes.

21 Q Then are you still saying it's going to
22 happen?

23 A Have you ever heard a scientist say anything
24 is a hundred-percent certain?

25 Q I'm not asking for 100.

1 A Okay.

2 Q I just want to make sure I understand your
3 theory, that's all. So do you need immune
4 suppression, in your theory, to be of the opinion
5 that, more likely than not --

6 A Yes.

7 Q -- that MMR vaccine caused ASD.

8 A That measles virus persisted, and this
9 caused problems associated with ASD, yes.

10 Q What level of immune suppression do we need?

11 A From my assessment, I think that's probably
12 best left for a clinical immunologist.

13 Q I understand from your testimony that there
14 are different kinds of immune suppression. What kind
15 of immune suppression do we need?

16 A I would say that anything that would affect
17 the induction of an effective vaccine, MMR-induced,
18 protective immune response.

19 Q Let's break that down.

20 A Okay.

21 Q What would prevent an effective vaccine
22 immune response?

23 A It could be holes in the innate immune
24 system. It could be holes in the adaptive, the
25 humoral immune response. It could be holes in the

1 cell-mediated immune response. It could be holes,
2 from the standpoint of the induction of any of the
3 products necessary. It could be a skewing of the
4 response that would not provide normal protective
5 immunity. It could be the lack of production. It
6 could be the overproduction.

7 Q So any of them may play a role. If any of
8 them are present, would you find that it's more likely
9 than not?

10 A Yes.

11 Q So it doesn't matter which one of them.

12 A No.

13 Q And you don't need more than one. You can
14 have any of them.

15 A You can have any of them, or you can have
16 several of them.

17 Q What if you have some of them working and
18 others not? Does that make it less likely that there
19 is immune suppression that would lead to viral
20 persistence of ASD?

21 A No. That's why the term "immune
22 dysfunction" or "dysregulation," meaning not
23 everything is operating properly.

24 Q Okay. So just any at all.

25 A Yes, but there are probably specific ones

1 that one would anticipate, based on the biology of the
2 measles virus, that might be occurring.

3 Q As I understand it, your testimony is about
4 somebody who does not mount an effective response to
5 the vaccine.

6 A Correct.

7 Q How do you measure an effective response to
8 the vaccine?

9 A There are a number of serologic, virologic,
10 and immunologic assays that can be used.

11 Q Do you want to name those assays?

12 A Viral neutralization assay.

13 Q Any others?

14 A Indirectly, a cytotoxic T cell assay
15 measuring the ability to lyse infected targets with an
16 MHC, Class 1, matched target.

17 Q Any others?

18 A Indirectly, an ELISA specifically for the
19 neutralizing epitopes found on the H gene product.

20 Q Any others?

21 A There's more complex ones. There's T cell
22 assays based on flow cytometry, the induction of
23 proliferative responses specific to a protective
24 epitope that is MHC Class 1 matched to one of the
25 measles virus proteins that are components thereof.

1 These tend to be very specialized assays, not
2 routinely used.

3 Q What's the one that we most commonly use to
4 test whether there has been an effective immune
5 response to a vaccine?

6 A We really don't measure that. What we tend
7 to measure is an ELISA where you use the whole virus
8 components, and you're measuring the antibody response
9 to it.

10 Q Antibody response.

11 A Antibody response. But we don't know if
12 those antibodies are protective or not.

13 Q And that would be a sign that there has been
14 an effective immune response to the vaccine.

15 A No. That would be a sign that there is an
16 immune response to the vaccine.

17 Q Now, why is it that you believe that there
18 is immunosuppression when someone is introduced to an
19 MMR, or when MMR is introduced to someone?

20 A There's citations from the literature.
21 There's a number of citations that deal with measles
22 virus being immunosuppressive. I pretty well think
23 that if it occurs in medical and graduate student
24 textbooks, that it's immunosuppressive, that it's
25 pretty much considered dogma by a majority of people

1 learned in the art and field.

2 Q You heard that my question was about measles
3 virus -- I think I said "vaccine virus." I'll
4 clarify.

5 What support is there that measles vaccine
6 virus causes immunosuppression.

7 A There's some publications in the literature.

8 Q Could you name the most recent?

9 A No, because I stuck with textbooks and older
10 ones, but I can certainly name one that I cite in my
11 report.

12 Q And what one is that?

13 A Fireman, 1969, I believe, but I would have
14 to get a copy of my report to verify that.

15 Q Was that MMR?

16 THE WITNESS: I would have to get a copy of
17 my report. If you can bear with me.

18 (Pause.)

19 THE WITNESS: In 1969, it would be very
20 difficult for it to be measles MMR vaccine, wouldn't
21 it? So that would be measles virus, the Fireman
22 article. I apologize.

23 BY MR. MATANOSKI:

24 Q Measles vaccine virus. Right? Is that what
25 it's about?

1 A The measles virus.

2 Q Measles virus itself?

3 A Yes. A 1969 publication would, most likely,
4 not deal with MMR being immunosuppressive. Would you
5 agree?

6 Q My question was to you, and I thought it a
7 little surprising when you --

8 A Measles virus. I stand corrected.

9 Q Any other literature support for the notion
10 that measles vaccine virus causes immunosuppression?

11 A Yeah. I would go ahead and cite the chapter
12 from the Plotkin and Mortimer Vaccine, 1994, the
13 chapter on measles MMR vaccine. Do I know the most
14 recent? No.

15 Q Anything else that you can think of today?

16 A That would be it right now.

17 Q Do either of those references tell us what
18 level of immunosuppression there would be after
19 measles vaccine virus?

20 A No. They really don't. This is with the
21 vaccine. They really don't discuss what levels.

22 Q Do either of them say whether those levels
23 of immune suppression are clinically relevant? Do you
24 know what I mean by that term?

25 A No.

1 Q Do they have any meaning for the host, for
2 the body that receives them, of any significance?

3 A I would counter that any level of
4 immunosuppression has some clinical relevance. Has
5 the clinical relevance for that been defined? I would
6 say no.

7 Q Does the immunosuppression that you say are
8 in these references; do they indicate that the body,
9 or the host that receives the vaccine, is unable to
10 mount a response to the vaccine virus itself?

11 A In some of the instances, it is not clear
12 what sort of vaccine-induced responses were generated.

13 Q I don't think I followed your response.

14 A So, in other words, the studies were not
15 done in a careful manner to address those questions.
16 So, in other words, they looked at total ELISA, but
17 they didn't look at responses to specific epitopes.
18 They didn't measure T cell responses from a specific
19 measles virus, recall response. They looked at
20 things like general overall immune function.

21 Q What did they find when they looked at
22 general overall immune function? Any problems with
23 clearing measles virus from the vaccine?

24 A They did not specifically examine to see if
25 the measles virus persisted anywhere. What they did

1 was they correlated the presence of an immune response
2 with that being an effective immune response, and,
3 therefore, getting rid of the measles virus.

4 As far as the normal situation in a healthy
5 host, the measles virus is cleared.

6 Q So these two references don't support the
7 notion that immunosuppression plays a role in
8 persistent measles virus infection in the body.

9 A These references actually do mention,
10 actually state, that immunosuppression, as the result
11 of exposure, can result in a persistent infection.

12 Q A persistent measles virus infection from
13 the vaccination.

14 A Okay. You're tripping me up.

15 Q No. I'm not trying to, Doctor.

16 A Okay. So from the MMR, does it persist as
17 the result of immunosuppression? That really has not
18 been addressed or looked at.

19 Q So they don't support that notion.

20 A They don't support that because the
21 technology, at that time point of that reference I
22 gave you, was not there to be able to look at it.

23 Q Is that what they said?

24 A No. That's what I said.

25 Q You see, because it seems that important to

1 your theory is that it not just immunosuppress but
2 that it immunosuppress in such a way that the vaccine
3 virus continues to persist. Correct?

4 A But I'm not saying that it's necessary for
5 the vaccine to cause the suppression. I'm saying
6 anything can cause the suppression, any environmental
7 trigger, and if you go back to the material I
8 presented, I mentioned that measles virus is an
9 environmental factor that can cause immunosuppression.

10 My premise does not deal with MMR being
11 responsible for immunosuppression; my premise deals
12 with any environmental trigger that can result in
13 immunosuppression can set up the potential for an
14 ineffective immune response that then leads to
15 persistence.

16 Q So the MMR vaccine itself, the
17 immunosuppression involved in it, is not integral to
18 your theory.

19 A No, but if it happens, it helps.

20 Q But we don't have support that it happens.

21 A We have support in that chapter that says
22 that --

23 Q -- that it allows the vaccine virus to
24 persist.

25 A -- that it can cause immunosuppression.

1 Q Of any clinical relevance to the vaccine
2 virus.

3 A Of clinical relevance to the vaccine virus?
4 So do you mean, is it clinically relevant to --

5 Q -- to the persistence of vaccine virus?

6 A That is not stated in there, no.

7 SPECIAL MASTER HASTINGS: All right. Just
8 for the record, before we leave this topic, the two
9 references that Dr. Kennedy cited -- the Fireman
10 article and the excerpt from the Plotkin text -- are
11 both cited at page 10 of Dr. Kennedy's report, and the
12 articles actually are in the record at Exhibit 112,
13 Tabs D and N. Go ahead, Mr. Matanoski.

14 MR. MATANOSKI: Thank you.

15 BY MR. MATANOSKI:

16 Q I have some further questions on the vaccine
17 virus. The MMR contains an attenuated measles
18 vaccine.

19 A Yes.

20 Q I'm sorry. Attenuated measles vaccine
21 virus. Right?

22 A Right.

23 Q And you mentioned briefly what was meant by
24 "attenuated." Could you explain to me what process is
25 involved to attenuate that virus?

1 A So, essentially, they take what was a wild-
2 type measles virus and passage it multiple times in
3 host cell tissue culture that allows it to replicate,
4 and the more that you allow it to replicate, it
5 becomes less virulent, less pathogenic, and
6 attenuated. It doesn't replicate as strongly.

7 Q What kind of host cell do they use?

8 A They usually use some sort of a monkey
9 kidney fibroblast as standard, hopefully not
10 contaminated with other viruses like SV 40.

11 Q You said it doesn't replicate as well.

12 A Right.

13 Q So if I'm understanding that part of your
14 testimony -- I'll take a step here -- it wouldn't
15 replicate as well as the wild virus.

16 A Right, as the wild type.

17 Q So it wouldn't proliferate as readily in the
18 body.

19 A It should not, no.

20 Q Turning back to your theory, after this
21 immunosuppression, there is an introduction of the
22 virus. What happens next?

23 A As far as --

24 Q -- your theory.

25 A -- this case or --

1 Q You've gotten the vaccine. What happens
2 next?

3 A The virus persists.

4 Q How does it persist?

5 A It's not killed by the normal host immune
6 response, and innate and adaptive immune responses
7 come into play. They are not effective at resolving
8 the infection, but they can be effective at causing
9 other problems affecting bi-standard cells, causing
10 inflammation, causing tissue destruction, causing
11 tissue damage, causing inflammation, so standard
12 things for an innate and adaptive immune response.

13 Q And this will happen in anybody who has any
14 kind of immune dysregulation because you had a whole
15 broad array.

16 A Right. It's individuals that have no
17 functioning immune system, it would be lethal. In
18 those that have a pretty good functioning immune
19 system, it may not be as severe.

20 Q We've got about 50 people, total, in this
21 courtroom, I would say, a rough guess, and if we were
22 to perform an immune test on them now, take all of
23 these functions that you were talking about, Do you
24 have an estimate of about how many would have some
25 abnormality and some immune function?

1 A I would kind of need to know other factors
2 like how much alcohol they had last night, what their
3 liver functions were. So, for me to make a real good
4 guess, I would need some other information.

5 Q So any of these could affect it.

6 A Any environmental trigger.

7 Q And they could --

8 A Did they eat a heavy meal? Can I get the
9 lymphocytes necessary to do the assay? I mean,
10 there's a lot of confounding factors. If you're
11 asking a statistically general question, in an adult
12 population that has an income over \$75,000 a year and
13 has good hygienic scenarios and lived past the age of
14 35, I would say that you probably wouldn't find
15 anybody who exhibited immunodysfunction.

16 Q They wouldn't have any IgG-2 elevation
17 amongst these 50 people.

18 A Well, you're looking, in general, at kids,
19 you know, one in 500.

20 Q One in 500 for what?

21 A For any sort of evident immunodeficiency.

22 Q By all of the tests that you were talking
23 about, it would be one in 500.

24 A About one in 500 for congenital.

25 Q For congenital?

1 A Right.

2 Q Okay. Because you said any environmental.

3 A I was referring to congenital. Okay? From
4 the standpoint of the secondary or acquired, it's
5 really not known.

6 Q Is it fairly common, or is it fairly rare?

7 A It really hasn't been looked at in a lot of
8 detail, from the standpoint of a large population. I
9 don't know how many people in here are HIV infected.
10 If they are HIV infected, there is a good chance that
11 they have some sort of immune dysfunction. I don't
12 know if people have Hepatitis C. So these are
13 confounding factors.

14 Q I just want to know, is that the level of
15 immune dysfunction you're talking about to be
16 significant for causing this problem?

17 A No. It could be anything. I thought I gave
18 you the example of pneumovax, where just a slight
19 alteration affects the ability to resolve an
20 infection.

21 Q Amongst these 50 people, do you think that
22 maybe -- I can tell you, all of my paralegals are
23 below the \$75,000 range in their --

24 A You guys are underpaid. I would demand
25 more.

1 Q Amongst these people, do you think every
2 single one of them is going to have every single panel
3 absolutely normal?

4 A No. There is going to be variation. There
5 is going to be some that are high and some that are
6 low, but will they fall in a median, plus or minus
7 standard error? I would need a biostatistician for
8 that, but I would say there is a good chance that they
9 would. They would fall into some sort of normal
10 range.

11 Q Now, we give MMR to -- I guess we've
12 vaccinated just about over 90 percent of our children.
13 Do you think every single one, except for the
14 autistic spectrum disorder, the kids who eventually
15 would have autistic spectrum disorder, would develop
16 that, whether or not related to MMR, have normal --
17 every single immune panel is normal when they get that
18 MMR vaccine?

19 A No. If you're giving me neonates versus
20 adults, that's a different question.

21 [Pause.]

22 MR. MATANOSKI: Fortunately, I'm moving
23 rapidly through my questions here, Doctor, so this
24 pause is good news for you.

25 THE WITNESS: I would say, take your time,

1 but please don't.

2 MR. MATANOSKI: It's good news for you.

3 BY MR. MATANOSKI:

4 Q Now, in your report, you mentioned
5 thimerosal as part of your thesis of
6 immunosuppression. I didn't see that in your
7 conclusion, your summary here, and I didn't hear it in
8 your testimony this morning. Is thimerosal a part of
9 your theory?

10 A I just mentioned mercury as being an agent
11 that is responsible for the immunosuppression. I
12 mentioned heavy metal exposure, some mercury.

13 Q And is it necessary to have exposure to
14 thimerosal, in your view, to have this
15 immunosuppression or immunodisregulation that could
16 cause a persistent measles virus infection?

17 A I would say that anything that would be an
18 environmental trigger that could cause
19 immunosuppression would be a reasonable part of the
20 immune dysfunction that's necessary for my --
21 actually, it's not really my hypothesis; it's more my
22 plausible explanation on the events that occurred.

23 Q What do you mean, it's not your hypothesis?

24 A As a scientist, my hypothesis would be
25 stated differently, so it's not a hypothesis. A

1 hypothesis is developed before you want to address a
2 question. I had the information, and from that
3 information, I came up with a plausible scientific
4 explanation.

5 Q Okay.

6 A I wouldn't call that a hypothesis.

7 Q So you were actually working from a result
8 and working back to how that result could happen.

9 A Correct.

10 Q So the result was autistic spectrum disorder
11 after exposure to measles/mumps/rubella vaccine.

12 A No. Was measles virus in the gut at a
13 timeframe when I wouldn't anticipate that it would be
14 there if the child mounted an effective immune
15 response to the MMR vaccine?

16 Q And resultant autistic spectrum disorder.

17 A And that, subsequently, based on what
18 measles virus and morbilliviruses can do, could lead
19 to neurologic manifestations.

20 Q So you reasoned back from those facts to an
21 explanation that you think is plausible.

22 A Correct. I didn't have an overriding
23 hypothesis when I looked at this.

24 Q As far as your explanation, is thimerosal
25 necessary?

1 A No, but it is helpful. Anything that is an
2 environmental trigger that causes --

3 Q So we could take out, in this instance, we
4 could take the thimerosal-containing vaccine -- assume
5 that Michelle Cedillo never got it, and your
6 conclusion would be the same.

7 A Mercury and thimerosal is not my area of
8 expertise. So the only thing I know is from the
9 textbooks, that heavy metals cause immunosuppression,
10 and apparently thimerosal is composed of mercury. So
11 as far as relying on what she got at what time point
12 and how that affects, that's not my area of expertise.
13 I would leave that to other experts.

14 Q Okay. So you have no idea what level of
15 immunosuppression you could expect after exposure to
16 thimerosal.

17 A No, I don't, but I know the level that you
18 can expect on general exposure to heavy metals.

19 Q You said in your report, "a potent
20 immunosuppressive." Was it potent in this case?

21 A Potent immunosuppressive from a standpoint
22 of this child? I think I just used it in a general
23 context of thimerosal containing mercury, in general,
24 heavy metals, are potent immunosuppressants. I don't
25 think I used it specifically to say, for this

1 particular individual.

2 Q At what level, in your view, what level of
3 exposure to thimerosal would be necessary for it to be
4 a potent immunosuppressive?

5 A As I stated, I'm not an expert in inorganic
6 chemistry or in heavy metals, per se. The information
7 that I obtained to state that heavy metals are a
8 potent immunosuppressant are textbooks and general
9 knowledge of the field that these do cause
10 immunosuppression, to point out that this is another
11 environmental factor or trigger that could cause it.

12 Q I don't want to belabor the point. So I can
13 take it, then, that what you did is you read some
14 textbooks in preparing your report. Is that right?

15 A Actually, I took my lecture notes from
16 medical school, graduate school, and read some
17 additional textbooks, and made some slides from that.

18 Q So, in preparing your report, in preparing
19 your slides, you took some notes that you had taken
20 back when you were in medical --

21 A No, I wasn't in medical school. No. I
22 teach medical students.

23 Q So, in terms of your statement -- I was just
24 dealing with your statement on potent
25 immunosuppressive -- that's taken from some textbook

1 references.

2 A Yeah, and general knowledge that heavy metal
3 exposure is a potent immunosuppressive, much like
4 chemotherapy is a potent immunosuppressive, much like
5 steroids to prevent transplant rejection are a potent.

6 Q Steroids are potent immunosuppressives? I'm
7 sorry. Did you say --

8 A No. I didn't say steroids. I said,
9 immunosuppressive agents used to prevent transplant --
10 costicosteroids. Sorry.

11 Q Now, I want to make sure I'm clear. You
12 don't feel that you have expertise, though, in the
13 area of heavy metals. Is that right?

14 A From the standpoint of how they function and
15 work, no.

16 Q As an immunologist, do you feel like you
17 have expertise to address how they work on the immune
18 system?

19 A A little bit.

20 Q Can you answer, then, how much, from that
21 standpoint, how much exposure to thimerosal would be
22 necessary to produce a potent immunosuppression?

23 A From a body standpoint, I couldn't. From a
24 test tube standpoint, I could.

25 Q And from a test tube standpoint, could you

1 tell us now how much --

2 A Yeah. You can get down in nanomolar ranges
3 in test tube to exposure to cause an aberration of an
4 immunologic function assay.

5 Q What nanomolar range?

6 A In the low nanomolar range like 10-to-100
7 range.

8 Q Per what?

9 A So nanomolar is a volume. So nanogram --

10 Q Expressed as what?

11 A As the amount added to the culture. So
12 nanomolar is nanogram per measure of volume.

13 Q Okay, exposed to culture.

14 A Exposed to culture.

15 Q And what's this based on?

16 A Publications in the literature that show
17 specific dendritic cell dysfunction.

18 Q Can you tell me right now what those are?

19 A No. I would have to refer to notes that I
20 didn't bring with me or a literature search that I
21 performed, or I'm sure you could ask the clinical
22 immunologist, who would be more in tune to looking at
23 this. This is just based on studies that we've done
24 from immunosuppressive agents in general in the
25 laboratory.

1 Q "We've done"? You've done these studies?

2 A I've done certain ones, not with mercury but
3 with other heavy metals.

4 Q These are published somewhere?

5 A No.

6 Q Why were you doing those studies?

7 A We were actually looking at proteins on HIV
8 that were immunosuppressive, which we did publish on.
9 We didn't include those heavy metal controls because
10 we were interested in what were the proteins of HIV
11 that were causing immunosuppression.

12 Q What heavy metals? I'm sorry. Go ahead and
13 finish.

14 A So we were using essentially lead because
15 that was extremely toxic to the culture, and we had to
16 have some sort of control as a baseline to show that
17 everything was dysfunctional, everything was potent,
18 so we used that as a control, but we never described
19 that in the publication because our interest was
20 looking at HIV, and these were in culture, so in test
21 tube culture.

22 Q Any other heavy metals that you were using
23 in this experiment?

24 A Lead was the easiest one we could get, and
25 we just threw it in there as a control.

1 Q And you knew that this was going to be
2 toxic.

3 A Yeah, at levels that we were also measuring
4 our HIV proteins at, and we found a wide array of
5 dysfunction, including affecting NK cells, lymphocyte-
6 activated killer cells, general immunosuppression, and
7 I have published those, if you go to my C.V., 1987-
8 1998, although if you asked me the details, my memory
9 is not --

10 Q Do you have any idea how long the
11 immunosuppression would last after thimerosal?

12 A In a person who is exposed, it would depend
13 on the amount, how often they got it, the additive
14 effects, age --

15 Q You've given me a number of factors --

16 A -- body fat. The answer is no, not without
17 those factors, and I wouldn't be the one to calculate
18 that. I would ask an expert in that area to calculate
19 that. But there are a number of factors that
20 determine.

21 Q So it's dependent on a number of variables.

22 A Yes.

23 Q And you're not competent, in your area of
24 expertise, to comment on this.

25 A No.

1 Q Now, it's important to your theory that
2 measles virus RNA be detected in the host that is
3 supposed to be harboring the persistent measles virus.

4 A Yes.

5 Q Where do we need to find that measles virus
6 RNA for purposes of your explanation of --

7 A I would say PCR is a good start.

8 Q Not how. Where?

9 A Where?

10 Q What part of the body?

11 A I would say --

12 Q What material from the body?

13 A I would say, in this particular instance
14 that we're focusing on, I think a gut sample, gut
15 tissue, gut biopsy, from the area of where the
16 inflammation was noticed, so at the site.

17 Q Why the gut?

18 A Because that's where the problems were
19 caused. That's where they did the biopsy, and that's
20 where they found it, and that's where I would not
21 expect it to be found.

22 Q Why wouldn't you sample the brain?

23 A Pardon?

24 Q Why wouldn't you sample material from the
25 brain?

1 A For ethical reasons. It's pretty tough to
2 do a brain biopsy.

3 Q I understand that. I understand that.

4 A But if you do it on dogs, you can find it in
5 canine distemper.

6 Q If your theory is that the virus is causing
7 autism, where would you expect the virus to persist to
8 cause a problem?

9 A I think the fact that it persists and causes
10 inflammation in a specific area and that the immune
11 response is a global, systemic issue, that problems in
12 one area can manifest themselves as problems in other
13 areas.

14 Q So, in your theory, then, if you have a
15 problem in your gut, you're going to have a problem in
16 your brain as well.

17 A You could, yes, because these are
18 neurotropic viruses.

19 Q But that means that they are in the brain.

20 A That's neurotropism, yeah.

21 Q Yes. So you need the virus to be in the
22 brain to cause the problem.

23 A I didn't say that. I said they are
24 neurotropic viruses. I'm saying if --

25 Q I'm just trying to understand your theory.

1 If it's just in the gut, and I'm trying to figure out
2 how now that's going to be infecting -- giving a
3 problem that's going to affect -- in the brain.

4 A Inflammation, the inflammatory response, can
5 be local, but it can also be systemic. The immune
6 response can be local, but it also is systemic. If
7 you didn't get a systemic immune response, then you
8 wouldn't be able to clear the MMR vaccine because it's
9 found in multiple places as it goes through the
10 process of inducing an effective immune response.

11 Q So, just to understand, in your construct,
12 we just have the persistence in the gut, but it
13 creates an immune effect that is felt throughout the
14 body.

15 A That's one possibility.

16 Q Is this your explanation, though?

17 A No.

18 Q What's your explanation?

19 A My explanation is that if it's found in an
20 unlikely place, such as the gut, there is evidence
21 that suggests that it can be found in other places,
22 like the CSF and the brain, and then that can cause
23 more neurologic manifestations than one may see just
24 as a result of the systemic immune response gone
25 haywire.

1 Q So, in other words, really, your explanation
2 is that it's not just in the gut, but it's, at least,
3 throughout the body to your brain.

4 A What I'm saying is this virus, and group of
5 viruses, has the capability of doing that.

6 Q And do you think that that's what's, more
7 likely than not, happening, as far as your
8 explanation?

9 A Without having the information and data on
10 Michelle, from a standpoint of the presence or
11 absence, I can't say that specifically, but I know of
12 instances where that can occur, and that can result in
13 neurologic disorders.

14 Q Now, we'll turn to those instances, and I'm
15 still just trying to figure out, to make sure that I
16 understand your theory. Is your theory, overall, that
17 the virus has disseminated through the body? Is that
18 what you think is happening, more likely than not,
19 after this immune immunosuppression or immune
20 dysfunction that permits the virus to persist?

21 A That's a plausible explanation but not an
22 absolute explanation.

23 Q Is that your explanation?

24 A My explanation would be dependent on what
25 information was available. The information I have

1 available suggests that it's a plausible explanation.

2 Q If I take that way, if I tell you that it's
3 only in the gut, and it's nowhere else in the body, do
4 you still feel that it's, more likely than not, that
5 the persistent measles virus is causing autistic
6 spectrum disorder?

7 A I would say, yes.

8 Q And what's the basis for that opinion?

9 A The immune response and the systemic
10 inflammatory response. I'm saying it's a possibility.
11 Is it the most likely possibility? No. Is it more
12 likely than not? Yes.

13 Q So, for you, just recovery -- if I could
14 show you that it wasn't in the brain, it wouldn't
15 change your opinion, if you could recover measles
16 virus from the gut.

17 A I would say that there is a possibility that
18 that set up the overall systemic response that caused
19 --

20 Q More likely than not, would you say that it
21 caused the autistic spectrum disorder, if I told you
22 that I could not recover measles virus, or I had no
23 evidence that it was in the brain?

24 A More likely than not, I would say yes, but
25 if you gave me measles virus in the brain, then I

1 would say, "Guess what? I'm right."

2 Q So you still would say, even if there was no
3 evidence that it was in the brain, you would say it's,
4 more likely than not --

5 A There's a lot of things that can go on from
6 the presence of having foreign viral RNA activation of
7 toll-like receptors. There's all sorts of
8 inflammatory conditions in cytokines that can be
9 secreted that cause systemic issues, just from a
10 standpoint of having the presence of foreign RNA,
11 viral RNA.

12 Q Doesn't that happen in numerous occasions?

13 A But in normal people, it's not an issue. In
14 people that have a skewing immune dysfunction, then it
15 can become an issue.

16 Q Okay. Do you have any examples of viral
17 persistence of measles virus, other than when you've
18 recovered it from the gut biopsy tissue? Are you
19 aware of any? Are there other examples?

20 Let's say you don't have any recovery of
21 measles virus from gut biopsy tissues. Are there
22 other examples of persistence of measles virus?

23 A Yes.

24 Q And you were talking about the other
25 morbilliviruses.

1 A Yes.

2 Q Are there examples of persistence in those
3 instances?

4 A Yes.

5 Q In those instance, turning to the
6 morbilliviruses, what's the outcome when the virus
7 persists?

8 A Usually, from the standpoint of a dog, it
9 has a temper problem, issues. It's deranged, and it's
10 usually put to sleep.

11 Q And if it weren't put to sleep, what would
12 happen?

13 A Probably those neurologic manifestations
14 would result in death.

15 Q And in the other instances, the phocine?

16 A That was found in seals that had died.

17 Q And in the morbillivirus that was affecting
18 dolphins, what happened?

19 A Again, found in dolphins that died, but,
20 again, you usually don't do autopsies on live animals.

21 So if they are floating around in the ocean, it's
22 kind of hard to know what the level of infection is.

23 There are examples of North Sea seals that recovered
24 from exposure to morbilliviruses.

25 Q Is it persisting when they have recovered

1 from it?

2 A They actually resolve the infection.

3 Q So when they have resolved the infection,
4 there is not a problem.

5 A I'm not a behaviorist, from the standpoint
6 of what the long-term effects would be on dolphins and
7 other animals like that, but I can imagine that, in
8 some instances, there may not be issues; in others,
9 there may be issues. The brain is a very touchy
10 thing.

11 Q What do you know about measles virus, what
12 it does on a cellular level?

13 A Quite a bit. It activates cell-mediated
14 immune responses. It activates T cells. The studies
15 that have been done that really well characterize
16 measles virus have been done in experimental animal
17 model systems, such as mice, and when they have set up
18 the infection in mice to examine what is required for
19 protective immunity, they having injecting measles
20 virus at doses that cause CNS disease, and they
21 eventually have to euthanize the animal, and they use
22 some sort of scale on when to euthanize the animal,
23 because the legs are dragging or some other issue.
24 But certainly it's shown clearly that cell-mediated
25 immune responses to measles virus are important for

1 protective immunity.

2 Q How long will it take for this persistent
3 measles virus to begin damaging the brain?

4 A I don't know. In a human? In a mouse, I
5 know.

6 Q I'm sorry?

7 A In a mouse, I know. I don't know in a
8 human. Ten days is standard in a mouse, depending on
9 the route. You can get it faster, if you want to give
10 a higher dose.

11 Q And how long before the mouse has to be
12 sacrificed because of the level of neurologic damage?

13 A Actually, it's really dependent on the
14 institution and what is required by their
15 institutional animal care and utilization committee
16 for pain and suffering of animals. So it's pretty
17 much dependent. Some people can --

18 Q What's the longest amount of time?

19 A Depending, if you give a low dose, and you
20 want it to persist, you can bring it out to 40 days.
21 If you want to give a high dose and want it to
22 manifest quickly with CNS disease, 10 days.

23 Q Ultimately, the course for those mice would
24 be death soon after --

25 A Yes, convulsions, death, yeah.

1 Q -- whether you sacrifice them or not.

2 A Whether you euthanize them or not, yeah.

3 Q You can't tell me how long the process
4 would be of neurotropism in a human.

5 A No, I can't.

6 Q Do you still have your expert report in
7 front of you? I'm sorry, not your expert report; your
8 slide presentation?

9 A Yeah. I sure do.

10 Q Could you turn to page 15 of that? You're
11 talking about PCR and recovery of measles virus. When
12 we're doing the PCR, what part are we amplifying here,
13 the red, the blue, the green?

14 A The blue.

15 Q And that's the blue at the top line would be
16 numbered right with the red at number one.

17 A Yeah.

18 Q And you said that that was positive.

19 A A positive stream.

20 Q In your view, as an immunologist, is a
21 single test enough to determine whether or not there
22 has been an immune dysregulation?

23 A Yes.

24 Q And in your view, just having the IgG-2 is
25 enough for immune dysregulation, that that will permit

1 persistent virus.

2 A Not only the IgG-2, but the fact that it
3 still persists tells me that whatever immune response
4 was made wasn't effective.

5 Q If I take away the evidence that there is
6 still persistence, if I were to take that away, and
7 you just had the IgG-2, would you think that there is
8 still persistent measles virus?

9 A If you took away the persistence, no, then
10 there wouldn't be persistence measles virus.

11 Q If I took away the recovery of measles virus
12 genomic material?

13 A I would have to know if that IgG-1 and IgG-2
14 are viral neutralizing. If they were viral
15 neutralizing, then I would say there is not a problem.

16 Q So you said the IgG-2 level was off in this
17 case.

18 A Was elevated.

19 Q Was elevated. If you didn't have recovery
20 of measles virus genomic material, if that was called
21 into question, would you still be of the opinion that
22 there is persistent measles virus?

23 A So if you don't have measles virus RNA
24 there, and you had no evidence of measles virus being
25 there, but you have an elevated IgG-2A response, which

1 I'm not even sure is specific toward measles virus,
2 would I still think that's persistent?

3 Q That's right.

4 A No. If you don't have measles virus RNA
5 there, why would you anticipate that it has anything
6 to do with measles virus?

7 Q So you really do need recovery of measles
8 virus RNA.

9 A Yes.

10 Q Now, in the one article that you published
11 in 2004 on MMR vaccine -- again, that was your first
12 article on MMR and measles virus. Right?

13 A Correct.

14 Q At the time you published that, you were a
15 consultant in the U.K. litigation, MMR litigation. Is
16 that right?

17 A No. That was over with.

18 Q Okay. So you had been a consultant before
19 then.

20 A Correct.

21 Q Was the litigation over with, or was your
22 involvement with it over with?

23 A My involvement was over, and I think -- I
24 don't remember the timeframe, but I think the
25 litigation was over with. I would have to refresh my

1 memory.

2 Q When did your involvement end?

3 A When I filed my expert report.

4 Q When was that?

5 A Again, I would have to look, but I would say
6 the timeframe would be 2002-2003.

7 Q You took parts of that report that you
8 published and used it in your expert report in this
9 case. Is that right?

10 A I used information that I obtained to do my
11 expert report to provide certain aspects from my input
12 and also from the other co-authors, their input, to
13 provide that article, yes.

14 Q I'm talking about the expert report. You
15 took parts of your article in 2004 and placed them
16 into the expert report that you filed in this case.
17 Correct?

18 A The article in 2004 was actually published
19 after the expert report.

20 Q I'm sorry. I can see the confusion. Not
21 your expert report in the U.K. litigation, your expert
22 report here in this case.

23 A Actually, to be honest with you, I couldn't
24 find any reprints of that paper because my secretary
25 had moved my office. So I had to go without having

1 access to that paper to do this. So --

2 Q So you didn't take parts of your 2004 paper
3 and use them in this report.

4 A I didn't have access to it because the
5 reprints that I had were in a file somewhere, but I
6 had excerpts that I used to essentially develop. So,
7 to answer, did I use that manuscript, the published
8 paper, word for word, I used excerpts that I had,
9 which were probably from formulating the opinion or
10 opinions or information from those, for instance, the
11 section on morbillivirus.

12 Q You used those word for word. Right?

13 A I would have to look to see if I used it
14 word for word. Hopefully, I would have changed it
15 little bit. I think the common premise would be
16 identical, that they are neurotropic.

17 Q In your paper in 2004, you had parts in
18 there that you didn't use in your report here.

19 A Right.

20 Q Actually, I guess we can show you, if you
21 would like, side by side, word for word, where parts
22 of your paper are repeated in the report here.

23 A Is that illegal? I mean, it's my reports.

24 Q No, it's not illegal, sir. Actually, it's
25 the next point that I want to make that this becomes

1 important because it's the parts of your paper that
2 you left out that I think we want to focus on now,
3 that you left out of this report.

4 In your paper, you acknowledge that, based
5 on your review of published research --

6 MS. CHIN-CAPLAN: Would you give him a copy
7 so he could follow along?

8 THE WITNESS: Do you have a copy? I don't
9 have a copy. How long was the paper? How many pages
10 was the paper? Twenty-eight?

11 MS. CHIN-CAPLAN: He is reading from the
12 publication.

13 MR. MATANOSKI: Actually, I'm reading from
14 my notes.

15 (Pause.)

16 SPECIAL MASTER HASTINGS: Mr. Matanoski, is
17 this paper in the record?

18 MR. MATANOSKI: I believe it was submitted.

19 THE WITNESS: It's cited in my expert
20 report.

21 MR. MATANOSKI: It would have been a
22 Petitioner exhibit.

23 (Pause.)

24 MR. MATANOSKI: We're going to pull it up on
25 the screen so that you can take a look at it there.

1 BY MR. MATANOSKI:

2 Q In your paper, you acknowledged, based on
3 your review of the published research, population-
4 based studies had not been able to detect a link
5 between MMR vaccination and autistic spectrum
6 disorder. Is that right?

7 A Correct.

8 Q That didn't find its way into your report
9 here.

10 A Was it relevant to my report? I don't
11 believe my report says anything about autism, does it?

12 Q That's the issue that we have to decide
13 here.

14 A Well, I'm the immunologist and the
15 virologist, so I focused on the immunology and the
16 virology. I agree to that statement. That statement
17 is supported by the literature and by the IOM/NAS
18 report, so I agree with that statement.

19 Q So do you hold an opinion as to whether or
20 not MMR vaccine is causing autism?

21 A Yes. I have an opinion.

22 Q Is it?

23 A I don't know. Is it plausible? Yes. Is it
24 absolutely? No.

25 Q Is it, more likely than not, causing autism?

1 A It would have to be examined on an
2 individual-to-individual instance, if that makes
3 sense.

4 Q So you believe it is.

5 A I believe it might and can, under the
6 appropriate conditions. Do I believe it is in all
7 instances? Absolutely not.

8 Q You believe that it is, or do you believe
9 it's not?

10 A On an individual-to-individual situation,
11 that it could.

12 Q So that it could. Is it, more likely than
13 not, doing it?

14 A Yes, in an individual-to-individual
15 situation. Do I say MMR causes autism? Absolutely
16 not. Can it, in an individual that has the following
17 considerations: persistent measles virus infection,
18 present in the gut? Yes, it could.

19 Q So, in your opinion, is MMR responsible for
20 an epidemic of autism?

21 A No.

22 Q In your opinion, is it responsible for 5,000
23 cases of autism?

24 A I'm not an epidemiologist, so I can't --

25 Q In your opinion, would it be, then, you only

1 could tell on a case-by-case basis?

2 A I would say that you would need to look at
3 instances on a case-by-case basis.

4 Q Would it be rare?

5 A You know, I'm not really an expert in
6 neurology or autism, so I don't know what you mean by
7 "rare."

8 Q Well, you're offering an opinion that, on a
9 case-by-case basis, it can.

10 A Yeah.

11 Q How often will we see it?

12 A It would depend on the conditions of that
13 case. You would have to find measles virus. They
14 would have to have an immune response to the measles
15 virus, and that immune response didn't clear it.

16 Q So you would have to find a measles virus in
17 the individual.

18 A You would have to have a set of
19 characteristics and criteria that I would need to
20 state that, yes, it's possible for, in this situation,
21 measles/mumps/rubella vaccine to cause neurologic
22 disorders.

23 Q In your view, would one of those criteria
24 that have to be fulfilled be recovery of measles virus
25 genomic material from the individual?

1 A I would say --

2 MS. CHIN-CAPLAN: Special Master, I think
3 Dr. Kennedy has answered this question several times
4 in different manners and reformulated and reformulated
5 it again. It's been asked and answered.

6 SPECIAL MASTER HASTINGS: I'm not confident
7 that that's the case, so I'll let them go on.

8 THE WITNESS: So, yes, you need to find
9 measles virus. RNA is a good start.

10 BY MR. MATANOSKI:

11 Q Is it necessary to do that, in your opinion,
12 to reach the conclusion that MMR caused autism
13 spectrum disorder?

14 A In my opinion, from the standpoint of
15 measles virus vaccine being directly involved, yes.
16 Indirectly, I can't say that.

17 Q I'm not sure I understand you, sir.

18 A So all of the other circumstances that we've
19 talked about -- the presence of immune dysfunction,
20 the presence of nonspecific inflammation -- I would
21 have to look and see how did the individual respond to
22 the vaccine, how much other factors were involved.

23 So you're asking me to give a global
24 statement that I can't give. The best that I can say
25 is, on an individual basis, that direct evidence is

1 the best.

2 Q It's just an easy question. Do you need it,
3 or do you not need it?

4 A You need it for direct evidence. Is it
5 possible that it could occur in another manner,
6 indirectly, through inflammation, et cetera? Yes.
7 It's also possible that way.

8 Q How does the inflammation occur without
9 persistent measles virus?

10 A Through a number of mechanisms.

11 Q And this would not be related to MMR, then,
12 without persistent measles virus.

13 A Persistent measles virus is one form that
14 can cause inflammation. MMR that doesn't persist,
15 when it was resolved, could have set up an
16 inflammation, could have set up a fever, could have
17 set up an issue, been resolved late, if no longer
18 persistent, but it set up a scenario that allowed the
19 immune system to get haywire, so that's another
20 possibility. Less likely, but it's still a
21 possibility, indirect cause.

22 Q Is it more likely than not?

23 A I would say it's less likely. That's the
24 opposite of what you're saying.

25 Q So it's not more likely.

1 A It's not more likely. The indirect --

2 Q It falls below the 50-percent threshold.

3 A Correct, but it's still possible.

4 Q So in your view, MMR -- it's possible that
5 MMR can cause autistic spectrum disorders even if the
6 virus doesn't persist?

7 A In some circumstances, it's possible. But,
8 again, it's the -- that it's not the most likely than
9 most, though, the low side?

10 A It's below 50 percent?

11 Q Yes.

12 Q Now, turning back to your paper, you said in
13 your review that large gaps remain in our
14 understanding of the risk factors and etiologic
15 mechanisms of ASD; isn't that right?

16 A Correct.

17 SPECIAL MASTER HASTINGS: Where were you
18 reading from?

19 MR. MATANOSKI: This is page 132.

20 SPECIAL MASTER HASTINGS: 132 of the paper?

21 MR. MATANOSKI: The 2004 paper.

22 SPECIAL MASTER HASTINGS: Okay.

23 MR. MATANOSKI: These are all a series of
24 questions that I will asking about. Parts of the
25 paper, it didn't make their way into the expert

1 report, in this case.

2 SPECIAL MASTER VOWELL: Can you identify
3 that by exhibit number for us, Mr. Matanoski?

4 MR. MATANOSKI: Certainly.

5 (Discussion held off the record.)

6 MR. MATANOSKI: I am sorry. We thought that
7 this had been filed, but apparently it's not been
8 filed by the Petitioner, this report. We will file
9 this as a trial exhibit. I apologize. We assumed
10 that a report by the expert --

11 SPECIAL MASTER VOWELL: That is something
12 from my computer.

13 (Laughter.)

14 SPECIAL MASTER HASTINGS: No wonder we
15 couldn't find it. All right.

16 MR. MATANOSKI: I apologize.

17 SPECIAL MASTER HASTINGS: That's okay. Why
18 don't you file it as Respondent's Trial Exhibit 3.

19 MR. MATANOSKI: We will do so, sir.

20 SPECIAL MASTER HASTINGS: Okay.

21 (The document referred to was
22 marked for identification as
23 Respondent's Trial Exhibit
24 No. 3 and received into
25 evidence.)

1 THE WITNESS: Yes, I agree with that
2 statement.

3 BY MR. MATANOSKI:

4 Q And you, also, state in your report that
5 'investigators have also failed to demonstrate
6 causality between MMR vaccination and autism?'

7 A Yes.

8 Q Do you agree with that statement?

9 A Absolutely.

10 Q You stated in your report that 'conflicting
11 data exists regarding the studies reported by
12 Wakefield and colleagues' and 'purported to find
13 persistent measles virus' --

14 A It's straight from the published literature.

15 Q And so, you agree with that statement?

16 A Oh, absolutely.

17 Q And you wrote 'there is little evidence to
18 support persistent infection by the vaccine strain of
19 measles virus except for individuals with a
20 compromised immune system, where all immune
21 dysfunctional.' You agree with that?

22 A Yes.

23 Q You, also, wrote 'the biological model
24 linking MMR and autistic spectrum disorder remains
25 incomplete;' correct?

1 A Correct.

2 Q And you agree --

3 A We don't have any good animal models that
4 would allow us to approach these issues in a direct
5 manner.

6 Q You agree with that -- and you agree with
7 that statement?

8 A Oh, absolutely.

9 Q And you still agree with that statement?

10 A Yes. You guys didn't find my typing errors
11 in there?

12 (Laughter.)

13 Q Just moving right along here.

14 A Getting back to Lubbock, my airlines is not
15 easy, so I've got plenty of time.

16 Q In your 2004 report, you stated, you would
17 need to find 'detection and isolation of the vaccine
18 strain of measles virus in the gut of inflicted
19 children' to be persuasive evidence of a biologic
20 model linking MMR to ASD.

21 A Right. That was in reference to trying to
22 develop an animal model that would closely mimic the
23 human scenario and also was related to Cox's
24 postulates.

25 Q In review today, is that what is necessary

1 to find persuasive evidence of a biologic model
2 linking MMR to ASD?

3 A For something to say that this is a relevant
4 model of the human situation and disease, yes.
5 Without having that animal model, without being able
6 to show that, then you would have some issues.

7 Q So, other animal models --

8 A Just don't do it.

9 Q And other models, we don't have other
10 models; correct?

11 A You know, canine distemper, I don't know too
12 many dog psychologists that figured out if they've got
13 canine distemper, they're autistic dogs or not. So,
14 that's why I kind of stick with neurologic
15 manifestations, if it affects the brain, and it can
16 cause a number of manifestations.

17 Q You referenced in your opinion in this case
18 personal communications between you and Dr. O'Leary?

19 A Yes.

20 Q Were those the -- is that the meeting that
21 you discussed earlier in your testimony?

22 A Yes. In fact, can I take this time to
23 clarify? I called Dr. Sheils, the pathologist. She
24 is actually not the pathologist. It's Dr. O'Leary.
25 He's the pathologist. Thank you.

1 Q You said that that meeting took place in
2 2002 in the United Kingdom?

3 A Yes, to the best of my memory, 2000 -- early
4 2002, could be late 2001. I'm trying to remember my
5 transition from Oklahoma to Texas Tech. It was in
6 that time frame.

7 Q Where in the United Kingdom did that meeting
8 take place?

9 A It was in London.

10 Q Do you recall when in 2002, 2001 that would
11 have been?

12 A I would have to look back at my travel. I
13 was doing a lot of travel in transition, so I can look
14 back and report back to you on the specific dates. I
15 tried to jog my memory. And I've been away from
16 Lubbock for about two-and-a-half weeks now, so I
17 haven't had a chance to go back and look and check to
18 see specifically. But, it was in 2001, fall 2001,
19 spring 2002, somewhere in that time frame.

20 Q Did you meet with him as part of the U.K.
21 litigation of MMR?

22 A Actually, I was invited over there by a
23 colleague to just evaluate the data. And as a result
24 of my evaluation and criticism of the data presented
25 by Dr. Sheils, the U.K. litigators decided that they

1 liked a guy from Texas, who wore a Texas shirt and
2 wore cowboy boots, and they asked me at that time
3 would I be interested.

4 Q So, the litigators were at this meeting?

5 A The litigators did not attend the meeting.
6 They were not at that particular meeting. But after
7 the meeting, that's when we had conversations.

8 Q Did they set the meeting up?

9 A To my knowledge, yes, but they did not offer
10 the invitation.

11 Q I see. They set the meeting up, but the
12 invitation was offered through an intermediary?

13 A Right.

14 Q And who was that intermediary?

15 A John Marchulonis. He is professor in chair
16 at Microbiology-Immunology at the University of
17 Arizona.

18 Q He was working with the litigators?

19 A Yes. I found out later on, yes. But, then,
20 so did I, so --

21 Q When you were meeting with Dr. O'Leary, did
22 he discuss with you any criticisms of the Unigenetics
23 Lab?

24 A Actually, Dr. O'Leary was not physically
25 present at the meeting. It was Dr. Sheils, who was

1 presenting the data, and Dr. O'Leary was available
2 later on for teleconference. So, she presented the
3 data in a scientific format. And over four-and-a-half
4 to five hours, she was asked a number of questions
5 relative to the technology, the standard operating
6 procedures, the immunohistochemistry that was shown,
7 how she detected what were her primers, what were the
8 sensitivity, how was isolation done, what were
9 controls, what were positive controls, how did she
10 know that this was not contamination, what was the
11 samples. She was essentially taken apart by, I would
12 say, three or four extremely good microbiologists.

13 Q Okay. Yourself included?

14 A No. I took her apart, not that I'm an
15 extremely good microbiologist, just from a standpoint
16 of, you know, I wanted to know, you know, is this real
17 or not.

18 Q And Dr. Sheils, she is the colleague of Dr.
19 O'Leary?

20 A Yes.

21 Q And she worked in the Unigenetics Lab at
22 Coombe Hospital?

23 A Yes. I never understood the connection
24 between Trinity, because she was also at Trinity --
25 they were both at Trinity -- and Unigenetics until

1 later on.

2 Q Okay. And what was the connection?

3 A My understanding was that Unigenetics was
4 set up as a for-profit laboratory to do testing for
5 measles virus specifically for this situation to
6 alleviate any legalities and issues and problems that
7 may occur with Trinity College.

8 Q 'This situation' being litigation, correct?

9 A No, just from the standpoint of universities
10 don't like to be involved with biotech companies or
11 whatever, other than to own a little stock on the
12 side. So, they like to set up foundations. That's
13 how they do it in the States. So, if they're a state
14 institution, you would set up a foundation, so you
15 don't break state guidelines from the standpoint of
16 ownership and things related to that. So, I thought
17 it was kind of a standard situation that Trinity
18 College being an old college in the U.K., this was the
19 way that they were going to set up. Instead of
20 setting up a foundation to do it first, they just set
21 up a separate laboratory.

22 Q So, is it your understanding, then, that
23 Trinity College set up Unigenetics or did Dr. --

24 A Dr. O'Leary set it up.

25 Q Because it was a for-profit --

1 A Because it was a for-profit and that is a
2 non-for-profit entity.

3 Q So, he wasn't doing it for the University?

4 A He was -- so, I guess the best way to say
5 it, this is a common practice that universities and
6 institutions keep productive investigators around. So
7 what they do is they allow them to be entrepreneurial,
8 to set up, start up biotech companies, to have
9 ownership in that. The university, also, figures out
10 to have ownership by setting up a foundation. It's
11 not really a university-sponsored company, but the
12 university is aware of it.

13 Q Did the university have any stake in
14 Unigenetics, Trinity College?

15 A I'm not aware of that. I'm not aware how
16 Unigenetics was set up.

17 Q Where --

18 A Lawyers at Chadwick and I don't know how
19 they set up corporations. I don't know if it was a
20 Chapter S, an LLP or --

21 Q So, when you were at this meeting, you were
22 provided with protocols, test results, all the
23 information regarding how Unigenetics was operating
24 and what their results were that were coming out of
25 that lab?

1 A Correct.

2 Q Did they discuss with you the equipment used
3 in the lab?

4 A Yes.

5 Q Did they discuss with you how the lab was
6 laid out?

7 A Yes.

8 Q Did they describe how the samples were
9 prepared?

10 A Yes.

11 Q Did they describe the primers that they used
12 --

13 A Yes.

14 Q -- and their specificity?

15 A Yes.

16 Q Can you think of any information regarding
17 how they were conducting their testing that they left
18 out of that, at that meeting, that they left out of
19 their presentation at that meeting?

20 A We didn't have -- there was one individual
21 at the meeting, who had visited the Unigenetics
22 Laboratory, but we didn't see, for instance, a visual
23 on the layout. We had a description of how isolation
24 was done in this laboratory, the PCR was done in this
25 laboratory, our reverse transcriptase was bought from

1 here, here's how we bought the isolation kits for the
2 RNA, here's how we prepared the samples. So, they
3 actually had standard operating procedures, a list for
4 a lot of the things that they were doing, because I
5 think the anticipation was that this was going to be a
6 long-term testing scenario.

7 Q Was there any information that you felt was
8 lacking that you would need to understand whether or
9 not their test results were reliable?

10 A Actually, we, a number of us asked for a lot
11 of things that weren't presented initially, because
12 they're standard stuff and why aren't they in there.
13 And they were provided to us.

14 Q They were provided to you?

15 A Yes, not necessarily during the initial
16 presentation, but we were able to see a number of
17 things that were of concern about primer specificity,
18 about things like cross contamination, about things
19 like isolation, how you dealt with the tissues, the
20 difference, the dyes used, the machine, the
21 reproducibility of the machine, the standard curves
22 used. So, we got a lot of information that is not
23 necessarily available through the Uhlmann paper.

24 Q Were you satisfied you had everything you
25 needed to determine whether or not it was reliable?

1 A We still had scientific discussions from a
2 standpoint of in some of the assessments relative to -
3 - a lot of us felt very uncomfortable from a
4 standpoint that what they were doing was essentially
5 using cycle number to determine faults -- I mean, to
6 determine a positive versus a negative reaction. We
7 were happy with they were doing quadruplicate samples.
8 So, that was not an issue. But, where we had a
9 problem is that they were pushing the sensitivity of
10 this to the very low end and they were using cycle
11 number, which was not really a standard at the time,
12 which was not used at the time, to determine a
13 positive versus negative. And some of the samples
14 that were on the verge of the limit of sensitivity,
15 there was a large amount of argument over whether
16 these were truly positive or truly negative. And a
17 number of suggestions were made on how do we prove
18 that from a statistical standpoint.

19 Q Who else was at the meeting?

20 A There was Dr. Richard Tedder from the U.K.
21 He's a professor of clinical virology. There were no
22 lecturers present. So, it was all pretty senior
23 people. There was a program chief from the National
24 Institutes of Health, National Institutes of
25 Neurologic Disorders and Stroke, Dr. Steven Jacobson,

1 myself, Dr. Marchulonis, Dr. Sheils. There was a
2 pediatrician molecular biologist, whose name escapes
3 me right now, who was real sharp -- I can come up with
4 that name later -- and one or two others. It was a
5 fairly small, small, intense session.

6 Q How much do you know about PCR?

7 A We do it in my laboratory.

8 Q Do you know enough about it to answer some
9 questions as an expert?

10 A Well, it depends how technical you get.

11 Q Because you were offering an opinion earlier
12 about the validity of the Unigenetics Lab result via
13 Uhlmann.

14 A No. About the Uhlmann --

15 Q The Uhlmann paper.

16 A No. My opinion is based on the fact that it
17 was detected. I relied --

18 Q And you've had --

19 A -- relied my opinion on the O'Leary and
20 Sheils Lab, based on the information that I obtained
21 at this and, also, through PubMed searches, to see
22 that they are being productive in 2006 and 2007 with
23 12 joint co-authored papers in some excellent high-
24 impact peer review journals, such as cancer research,
25 the Journal of Micropathology, et cetera. So, the

1 laboratory is doing their thing.

2 Q So, well, I will ask you some questions
3 about PCR. Before you conduct an experiment doing
4 PCR, do you develop a standard operating procedure?

5 A Yes.

6 Q Would you follow that standard operating
7 procedure throughout the experiment?

8 A There is -- if we're going to do a large
9 amount of samples, we have to set up the conditions
10 first. When we set up the conditions, that, then,
11 allows us to determine what our standard operating
12 procedure will be.

13 Q Okay. If you're going to operate the lab as
14 a diagnostic, essentially a diagnostic lab, would you
15 need a standard operating procedure?

16 A Absolutely.

17 Q Would you vary from that standard operating
18 procedure, if you were doing it diagnostically?

19 A No. And if you did, there would be adequate
20 notes taken as to why it occurred, why it happened,
21 and what was the issue.

22 Q Would it make a difference to you, in terms
23 of the reliability of a result that came out using a
24 RT-PCR, if the quality of the probe varied from batch
25 to batch?

1 A Absolutely.

2 Q And would you think the reliability of the
3 results were less if the probe varied from batch to
4 batch?

5 A So, the question was if the probe was messed
6 up --

7 Q If the probe -- the quality of the probe
8 varied from batch to batch, would you expect the
9 results to be less reliable?

10 A So, your question is, if the probe is a
11 problem, do you expect more variability?

12 Q No. My question is --

13 A Is that the question? I'm confused, sorry.

14 Q It's actually a lot simpler.

15 A Okay.

16 Q Would you call into question the reliability
17 of a PCR test result, if the probe quality varied from
18 batch to batch?

19 A So, if you ran out of probe during --

20 Q No.

21 A -- a large-scale testing, then -- and you
22 substituted new probe from another place without going
23 back and setting up your standard conditions, would I
24 question that? Absolutely.

25 Q No. If you had the same -- if you were

1 using a probe and from batch to batch, the quality of
2 your probe varied --

3 A The same probe?

4 Q Yes, the probe.

5 A The only way it would vary is if you had to
6 have new probe synthesized.

7 Q Okay.

8 A So, that would mean you ran out. So, you
9 would have to reset your conditions, have to order
10 more. So, yes, that -- you don't do that, without
11 resetting your conditions and --

12 Q Would the reliability of the PCR result --
13 would your assessment of the PCR result be less, if
14 the amplification efficiency varied from batch to
15 batch?

16 A So, batch to batch, what are you talking
17 about? Your Taq preliminaries? Your -- what is a
18 batch-to-batch variation?

19 Q If you run one batch --

20 A Right.

21 Q -- and you have a certain amplification
22 efficiency and then you run another batch and your
23 amplification efficiency is different, does that call
24 into question the reliability of the results overall?

25 A Are you running a standard curve at the same

1 time and do you have proper internal controls and have
2 you standardized that --

3 Q So, these are the things that you would need
4 to know?

5 A Right.

6 Q And could you go through, please, all the
7 things that you would want to know, in order to assess
8 whether it was reliable?

9 A I would want to know how it was run. All
10 samples should be run at the same time with a positive
11 and a negative control. It has to have a standard
12 curve in there, has to have the number of internal
13 controls. I know when I publish my PCR technology in
14 peer review publications, I don't write down all of
15 this stuff that's necessary for --

16 Q So, what is it that you would need --

17 A -- people learned in the art normally
18 include, because it's a given.

19 Q If there was amplification variability, what
20 is it that you would want to look at?

21 A I would say the Taq preliminaries is bad. I
22 would want to look at a new Taq preliminaries. It
23 could be primer is bad. It could be a number of
24 different things.

25 Q These are all things that would go to the

1 reliability of the results you would get?

2 A Correct.

3 Q Would you want standardization, that you
4 would use the same amount, extract the same amount of
5 RNA each time for testing?

6 A It depends. So, a number of us use
7 different techniques for quantitation. Some of use a
8 standard housekeeping gene. So --

9 Q I'm talking about the amount that you
10 extract for testing.

11 A So, you mean, the size of the tissue or the
12 --

13 Q The amount of RNA that you're testing.

14 A Do you want -- well, it depends what you are
15 trying to set up.

16 Q If there is variability from time to time --
17 now, you're using the procedure diagnostically and you
18 vary the amount of RNA that you're extracting for
19 testing.

20 A So, you're varying the amount that you add
21 to each of the well?

22 Q Yes.

23 A Is that what you're asking?

24 Q Yes.

25 A That's how you generate the standard code,

1 okay, and that's how you, also, can quantitate,
2 because you have to hit the linear portion of the
3 standard curve to be able to tell how much is there.
4 And that's a standard procedure. So to get the actual
5 quantitation, you need to do --

6 Q I understand you, sir.

7 A -- by different lengths.

8 Q If you vary from your SOP in the amount that
9 you extract, your SOP is set up to tell you how much
10 to extract and you vary from that, does that call into
11 question the result that you receive?

12 A So, the initial amount you extracted is
13 different or the initial amount you had is different?
14 I'm confused.

15 Q Never mind. It's fine, sir. Would you want
16 to see that the test results for negative test
17 consistently came back negative?

18 A It better, yeah.

19 Q And if they didn't, would that call into
20 question the result?

21 A I would say, absolutely, and you would need
22 to run them again and you would need to find out why
23 that negative result became positive. It's part of
24 the aspect of troubleshooting, which, you know, is
25 with the very sensitive assays, is something that's

1 routinely done.

2 Q What is the gold standard PCR testing?

3 A As far as quantitative PCR, you mean?

4 Q What would be -- in terms of the accuracy of

5 -- let's make it specific to this case. You want to

6 get -- you are trying to identify measles virus RNA.

7 What would be the best possible way of doing that,

8 from PCR standpoint?

9 A So, you would have quantitative PCR and you

10 would use multiple primer peers.

11 Q That, you believe, is the best way to do

12 that?

13 A Yeah, for PCR.

14 Q You wouldn't sequence?

15 A Oh, you're asking me after you detect it.

16 You're saying for a PCR, in general?

17 Q Yes.

18 A All right. Once you've got a positive, yes,

19 you want to sequence. In fact, Dr. Sheils was asked

20 had they sequenced and verified that this was indeed

21 vaccine strain and they had done sequencing of H and

22 F.

23 Q Specific to vaccine strain?

24 A Specific -- an allelic discriminator from

25 the F. They had actually sequenced the product that

1 came out.

2 Q Specific to vaccine strain?

3 A Specific to vaccine strain.

4 Q And they didn't publish this?

5 A This was in -- I had assumed that the
6 Uhlmann paper had already been submitted. So -- and
7 the Uhlmann paper came out --

8 Q And the Uhlmann paper was criticized --

9 A -- in 2002.

10 Q -- and they had this data and it was never
11 published.

12 A I think it was criticized after the fact,
13 that it was accepted for publication, that it was
14 published. So, it went through peer review. Those
15 criticisms were not levied. The criticisms came
16 afterwards, after the paper was published, you should
17 have done this, you should have done this. And I
18 think if you take a look at anyone that was doing PCR
19 at that time, that, you know, you knew that you needed
20 to sequence to verify it. But, did you always do it?
21 If the paper you submitted it to and those reviewers
22 came back and said you need to sequence it, you did
23 one of two things. You sequenced it to get it
24 published or you submitted it to another journal and
25 saw what the issues were then.

1 Q Okay. So, you could get it published
2 without sequencing by just going to another journal?

3 A That's correct.

4 Q And after --

5 A Depending on the reviewers.

6 Q Okay. You have to sort of shop the paper
7 around to find somebody, who didn't require
8 sequencing?

9 A Actually, it's more the luck of the draw.
10 You get a reviewer, who is out of town, who is in a
11 hurry and is not, you know, as stringent as he might
12 be.

13 Q Somebody --

14 A Or you get someone, who, you know, is not
15 familiar with certain aspects --

16 Q And doesn't know to ask?

17 A Correct.

18 Q And in the five years since you saw this
19 data, none of this has been published?

20 A To my knowledge, no.

21 Q Now, you said that Dr. O'Leary has been
22 involved in this?

23 A Yes.

24 Q Okay. Does Dr. O'Leary believe that the
25 data generated by his lab supports a causal

1 association between MMR vaccine and autism?

2 A He never made that statement to my
3 knowledge.

4 Q So, he, presumably, has this data that you
5 have seen, this data that no one else has seen and,
6 yet, he doesn't believe --

7 A Well, the point is, it wasn't in every
8 individual.

9 Q Which would mean that there could be a
10 problem with the data, correct?

11 A No. It means that their positives were
12 positives and their negatives were negative and not
13 everybody was positive and there were negatives. So,
14 not everybody had the same --

15 Q So, he didn't believe --

16 A -- situation --

17 Q -- there was enough there, though, to
18 conclude that MMR was causing autism?

19 A I would say that anybody that would say that
20 based on any sort of data, that would be very
21 problematic in most scientific opinions and
22 assessments.

23 Q We're talking about Dr. O'Leary.

24 A Oh, Dr. O'Leary, I can't speak for him, but,
25 no, he never made that statement and I can't --

1 Q And he has this data?

2 A He has the data.

3 Q And he was the one that you said, I believe
4 in your report, is well regarded and well renown.

5 A Yes. And he is, also, a pathologist by
6 training, so he did a lot of the immunohistochemistry
7 to prove that there was an N protein product present
8 in these autistic enterocolitis children.

9 Q All right. You were asked a question, you
10 were giving some testimony and you were asked a
11 question by one of the Special Masters, I believe it
12 was Special Master Campbell-Smith, to clarify your
13 statement on -- she asked you a question on your
14 statement on high copy number and what the
15 significance of that was. Could you clarify what the
16 significance of a high copy number in the measles
17 virus RNA, what the significance is?

18 A Essentially to me, it suggests that the
19 amount that's there is high compared to what you would
20 anticipate with a natural measles virus infection.
21 And if it's high and if it's there, then it had to
22 amplify in some manner. And the only way it would
23 amplify is to have it transcribed into the positive
24 strand and have some evidence of replication. Does
25 that make sense?

1 Q No.

2 A Okay.

3 Q It doesn't.

4 (Laughter.)

5 MR. MATANOSKI: Maybe it does to the Special
6 Master, but it doesn't to me. I'm sorry.

7 BY MR. MATANOSKI:

8 Q I'm trying to figure out how you have higher
9 copy numbers than you have in natural infection.

10 A So what happens is when you usually look at
11 copy numbers for natural infection, you've got an
12 effective immune response that is trying to clear
13 things, okay. And as it clears things, the amount
14 goes down. It's a natural process. So, the immune
15 system is supposed to get rid of this. As it gets rid
16 of this, the number, copy number would go down. As a
17 result of the infection and there is no more measles
18 virus there, then the copy number should be
19 undetectable or zero.

20 Q Okay.

21 A In this situation, to get a higher copy
22 number, I would state that in light, there's an IgG
23 antibody response, that that is not effective at
24 clearing, resolving the infection. So, you're getting
25 an amplification of the measles virus RNA to higher

1 copy numbers than you would anticipate just through a
2 natural infection, because it's amplifying and
3 replicating. So, the measles virus enzyme is making
4 more.

5 Q What if it was higher than in the peak times
6 of an infection, what -- if the copy number is here or
7 higher than in the peak times of an infection?

8 A I would anticipate that you've got a lot of
9 inflammation then going on.

10 Q If the copy number is here exceed what you
11 see at the peak of a natural infection, why would that
12 be?

13 A Because you're pulling the copy numbers from
14 a biopsy material where inflammation is ongoing and
15 that high copy number, as the result of amplification,
16 is probably resulting in that inflammation. When you
17 look for copy numbers in a natural measles virus
18 infection, you are not going to a specific site where
19 it's inflamed. The individual would actually
20 eventually resolve it.

21 Q So, it's your belief that there's more
22 measles virus there, is that what you're saying?

23 A There is more measles RNA there.

24 Q Okay. And not necessarily more virus, is
25 that what you're saying?

1 A There is virus there, because there's
2 protein there. So, the process is going on. But, I
3 can't argue that there is more measles virus there.
4 There could be. There might not be.

5 Q So, are these copy numbers, in any way,
6 related to the amount of measles virus that are there?

7 A No. As I mentioned before, it's not a one-
8 to-one situation where you get one message producing
9 one protein producing one virus particle. It's more
10 stoichiometric than that. And you can get, you know,
11 RNA without getting the protein. So, the RNA can
12 accumulate and not necessarily get protein.

13 Q Okay. So, there's just this RNA, but
14 without actually virus?

15 A Well, we know at least in the autistic
16 enterocolitis kids, that there is nuclear protein by
17 immunohistochemistry. So, there's protein. Did they
18 isolate the virus?

19 Q I'm just trying to figure out how we get
20 these high copy numbers.

21 A So, I would simply say, I would say that
22 it's evidence that there is amplification in the
23 infected cells that produces more RNA.

24 Q Amplification of RNA or amplification of
25 virus?

1 A Of the negative strands and the positive
2 strands.

3 Q In your report, in this case, you mentioned
4 that you had been a reviewer for the IOM for the
5 Immunization Safety Review Committee --

6 A Yes.

7 Q -- on MMR vaccine and on thimerosal
8 containing vaccines?

9 A Yes.

10 Q What were your conclusions to that? What
11 did you tell them about the issue?

12 A That essentially more studies needed to be
13 done. My conclusions were not that different from
14 theirs. But bottom line was, if this is such an
15 important issue, we need to be putting more money into
16 it from the federal government standpoint to resolve
17 this issue and it needs to be a multi-front scenario.
18 The limitations of what the report found from a
19 standpoint of being able to say it causes or it
20 doesn't cause, those limitations need to be examined
21 from an experimental standpoint and need to be
22 supported through funding agencies, like the National
23 Institutes of Health, because it's an important issue
24 and it needs to be resolved, including things like
25 developing animal models, looking at sensitivity

1 issues from the standpoint of are there more and
2 better assays that can be brought to bear, which are
3 presently being used in cancer treatment and cancer
4 diagnosis, which are very sensitive and work very
5 well. Can we bring those expensive assays into the
6 arena and address this issue?

7 Q Did you tell them that you believed that MMR
8 vaccine and thimerosal containing vaccines acted
9 together caused autism?

10 A Not to my knowledge.

11 Q Did you tell them that thimerosal vaccines
12 caused immunosuppression in a vaccine recipient?

13 A I was not at that level of critique. So
14 what I was, was essentially a reporter in there and I
15 essentially evaluated what was going on and gave my
16 opinion of where things need to go.

17 Q Did you agree with their conclusions?

18 A Yes, except for one.

19 Q What conclusion was that?

20 A The conclusion was that they didn't
21 specifically come up with a recommendation for the
22 National Institutes of Health to examine this as a
23 priority issue.

24 Q Okay. So, you agreed with their conclusions
25 favoring the rejection of a link between an MMR

1 vaccine and autism?

2 A It was -- they rejected the causal, but they
3 didn't reject the possibility, if I'm correct.

4 Q And you agreed -- you agreed with their
5 conclusions, but just you thought that there should be
6 more research done; is that right?

7 A Absolutely. And let's address the question.

8 MR. MATANOSKI: I have no further questions
9 at this time.

10 SPECIAL MASTER HASTINGS: All right. Will
11 there be any redirect?

12 MS. CHIN-CAPLAN: Yes, Special Master, but
13 could we take a break?

14 SPECIAL MASTER HASTINGS: I was going to say
15 if there is going to be redirect, let's take a break.
16 Take a 15-minute break.

17 (Whereupon, a short recess was taken.)

18 SPECIAL MASTER HASTINGS: Back from our
19 break here. And I'm going to ask Ms. Chin-Caplan, if
20 she has a redirect? Do the Special Masters have any
21 questions?

22 SPECIAL MASTER VOWELL: Yes, we do.

23 SPECIAL MASTER HASTINGS: Special Master
24 Vowell has some questions.

25 SPECIAL MASTER VOWELL: Okay. Doctor, would

1 you look at your slides. And I just have a couple of
2 questions, one at least about the slide. And that is
3 on page three of your slide, you refer to the products
4 of the innate immune system and you refer to something
5 called NO, but you didn't define that for us.

6 THE WITNESS: Yes. That's nitric oxide.

7 SPECIAL MASTER HASTINGS: Okay.

8 THE WITNESS: It's a mediator of
9 inflammation.

10 SPECIAL MASTER VOWELL: Got it. And I may
11 be misquoting you, so, please, correct me. I thought
12 you testified, this was that long ago, that effector T
13 cells provide immunity. Is that what you said?

14 THE WITNESS: Correct.

15 SPECIAL MASTER VOWELL: Could you explain
16 that to me?

17 THE WITNESS: Okay. An effector T cell is
18 one that has gone from being naive, has matured, has
19 become activated, and is now armed and ready to
20 destroy infected cells. There's also effector T cells
21 that differentiate into the TH-1 and TH-2 types that
22 secrete different cytokines to help arm, in the case
23 of TH-2, B lymphocytes, activate them to produce
24 antibodies. And in the case of CD8 cells, turn them
25 into the cytotoxic T lymphocyte, which is then the

1 effector cell of the cell mediator immune.

2 SPECIAL MASTER VOWELL: So, do we have any
3 particular term that we use for an effector T cell
4 that is involved in the process of providing immunity,
5 as opposed to --

6 THE WITNESS: So, examples of effector T
7 cells involved in immunity would be the CD4 --

8 SPECIAL MASTER VOWELL: CD4, CD8?

9 THE WITNESS: CD8.

10 SPECIAL MASTER VOWELL: Okay. Then looking
11 back to your slide, and this is your cell's picture at
12 page 15, when the genes, the N, P, M, F, H, and L are
13 produced, do you need all of those genes before a
14 protein is made or will the N gene encode a particular
15 protein?

16 THE WITNESS: The N gene will code a
17 particular protein?

18 SPECIAL MASTER VOWELL: Yes.

19 THE WITNESS: Yes.

20 SPECIAL MASTER VOWELL: Okay. So, you can
21 get proteins produced within the cell, if you only had
22 the N gene replicated?

23 THE WITNESS: Correct, but you wouldn't get
24 F protein off the N gene.

25 SPECIAL MASTER VOWELL: All right. And when

1 you are -- what you are measuring in the PCR is the
2 genes, themselves, not the protein?

3 THE WITNESS: Correct.

4 SPECIAL MASTER VOWELL: Okay. And you
5 testified on cross-examination about some knowledge
6 about the interactions, both in the United Kingdom
7 between universities or government entities and
8 private firms, and within the United States; is that
9 correct?

10 THE WITNESS: Yes.

11 SPECIAL MASTER VOWELL: Does the term
12 'CRADA' mean anything to you?

13 THE WITNESS: Yes.

14 SPECIAL MASTER VOWELL: Okay. Do they have
15 in the United Kingdom CRADAs?

16 THE WITNESS: Not per se. What they do is
17 instead of going -- the companies going outside and
18 supporting -- so, they bypass their government, in
19 their situation. So, the companies will specifically
20 support laboratories.

21 SPECIAL MASTER VOWELL: So, that there is no
22 -- there is not that linkage that we have within our
23 system?

24 THE WITNESS: Correct.

25 SPECIAL MASTER VOWELL: Now, I hate to get

1 back into -- and CRADA would be -- it's an acronym --

2 THE WITNESS: Now, that was as of 2002, so

3 it may --

4 SPECIAL MASTER VOWELL: And I noticed that
5 my colleague just asked me to spell CRADA and it's C-
6 R-A-D-A. It's an acronym. I hate to go back there in
7 one sense, but in your report, when you talked about
8 the high level in, and I'm leaving the object there
9 blank, within Michelle, can you explain to me what
10 specifically you are referring to?

11 THE WITNESS: It is the copy number I'm
12 referring, the amount of RNA that's present.

13 SPECIAL MASTER VOWELL: Okay.

14 THE WITNESS: And it's measured as an amount
15 per volume.

16 SPECIAL MASTER VOWELL: An amount per volume
17 of what?

18 THE WITNESS: Of RNA that they pick off the
19 standard curve.

20 SPECIAL MASTER VOWELL: Okay. And does that
21 amount -- is that amount dependent on the amount of
22 time in a replicator?

23 THE WITNESS: No.

24 SPECIAL MASTER VOWELL: Okay.

25 THE WITNESS: The amount is dependent. So,

1 everything is set at a threshold. The amount is
2 dependent on the standard curve.

3 SPECIAL MASTER VOWELL: So, you plug
4 something into the standard -- you plug the results
5 into the standard curve and that tells you?

6 THE WITNESS: You have to find a straight
7 line portion of the standard curve.

8 SPECIAL MASTER VOWELL: Okay.

9 THE WITNESS: So, that's why you do
10 different dilutions or different amounts. You find a
11 point on the straight line and that, then, tells you
12 the amount that's present.

13 SPECIAL MASTER VOWELL: Okay. Let's
14 suppose, and I am going to pick on folks here, Mr.
15 Conway has an active measles viral infection and we
16 take some tissue from his body and we measure the
17 amount of measles virus RNA through a PCR method. Are
18 you saying that the level in Michelle was higher than
19 someone with an active -- that Mr. Conway -- with an
20 active measles virus infection?

21 THE WITNESS: Yes.

22 SPECIAL MASTER VOWELL: Okay.

23 THE WITNESS: With the caveat, in, Mr.
24 Conway, you are not measure his gut.

25 SPECIAL MASTER VOWELL: Okay. Well, let's

1 assume -- well, let's not even assume that we're
2 measuring his gut. We won't go there, Mr. Conway.

3 (Laughter.)

4 SPECIAL MASTER VOWELL: Are you volunteering
5 for a brain biopsy? I don't think so. All right.

6 So, would it matter, then, what tissue we were
7 measuring? You talked about --

8 THE WITNESS: No, it wouldn't. That would
9 consider a high level --

10 SPECIAL MASTER VOWELL: Okay.

11 THE WITNESS: -- and the amplified level.

12 SPECIAL MASTER VOWELL: And it wouldn't
13 matter how much tissue we started with, because you're
14 using standard dilutions?

15 THE WITNESS: Correct.

16 SPECIAL MASTER VOWELL: So, it would mean
17 that there was a high amount -- there was a high viral
18 load; is that correct?

19 THE WITNESS: Yes.

20 SPECIAL MASTER VOWELL: Would that be a
21 correct term?

22 THE WITNESS: Yes.

23 SPECIAL MASTER VOWELL: Okay. I think I
24 have it now. Thank you.

25 SPECIAL MASTER CAMPBELL-SMITH: Further

1 related to Special Master Vowell's question, you
2 indicated several times during direct examination that
3 you were surprised, you would not have expected to
4 have found replicating measles virus in the gut?

5 THE WITNESS: Correct.

6 SPECIAL MASTER CAMPBELL-SMITH: Is there
7 someplace you might have expected to find replicating
8 measles virus?

9 THE WITNESS: In this particular individual
10 or in general?

11 SPECIAL MASTER CAMPBELL-SMITH: I'm asking
12 you. It was your testimony.

13 THE WITNESS: Okay. So, no, I wouldn't
14 expect to find it in the gut of this individual. I
15 would have expected that the immune response would
16 have taken care of that and you wouldn't see it. In
17 an individual, who has an infection, then --

18 SPECIAL MASTER CAMPBELL-SMITH: A natural
19 infection?

20 THE WITNESS: A natural infection, you can
21 find it at various sites. And it depends on the
22 cycle. So, the measles virus has a specific cycle,
23 how it infects a host. It's got a viremia stage where
24 it's found in the blood. It's got a droplet stage on
25 how it can be spread through coughing and upper

1 respiratory. You can potentially isolate it from the
2 legions that occur, as a result of an active measles
3 virus infection. So, depending on the site, you can
4 get amounts. But, I wouldn't anticipate on what's
5 been reported to see the amount that you saw in
6 Michelle at any one of those sites.

7 SPECIAL MASTER CAMPBELL-SMITH: Okay. Your
8 answer there almost anticipates my next question,
9 where you didn't expect to see it. You talked about
10 the blood. And I was going to ask, based on what was
11 discovered, what is alleged to have been discovered in
12 the gut tissue here, would you expect to find the
13 presence of measles virus in blood cells?

14 THE WITNESS: Not necessarily.

15 SPECIAL MASTER CAMPBELL-SMITH: That depends
16 on the stage?

17 THE WITNESS: It depends on the stage. And
18 viremia could have been cleared. The virus could be
19 gone, but it's persisting somewhere else. And because
20 it's there, that's another one of my arguments for
21 persistence. If you found it in the blood, then it
22 would be still trying to find its place, going to
23 other places.

24 SPECIAL MASTER CAMPBELL-SMITH: Okay. One
25 more, just to be certain that I understand this piece.

1 In a normal, meaning normally operating, with whatever
2 range that means for an immune system, you would
3 expect a natural virus to be cleared or at least to --
4 to be cleared entirely or to be cleared to the extent
5 that it could not be detected, it either didn't exist
6 or could no longer be detected even by PCR methods?

7 THE WITNESS: Cleared such that it couldn't
8 be detected.

9 SPECIAL MASTER CAMPBELL-SMITH: So, it's
10 possible that you could still have some remnant?

11 THE WITNESS: It's possible.

12 SPECIAL MASTER CAMPBELL-SMITH: Okay. Even
13 in, I'm making sure, in a normally operated --

14 THE WITNESS: It's possible.

15 SPECIAL MASTER CAMPBELL-SMITH: Okay.

16 THE WITNESS: But the studies have not
17 really looked at that to say if you can't detect it,
18 the assumption is it's clear. But, if you can't
19 detect it is it because you're not sensitive enough or
20 is it because it's not there.

21 SPECIAL MASTER CAMPBELL-SMITH: Thank you.

22 THE WITNESS: And just let me clarify.

23 SPECIAL MASTER CAMPBELL-SMITH: Yes.

24 THE WITNESS: For the type of viruses that
25 measles virus is grouped in, it is virologic dogma,

1 that once you amount an effective immune system -- an
2 immune response and it's no longer there, then it is
3 cleared, gone.

4 SPECIAL MASTER CAMPBELL-SMITH: Thank you.

5 THE WITNESS: Put the new sensitive
6 techniques in, it may throw that dogma out.

7 SPECIAL MASTER CAMPBELL-SMITH: Got it.

8 SPECIAL MASTER HASTINGS: All right. Ms.
9 Chin-Caplan, you had some redirect for this witness.
10 Go ahead.

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

12 REDIRECT EXAMINATION

13 BY MS. CHIN-CAPLAN:

14 Q Dr. Kennedy, you were asked a series of
15 questions about the meeting that took place in the
16 United Kingdom; is that correct?

17 A Yes.

18 Q Did you identify the people, who were
19 present at that meeting?

20 A Yes, I did, to the best of my knowledge.

21 Q And could you just remind the Court who they
22 were?

23 A So, Dr. Richard Tedder, who is a professor
24 of clinical virology from the U.K.; myself; Dr. John
25 Marchulonis; Dr. Orla Sheils; Dr. Steven Jacobson, who

1 is a branch chief at National Institutes of Neurologic
2 Disorders and Stroke; a pediatrician, who I can't
3 remember; and a couple of other individuals from the
4 U.K.-Ireland, who are molecular biology expertise that
5 I would have to dig back and remember who they were.

6 Q And you, also, indicated that you grilled
7 Dr. Sheils for about four hours; is that what you
8 stated?

9 A Yes, four-and-a-half, five hours.

10 Q Okay.

11 A We made her cranky.

12 Q When you say you grilled her, did you
13 question her about the procedure that subsequently
14 constituted the Uhlmann paper?

15 A There were several of us that went in with
16 the opinion with the null hypothesis. We don't
17 believe you're data, prove it to us. So, we took a
18 very strong ground and made her show and try to
19 convince us that this was, indeed, what was going on.

20 Q And when you said that you tried to make you
21 show that this is what was going on, did you ask her
22 questions about whether cross contamination could
23 occur?

24 A Yes.

25 Q And what was her response to that?

1 A That she deals with cross contamination the
2 way that most laboratories do. She has a standard
3 operating procedure. If she sees cross contamination,
4 the run is invalid, nullified. She tries to figure
5 out where the cross contamination occurred and the
6 laboratory is set up, in that isolation takes place in
7 one area, the actual PCR reaction takes place in
8 another, and the analysis takes place in a third.

9 Q So the possibility of cross contamination
10 after she explained that to you, how would you
11 characterize the possibility of cross contamination?

12 A We didn't feel that cross contamination was
13 an issue relative to the data that she was showing us
14 in the final analysis.

15 Q Did you ask her about this issue of
16 controls?

17 A Yes.

18 Q And what specifically did you ask her?

19 A Everything.

20 Q When you say, 'everything,' could you state
21 to the Court what everything is?

22 A Why did you not run a no-template control.

23 Q And what was the response?

24 A We ran a no-template control. When you
25 publish your PCR data, do you put that you ran a no-

1 template control in your materials and method section.

2 Q And what other questions did you ask her?

3 A Asked her things like what were your
4 controls for your primers; how did you determine
5 whether something was positive; why did you substitute
6 in your consent to sequence a T for a C, because that
7 would lower the potential melting curve and cause some
8 issues. She explained that.

9 Q And what was her response to that?

10 A That she was looking for allelic
11 discrimination, that this was an easy way to show the
12 difference between wild type measles virus and the
13 vaccine strain.

14 Q And were you satisfied with that
15 explanation?

16 A Absolutely, because she showed us sequence
17 data on the primer products that suggested that it was
18 directly related to the vaccine strain.

19 Q And you indicated earlier that, and correct
20 me if I'm wrong, that they had found vaccine strain in
21 the F gene; was that it?

22 A Yes. That's where they look for the allelic
23 discriminator.

24 Q And is that specific to vaccine strain?

25 A The primers that they used for that

1 particular thing, yes.

2 Q And Doctor, when you addressed the issues of
3 control, were you satisfied that every positive that
4 was supposed to be positive was positive and every
5 negative that was supposed to be negative was
6 negative?

7 A Yes.

8 Q And, Doctor --

9 A Was I positive with her interpretation of
10 when something was positive versus negative from a
11 standpoint of overall? We had a lot of discussion
12 about that.

13 Q And did some of that discussion involve copy
14 numbers?

15 A No. When we talked about copy numbers and
16 she had high-level copy numbers, that was not a
17 concern. It was when she was stretching the limits of
18 sensitivity of the assay and trying to pick up these
19 very low copy numbers, that there was a lot of
20 discussion what she determined was a positive result
21 versus a negative result.

22 Q And what did you consider a low copy number?

23 A Anything that was below 1,000.

24 Q Doctor, I am going to show you a lab result
25 from Petitioners' Exhibit 28, page 179. Doctor, would

1 you take a look at this document and tell me what this
2 document represents.

3 A Okay. It's a report on measles virus
4 detection from Unigenetics Limited. It's from
5 Michelle Cedillo.

6 Q And what is the date on this?

7 A The date of the test is 15/03/02, so that
8 would be March 15, 2002.

9 Q And does this test indicate that measles
10 virus was detected in this allele biopsy?

11 A Yes.

12 Q What was the copy number that was obtained?

13 A 1.67 times 10 to the fifth copies per
14 nanogram of total RNA.

15 Q Would that be considered -- how would you
16 characterize that copy number?

17 A I would consider that high.

18 Q And, Doctor, you had previously testified
19 that there was no dispute among the participants
20 present at this meeting that high copy numbers were
21 accurate?

22 A We had no issues when she had high copy
23 numbers.

24 Q Now, Doctor, you, also, indicated that Dr.
25 Sheils said she found some N protein; was that it?

1 A Yes. The group presented data on
2 immunohistochemistry, where they identified N protein
3 present in the biopsies and these were from children
4 that had autistic enterocolitis. In addition, they
5 provided some data from some individuals of the
6 presence of measles virus RNA in cerebral spinal
7 fluid.

8 Q And, Doctor, the presence of N protein, if
9 we go to your chart on page 15, we've now gone through
10 N gene, P gene, F gene, F gene, and H gene; correct?
11 And we're now down in the green part here, correct?

12 A Right.

13 Q And does that mean that we have replicating
14 virus here --

15 A Yes.

16 Q -- if you have protein present?

17 A In my assessment, yes.

18 Q And, Doctor, you've reviewed the report of
19 Respondent's expert, Diane Griffin, have you not?

20 A Yes, I have.

21 Q And, Doctor, do you recall whether Dr.
22 Griffin indicated that if protein was present, then
23 it's an indication that it's replicating and spreading
24 to other tissues?

25 A Not off the top of my head.

1 Q Okay. I am going to ask you to look -- I
2 will bring you over Respondent's Exhibit B, page
3 seven, number two on that page. Would you kindly read
4 that into the record, please?

5 A Okay. This is from page seven. It says
6 that 'viral protein production is also necessary for
7 virus to replicate and for new variance to be made.
8 Without this, there can be no virus replication and no
9 spread of virus within the host to other cells or
10 tissues.

11 Q When you read that statement, Doctor, is Dr.
12 Griffin indicating that if there are proteins present,
13 the virus is replicating?

14 A Yes, that's how I would read that statement.
15 And since Dr. Griffin is the expert, I'm certainly
16 not going to question anything that she says. I
17 believe Dr. Griffin has over one hundred publications
18 in measles virus, so --

19 Q Do, Doctor, at the end of this meeting, what
20 conclusions did the group come to?

21 A We were satisfied with the results of the
22 O'Leary and Sheils laboratory. We felt that they were
23 confident, that they were capable. They did not find
24 in every instance where they looked, measles virus.
25 In some instances, in some individuals, they found

1 measles virus. So, that made us feel a lot better, in
2 that it wasn't found in every instance. And in ones
3 that they would run, they would have a positive
4 individual and a negative individual. So, that argued
5 that there was no cross contamination. There was no
6 issue relative to that. And we felt fairly assured
7 that this was a very competent laboratory, that they
8 were skilled at what they did, that they were aware of
9 the problems with PCR, and that their conclusions were
10 supported for the presence of measles virus in gut
11 biopsies and in the instance where it was found in
12 cerebral spinal fluid.

13 MS. CHIN-CAPLAN: Thank you, Doctor.

14 SPECIAL MASTER HASTINGS: Mr. Matanoski, any
15 re-cross?

16 MR. MATANOSKI: Yes, sir, briefly.

17 SPECIAL MASTER HASTINGS: Go ahead.

18 RE-CROSS EXAMINATION

19 BY MR. MATANOSKI:

20 Q I'm sorry, sir, I'm just taking a look at a
21 document and unfortunately, it's very small print.
22 Hello, again, Dr. Kennedy.

23 A How are you?

24 Q In the exhibit that you were asked to look
25 at, the results from Unigenetics reported on Michelle

1 Cedillo, was there any positive result for protein,
2 measles virus protein?

3 A In that document, no.

4 Q Your discussion about positive results for
5 protein is based on the representations of Dr. Sheils
6 at a meeting?

7 A Yes.

8 Q Did you see any of the actual lab reports?

9 A I actually saw some lab reports and data and
10 we questioned her with regards to the use of the
11 specific reagents that she was performing the
12 immunohistochemistry with. One reagent, in
13 particular, we felt as a group was very problematic.
14 It was a polychronal antibody to the measles virus
15 that was used and there was a concern that that
16 polychronal antibody had issues related to cross
17 reactivity and may not be detecting measles virus
18 protein. She addressed this by providing us with
19 information that they used four different monochronal
20 antibodies, in addition to the polychronal, that were
21 specific for epitopes on the measles virus in protein.
22 And we felt confident with the monochronal antibody
23 that that dealt with the issue that we, as a group,
24 had over the concern with them using a polychronal
25 antibody.

1 Q In terms of presenting this to you, she told
2 you about it?

3 A No. We actually saw data.

4 Q Okay. Did you go to the lab?

5 A No, I did not.

6 Q Did you look at the lab notebooks?

7 A Yes, I did.

8 Q They had all of them?

9 A They had, I want to say four or five boxes.
10 I didn't look at every individual one and every
11 individual page, but there were certain ones that I
12 was interested ones, certain ones that other in the
13 group were interested in. And we looked at what our
14 interest was and then discussed that later on in
15 comparison what did we found, were we satisfied, was
16 there a control, when was this run, what was the
17 primer set. We were concerned about where were their
18 primers, when they ran out of primers, what was the
19 source of their Taq preliminaries, where did they
20 specific -- a lot of us not being from the U.K. didn't
21 know where they got the specific reagents to isolate
22 the RNA, where there issues related to that, sample
23 preparation. So, it was a pretty rigorous go at it.
24 I had a look at specific aspects that made me feel
25 comfortable, that in the instances that I was looking

1 for, that the notebooks were a true representation of
2 the data that she presented to us in general.

3 Q Okay. So, from your point of view, you had
4 a good idea of what was going on in the lab?

5 A Yes.

6 Q And this data, though, this meeting wasn't
7 to publish a scientific paper or anything like that;
8 correct?

9 A You know, it was set up, in my mind, the way
10 the presentation was, in that it was -- you know,
11 eventually that this information was going to be
12 published.

13 Q And it never was?

14 A I have never seen it in print.

15 Q That's five years ago that this took place?

16 A That's five years ago.

17 Q And this meeting was set up by lawyers,
18 correct?

19 A Yeah, it was set up by the U.K. -- I forget
20 what the firm was called -- what are they called --
21 they were representing --

22 Q The claimants?

23 A The claimants, thank you.

24 Q The claimants, who were alleging that MMR
25 vaccine was causing autism?

1 A Correct.

2 Q And does Unigenetics exist anymore?

3 A No, it does not.

4 Q And do you know what happened to the
5 litigation in the United Kingdom?

6 A To the best of my knowledge, what I heard
7 was that the judge dismissed the claimants and that we
8 were essentially no longer needed, so do not worry
9 about it, and that anything that related to
10 Unigenetics, I was not aware of until later on. I
11 assumed that they no longer operate, because they no
12 longer had a source of support for this activity.

13 Q Because the litigation was gone?

14 A The litigation was gone.

15 MR. MATANOSKI: Thank you.

16 SPECIAL MASTER HASTINGS: Anything further
17 from this witness, Ms. Chin-Caplan?

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

19 SPECIAL MASTER HASTINGS: Anything further?

20 (No response.)

21 SPECIAL MASTER HASTINGS: All right. Dr.
22 Kennedy, we thank you, very much.

23 THE WITNESS: Thank you.

24 (Witness excused.)

25 SPECIAL MASTER HASTINGS: You are done for

1 this afternoon. Should we -- do we have anything else
2 to talk about this afternoon? Is the plan to start
3 with Dr. Byers in the morning?

4 MS. CHIN-CAPLAN: That's correct.

5 SPECIAL MASTER HASTINGS: Anything we should
6 talk about before we adjourn for the day?

7 MR. MATANOSKI: No, sir.

8 SPECIAL MASTER HASTINGS: All right. Well,
9 we are adjourned for the day, then. We will see you
10 tomorrow morning at 9:00 for Dr. Byers' testimony.

11 (Whereupon, at 4:55 p.m., the hearing in the
12 above-entitled matter was recessed, to reconvene on
13 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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