UNITED STATES
COURT OF FEDERAL CLAIMS

pTHERESA 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: 1791 through 2071

Place: Washington, D.C.

Date: June 20, 2007

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IN THE UNITED STATES COURT OF FEDERAL CLAIMS

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

Wednesday,
June 20, 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

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C O N T E N T S

P R O C E E D I N G S

(9:02 a.m.)

SPECIAL MASTER HASTINGS: All right. Good morning to all here in the courtroom and at home.

We are going to start this morning with which witness?

MR. MATANOSKI: It will be Dr. Ward.

SPECIAL MASTER HASTINGS: This is Dr. Ward. All right. Dr. Ward is at the witness stand.

Who is going to do the examination, Mr. Matanoski?

MR. MATANOSKI: I'm sorry, sir?

SPECIAL MASTER HASTINGS: It will be Ms. Babcock doing the examination?

MR. MATANOSKI: Yes, sir, it will.

SPECIAL MASTER HASTINGS: All right.

MR. MATANOSKI: I apologize. I don't rise to speak to the Court just because I'm afraid the microphone won't -- I certainly would prefer to give you the courtesy.

SPECIAL MASTER HASTINGS: That's fine. I understand. We want the people at home to be able to hear everything.

All right. Ms. Babcock?

Dr. Ward, I'm going to ask you to raise your

right hand, please.

Whereupon,

BRIAN J. WARD

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

SPECIAL MASTER HASTINGS: Okay. Please go ahead.

DIRECT EXAMINATION

BY MS. BABCOCK:

Q Good morning, Dr. Ward.

A Good morning.

Q Could you please state your name for the record?

A Brian Ward.

Q And what is your profession?

A I'm a medical doctor at McGill University.

Q And could you briefly describe your collegiate and medical education?

A I started in the French junior college system in 1971 and didn't get a paying job until 1992, much to the distress of my parents.

I went through junior college, which is a peculiarity of Quebec, which allowed me accelerated access to medical school. I found myself in medical school at age 19 and three years later decided I

didn't want to be a doctor, won a little bit of an academic lottery and went to Oxford on a Rhodes scholarship where I did some research.

I then, holding my nose, came back to medicine, finished the medical degree in 1981 and then again left medicine and went to be an archeological dig doctor for a year. I came back to medicine and then left again to be a volunteer in refugee camps in Thailand for a couple of years.

I finally returned to Johns Hopkins where I completed my internal medicine residency, then infectious diseases, to be recruited back to McGill University, coming full circle, only to discover that Quebec didn't recognize my U.S. medical specialty, and I had to be a resident again for another year in microbiology. That was 1992, so 20 years later I had a job.

Q Now, to be clear, when you were at Hopkins you studied infectious diseases?

A Yes. I completed the internal medicine residency and infectious diseases, two years of which was devoted to research in Diane Griffin's lab.

Q So you studied the measles virus?

A Yes. She offered me the opportunity to go back to Peru to study measles, and I didn't think too

long about it.

Q Now, are you board certified?

A Board certified in internal medicine and infectious diseases in the U.S. and internal medicine and what in Quebec is called infectiology, which combines microbiology and infectious diseases in the province of Quebec.

Q And what responsibilities do you have at McGill University?

A Well, I'm currently the very happy retired chief of Infectious Diseases. I am the ex-chief of the Division of Infectious Diseases, returning to my lab just in 2006, actually the beginning of 2007.

My current responsibilities are as a member of the Division of Infectious Diseases. I'm the associate director of the Tropical Diseases Center, and I run a national reference lab for the country in parasite diagnostics, so anybody who has a weird parasitic disease in Canada we get the sample, and we work in close collaboration with the CDC in Atlanta.

Q And do you also hold teaching positions at McGill?

A Yes. I'm an active teacher at the graduate and undergraduate level.

Q And have you published in the field of

infectious diseases and virology?

A Yes. Yes. There are several areas of activity in virology, infectious diseases and vaccines.

Q And this includes book chapters and articles?

A Yes, that's right.

Q And what is the current focus of your research?

A The research is divided. What at first blush may seem an illogical pairing of viruses and intracellular parasites, but viruses are also intracellular parasites and so the immune response to those two classes of organisms is very similar so my research focuses on viruses and also the intracellular parasites like malaria, leishmania, diseases of the developing world and the immune response and strategies to treat and prevent those infections.

Q And have you ever testified in a legal case before?

A Four, including this one. One civil case in Canada. I've helped the Quebec Vaccine Injury Compensation Program. It's the only providence in Canada that has a similar program. One case there and one prior case in the U.S. Vaccine Injury Compensation

Program in Toledo.

Q Now, what materials did you review in preparation for your testimony today?

A I reviewed everything I was sent and some of the medical literature that I was directed to by the reports.

Measured in pounds or inches, it was a lot. Probably about four or five inches worth of documents at least.

Q And have you been here to listen to the testimony of Petitioners' experts or reviewed the transcript?

A I have been here all week. Yes.

Q Now, I wanted to start with immunology and basic biology. Dr. McCusker is going to talk about this in more depth, but do you also have experience with vaccine immunology?

A Yes. I've been very interested in vaccine immunology, particularly as it pertains to measles and respiratory viruses.

Q Okay. Is immune status a static entity?

A Immune status is absolutely not a static entity.

Q I believe we're switching to Slide 3 now.

A Immune status changes over time. It changes

from day to day, week to week and certainly over the lifetime of an individual.

Dr. McCusker is certainly going to address one of the comments made by Dr. Byers in her testimony that it was entirely acceptable to assess a child's immune status using adult normal ranges and so I just pulled one simple, single example that's I think relatively straightforward from a lovely paper done recently by the AIDS Clinical Trial Group, Pediatric AIDS Clinical Trial Group, where they look at CD4 cells, CD8 cells and B cells over time in children in the U.S.

I think it should be fairly obvious to all of you. You can see the CD4 cell counts in children. The first three red dots are at zero to three, three to six and six to 12 months. Obviously there's a wobble around each of these measurements, but you can certainly understand quite easily just visually how by taking a normal range as dictated by, for example, the 12- to 18-year-old values that that would be wildly inappropriate to use as a normal range for assessing a six to 12-month-old child.

Chris McCusker will certainly talk about this in greater detail.

Q Now, in broad strokes what types of

immunocompromise are there or immuno suppression, and can you immunize kids who have some sort of immunocompromise?

A Well, in listening to the testimony of last week it was quite clear to me that many of the expert witnesses were mixing terms and using equivalencies that don't really exist and so what I thought I would try to do, again quite simply and to be amplified by Dr. McCusker, try to distinguish between immune suppression, immune defects and what's being termed an unbalanced or dysregulated immune response.

SPECIAL MASTER HASTINGS: Let me interrupt for a minute.

I'll note for the record that Dr. Ward here is also showing a series of slides. He's now begun to talk about Slide No. 4.

Let's also mark this handout that contains paper copies of his slides as the Respondent's Trial Exhibit No. 12, I believe.

Go ahead. Sorry, Dr. Ward. I interrupted you. Go ahead.

THE WITNESS: No problem. I apologize at one level for the relative simpleness of some of these slides, but I think it is very useful to just get a broad understanding of some of the terms that we're

talking about.

Immuno suppression in a medical sense is a relatively crude phenomenon. If someone is immuno suppressed it's a big deal. Most immuno suppressive things target cellular immunity. Antibody type immunity is relatively spared, and there are many things that cause fairly powerful immuno suppression: high dose steroids, Azathioprine or Imuran, anti-TNF alpha therapies.

All of these things would be considered to be potently immuno suppressive and therefore from a medical point of view quite risky. It should not be lost on you that several of these agents were administered to Michelle Cedillo during her therapy.

There are other things as well that can be acquired naturally. For example, HIV is towards the end of its evolution powerfully immuno suppressive, as is wild-type measles. Prior to the discovery of HIV, measles was considered to be the most potent immuno suppressive virus known in its wild type form, but not measles virus vaccine.

BY MS. BABCOCK:

Q To just sort of follow up, I want to emphasize that. Does measles vaccine cause immuno suppression?

A Measles vaccine causes changes in immune cell phenotype and the functioning of some cells in the test tube -- that was my work in Diane Griffin's lab -- but it does not that anybody knows cause clinical immune suppression, so a state of suppressed immunity that is relevant to the child.

There are also many known immune defects and probably many to be discovered. We now know that there are individuals who have parts of their immune system specifically deleted. They're gone genetically.

These defects can involve the innate, the cellular or the antibody sections of the immune system, and in some cases they can involve multiple areas, the so-called severe combined immunodeficiency type children, the ones that we know in the lay press as bubble children where they're missing several parts of their immune system and they are horribly susceptible to a wide range of microorganisms.

What's very important to understand in the context of this case is that these immune defects rarely, if ever -- I broke one of my rules by putting an absolute onto this slide where I say never, but I'll modify it slightly and say rarely, if ever -- are they pathogen specific so that if you are

immunocompromised in some way because of an immunologic defect then you are generally susceptible to a range of organisms, similar organisms.

So, for example, if you're missing your antibody production capacity you are highly susceptible to one group of viruses, polio and the other enteroviruses, three encapsulated bacteria and, bizarrely, one gastrointestinal parasite, but none of these immune defects are pathogen specific. That really hasn't been reported.

Q Now, are you aware of any populations where individuals known to be immuno suppressed received the measles vaccine?

A Well, I've done a fair bit of work in Africa and have been involved with recruiting large cohorts of mothers and their babies in Zimbabwe over the last 10 years.

When we initiated this study in 1996 we were trying to prevent mother-to-child transmission of HIV using Vitamin A. It turns out it didn't work, although everybody thought it should work. It was a hypothesis that proved to be incorrect.

Q Now, this is kind of an obvious question perhaps, but is it fair to say that children in Africa have HIV?

A Well, Zimbabwe has one of the highest rates of HIV prevalence in the world, and in our study at recruitment the mothers were 33 percent positive for HIV across all socioeconomic strata so we recruited and followed 14,000 women and their babies to see the development of HIV in the children and see if we could prevent it.

In our study alone we watched unfortunately almost 1,400 babies become HIV positive, and there was nothing that we could do about it. The mortality rate in those HIV positive babies was almost 50 percent in the first two years of follow-up, which suggested that even though they started life with a relatively normal immune system that many of them became immunologically abnormal and susceptible to a wide range of infections at various times through their first two years of life.

Q Now, did these children receive vaccinations?

A All of these children received MMR unless they were clinically obviously ill at the time that they should have received it.

In Zimbabwe they receive measles, monovalent measles, at the age of nine months of age. That's the WHO recommendation in fact.

Q And to the best of your knowledge, do they also receive thimerosal-containing vaccines?

A Yes. To the best of my knowledge, the large majority of the EPI vaccines are distributed in multivalent vials containing thimerosal.

Q Now, according to your research --

A I'm sorry. EPI is Expanded Program on Immunizations, which delivers vaccines to the developing world at no or low cost.

Q Thanks. Now, is there any evidence for an increase in autism spectrum disorders?

A Well, you would think with the huge burst, the epidemic of HIV that is, really destroying a large part of the African population that if there was a true association between thimerosal-containing vaccines, measles vaccination and autism that we would have seen a huge burst in the incidence of autism.

As it turns out, autism is actually fairly rarely reported in Africa, probably because of societal acceptance of children who are a little bit different from the norm, but still one would have anticipated an increase in reporting of autism in Africa with this massive change in the number of children receiving what the Plaintiffs' experts are arguing is an immuno suppressive combination leading

to autism.

That hasn't been reported either in my experience or in the experience in the literature, and I wrote to one of my pediatric colleagues with whom I work. He thought it was an interesting question, and he sent a letter out to 17 of his Zimbabwean colleagues all over the country and said are you guys seeing any Zimbabwean children with autism? They all wrote back and said no, not us. No change.

So that's not in the literature, but there isn't a dramatic increase in the incidence of autism that's been reported in the literature.

Q Now, are you aware of populations where there would be questions about immune balance?

A Well, then we have to go back to this slide of the third category, a broad category of immune balance.

This idea of immune balance is relatively new. I can remember very specifically as a postdoctoral fellow in Diane Griffin's lab I walked into her office with Tim Mossman's paper when Tim Mossman was at DNAX, and I said this is the answer to measles.

You'll have a chance to meet Diane next week, and you'll understand that she is used to

postdoctoral fellows walking in with revolutionary ideas. She said yeah, yeah, maybe. We'll see.

That paper proved to be a paradigm changing paper where they had identified the counterbalance cytokine to gamma interferon where they demonstrated that Interleukin-4 and gamma interferon lived in balance with each other in mice to direct the immune responses to a wide range of organisms.

What's very important to tell you is that while pathogen specific abnormal immune imbalance can exist, and the best example is actually from measles and closely related viruses. If you have a general state of TH1 or TH2 predominance in one's ability to respond that is never pathogen specific. That means that that's how you respond to almost everything if you have a tendency to respond in a TH1 or TH2 way.

I'll just briefly digress to explain the measles vaccine imbalance that was described. In the early 1960s, in the mid 1960s actually, the first measles vaccine that was invented was an inactivated measles vaccine.

We now know a great deal about what happened with that vaccine in terms of the basic science, but the clinical science was that children who received that vaccine appeared to have a normal immune

response, but when they were subsequently exposed to the wild-type virus they made an aberrant TH2 deviated immune response to the measles, the wild-type measles that they were exposed to, and they developed a much more severe disease.

They didn't develop TH2 deviated immune responses to anything else; only to the measles, and so you can initiate an immune memory that is aberrant and immune deviated, but it is pathogen specific.

A very similar experience was replicated with the initial respiratory syncytial vaccine where there was pathogen specific immune deviation, but those children who had aberrant responses to RSV did not have aberrant responses to any other virus or pathogen they were exposed to.

I think we should go to the next slide.

Q Now, what determines the balance in any given immune response?

A Well, there's no denying that there are genetic influences on immune balance. Many, many people take the cartoon of TH1/TH2, the very simple thing, and say look at this. This cytokine is up. Therefore, this is a problem. Or, this particular immune parameter is down. Therefore, that indicates. That's just not true at all. The TH1/TH2 balance is a

very dynamic system with dramatic genetic influences.

The best example in basic science is the difference between the BALB/c mouse, which you see on the left side, the nice, little white lab mouse, and on the other side the black 6 mouse, which is taken from a recent *Nature* article, and the tail of the mouse is spinning out into a DNA molecule because they were discussing the influences on immunity.

These two mice, these two inbred mouse species, are known by investigators to be TH1 biased or TH2 biased, so you can cheat a little bit. If you are trying to develop a vaccine where you want to demonstrate a really good antibody, well, you would kind of be an idiot to use the black 6 mouse. You would use the BALB/c mouse because they are biased towards the production of really good antibody responses.

Similarly or oppositely, if you're trying to develop a vaccine that requires a good cellular immune response you can help your experimental design by using the black 6 mouse because you know that it is biased towards the production of cellular immune responses.

This does not mean, even in these powerfully immune deviated genetically inbred mice, that the

BALB/c mice do not generate cellular responses or that the black 6 mice do not generate antibody responses. They do. They just tend to lean one way or the other, but you can readily measure antibodies in black 6 and cellular immunity in BALBs when you're doing it.

This relative balance shifts in mice just as it does in human beings from day to day, week to week and with events. When you're stressed, when you are tired. I suspect that the paralegals on both sides have imbalances in their immune systems right now, and if we were to measure we could probably identify one or two -- probably the lawyers too -- abnormalities that would correct over a period of time with good food and some sleep.

However, there are some populations that are known to be kind of BALB/c-ish or black 6-ish. They tend to have antibody responses or responses that lean towards TH1 or TH2, and the classic examples would be asthma, allergy and, for example, parasitic diseases.

Children with asthma and allergies tend to respond more like BALB mice. They're kind of BALB type people, while other people, families that have absolutely no allergies, asthma and no parasitic infections, tend to respond a little bit more like black 6s. It's a little bit humbling to think of

yourself in terms of inbred mouse trains, but it tends to be true.

While there is in vitro evidence, you can show changes following measles immunization that look like TH2 bias. They are transient, and they are not known to have any clinical significance.

This was in fact the work that I did with Diane Griffin in her lab. We were the first to demonstrate that there was a TH2 response to measles and to measles vaccination, but we never claimed that there was any clinical significance to these events that we could measure in test tubes.

Q Now, Dr. Byers last week discussed extensively cytokine inflammation. Did you have a comment on her testimony?

SPECIAL MASTER HASTINGS: And now we're moving to Slide 6.

THE WITNESS: Slide 6. Sorry. I think it's useful just to take a step back again and try to put cytokines into perspective as sort of a reality check.

There's nothing mysterious about cytokines, although we're getting a lot of them. Cytokines are essentially messages sent from one cell to another so that individual cells can communicate with each other and tissues can communicate across large distances in

the body. Cytokines act locally and some act at great distances.

What needs to be put into perspective is that some cytokines, particularly the unnumbered ones and the early numbered ones like IL1, IL2, are actually produced in very large quantities, and they are not terribly well regulated. From the point of view of the body, they're kind of sloppily produced. They're produced fast, sloppy, and they elicit in many cases these proinflammatory responses.

The first one, IL1, was initially discovered because when you injected it into an animal you got fever, high fever. It was called endogenous pyrogen, subsequently called Interleukin-1.

The later numbered cytokines, the ones that we are now discovering today, all have higher numbers or mostly have higher numbers, and they are produced in much smaller quantities. They are much more tightly regulated, and they tend to do much more specific things. They're much more precise.

What I think is really important in the context of this case to understand is that these proinflammatory cytokines float around in our bodies in many, many circumstances, and as the father of three children and a physician I know that

inflammation is certainly not rare.

Many children have many kinds of inflammation starting with diaper rash that extends from the knee to the nipples, through eczema to the more severe medical conditions. Children who are scalded and burned can have inflammation for months. They have circulating proinflammatory cytokines that are readily measurable for weeks to months.

Children with recurrent otitis media, which is very common, children with food allergies, until you figure it out, have chronic inflammation of their gut with circulating proinflammatory cytokines. These are not unusual situations for a child.

Inflammation of the brain, specifically of the brain. It's fortunately much rarer, but in the medical context it's not that rare. We still see lots of children with viral meningoencephalitis or bacterial meningitis, and children with brain abscesses, subtle brain abscesses, can have inflammation right in the brain for periods of weeks to months before we discover what's happening.

As far as I'm aware, there's no known association between these intense and in some cases prolonged peripheral states of inflammation or even the CNS inflammation with powerful cytokine release

and production right in the brain and the development of autistic spectrum disorder with the exception of the very destructive lesions of herpes virus encephalitis. That's really the only exception.

BY MS. BABCOCK:

Q Now, Doctor, I wanted to shift back to immuno suppression for a moment. Now, we heard from Dr. Krigsman and other testimony that he had prescribed immuno suppressants, specifically Humira and Remicade, for Michelle, correct?

A Yes, that's true.

Q Would you consider these potent immuno suppressants?

A Yes. These are drugs to be greatly respected.

Q Now, if someone had a persistent measles virus infection and started taking Humira or Remicade what would you expect to happen?

A In most instances in an individual with a persistent viral, bacterial, fungal or parasitic infection that requires cellular immune responses to control it, the administration of these drugs, almost anyone would be fearful that these drugs would reactivate these organisms and make the disease worse.

Q How much worse?

A In many cases dead. Dead worse. There are many individuals in the medical literature who have received one or another of these drugs and have had their persisting infections -- viral, intracellular, bacterial or parasitic -- reactivate and kill them, and so these are drugs to be greatly respected.

Therefore, to me it seems powerfully illogical that in the case at hand we have the simultaneous argument that there is a persisting viral infection that is controlled by cellular immunity, and yet when very powerful drugs that target cellular immunity to suppress it are administered that Michelle got better rather than worse. This is logically inconsistent to me.

Q Now, during her testimony last week Dr. Byers recommended a paper by Ashwood as a good summary of some of the immunological issues here. Does Ashwood mention the MMR-ASD hypothesis?

A In my reading of the Ashwood article, which I found very interesting to read, I couldn't find a mention of the MMR-ASD hypothesis anywhere.

The only mention in this article of a possible association with measles, if I'm not mistaken, was prenatal or congenital infection with wild-type measles.

Q Does Ashwood talk about vaccines at all?

A Not to the best of my recollection. I don't believe he does.

Q Now, based on your review of the records is there any evidence that Michelle was immuno suppressed prior to her receipt of the MMR vaccine?

A Well, with the caveat that I'm not a pediatric immunologist, I see no evidence either in the frequency or severity of the infectious episodes that were reported in the first year or two of life that she had any degree of immuno suppression.

Q And again with the caveat that you're not a pediatric immunologist and being more general because it's a little unclear, but is there any evidence of an abnormal immune system after receipt of the MMR vaccine?

A Not from my point of view. Not at all.

Q Now I want to move on to viral pathology.

A I'm sorry. I'll take that back. From an infectious point of view, I think that children who develop autoimmune inflammatory conditions would be considered to have abnormal immune systems, but they're not immuno suppressed.

I think that her later evolution clearly demonstrated that she had abnormal immune responses,

but I would not consider those to be the same as immuno suppressed.

Q Okay. Now moving back to the measles virus, did you listen to the testimony of Drs. Kennedy and Byers?

A Yes, I did.

Q Did you have any concerns about how Dr. Kennedy presented measles virus biology?

A Well, yes, I do. I've been working with measles vaccine and measles virus for about 20 years.

Q We're now on Slide 7 for clarification.

A I've continued the tradition of Diane Griffin slapping my wrist whenever I confused measles virus with measles vaccine, and many of the Petitioners' experts tended to equate them. Measles virus. Measles vaccine. They're the same. They do the same. Everything that relates to measles wild-type is the same as measles vaccine.

If you would just go to Slide 8 for a second and then bounce back? This is really like comparing wild animals. You have the Egyptian wild cat from which all domestic cats are derived on the top and a lynx on the bottom and a cute little Somali cat on the right side.

I think that it's fair to say that the wild-

type measles virus and the vaccine strain measles virus are very, very different in their biological characteristics and that one shouldn't equate them. One can be informed by studies in one or the other, but one should be very specific about which virus one is talking about in this kind of proceeding.

Can you go back, please? Go back to Slide 7. Great.

There were just a couple of things that I thought because my understanding is that the Court is going to be hearing a lot about measles and measles vaccine over the next little while, and while Dr. Kennedy clearly has extensive knowledge in the area of HIV vaccine development and with some other viruses, far greater than my expertise, there were just a couple of errors of straightforward fact that I thought needed to be corrected.

Dr. Kennedy said that the measles virus was originally attenuated in monkey kidney cells. While that does apply to some other vaccines, it does not apply to measles. The original vaccines were attenuated in chick embryo fibroblasts.

He mentioned that the seed lots, the vaccines, were tested in mice. Neither the vaccine strength virus nor the wild-type virus actually infect

mice. They don't have the receptors for the virus and so they cannot infect mice, so seed lots for the vaccine are tested in dogs.

There's a very close genetic and protein relationship between canine distemper virus and measles and so dogs can actually be protected from canine distemper virus by measles vaccine, which is useful to know if you have a dog and have run out of canine distemper vaccine. It doesn't work in reverse, interestingly. You can't protect a child with canine distemper vaccine from measles. We don't know why.

He also stated that the viruses, the vaccine strains, were neurovirulence tested in mice, in neonatal mice. Again, this is not true. They're tested in monkeys. Lots of people make arguments that they shouldn't need to do this because this is really expensive, but they are still neurovirulent tested in monkeys, to the best of my knowledge.

Q I think that in the heat of cross-examination there may have been some mis-statements because his slides certainly suggested that he was aware that the virus was a single-stranded negative sense RNA genome and so his statement that individual genes can persist doesn't make sense.

The virus is not segmented. The viral

genome is not segmented, so if the genome is there all of the genes are there and so you can't have individual genes persisting. You can have relative excess of one gene or another being transcribed into mRNA, but you can't have individual genes persisting.

There was also some I think confusion that needs to be straightened out because of the importance of PCR in this proceeding of what's being amplified because Dr. Kennedy seemed to be of the opinion that the positive sense RNA, the antigenomic and the RNA, the messenger RNA, was being amplified, but this is not the case.

That is theoretically possible. You can do that, but in the case of the Uhlmann work and I believe almost all of the work done by Unigenetics both primers are added, which results in the amplification of all of the viral RNA present, whether it's positive or negative sense.

If we could just go to the next slide? Bounce one past the cute kittens.

Q If it's okay just for me to set this up?

A Sure.

Q Dr. Kennedy also spoke at length about how the measles virus replicates, so I wanted to give you an opportunity to comment.

A I've modified one of my slides to give you the same color coding that he used with the red and the blue strands.

If I may, so I can talk into this and still point, I'll just very rapidly go through the movement of the virus through any cell, and we believe this is the same in any cell that the virus infects where it goes from infection to release of virions.

The virus comes in, and I've had coffee so this is going to bounce a little bit. The virus comes in, and the viral genome is negative sense, okay, which means that the negative sense RNA cannot be used to make proteins. You can only make proteins in a human cell from positive sense RNA.

Some viruses have positive sense RNA. They go into the cell and they can use their own genome straight away to make protein. That doesn't happen with measles.

So Dr. Kennedy mentioned that they came into the cell through CD46, which is true. That is possible, but in fact many people believe that the more important cellular receptor, at least in the initial phases of the infection, is the so-called SLAM molecule, which is expressed on immune cells, and so most people believe that the virus enters the immune

cells almost exclusively through the SLAM receptor, not the CD46 receptor.

The Japanese investigators at the CDC in Japan believe they've found a third receptor, so this may get even more complicated later in the proceedings.

However, once the virus is in you have this red strand, the negative strand, and the virus begins to make positive sense RNA copies. One peculiarity of this virus is that it transcribes the front end of its genome to RNA much more successfully than it does the back end of the genome and so it has many, many more copies of the RNA, for example, of the N gene than it does for the F or the H gene.

This is essentially a virus strategy for building more copies of itself, and the best analogy is that of a factory that's trying to make a bicycle. If you're making a bicycle you only need one pair of handlebars, but you need 40 odd spokes and so the virus is making the spokes up at this end and the handlebars down at that end and so at the end of its process of producing proteins it will have the appropriate proteins in appropriate ratios to make viral particles.

Now, positive sense RNA molecules are

translated into the proteins which go up to the cell surface where they are assembled at specific locations on the cell surface into the new virions.

When you have a high enough concentration of these proteins, the virus then switches its strategy to make a copy, an antigenomic or positive sense, full-length copy of itself. This is the blue strand that Dr. Kennedy was talking about.

So these would be blue, and these would be blue. This blue strand is then used by the virus to again make a full-length copy of itself but now in the negative sense, so it photocopies itself and photocopies itself again to get into the right positive versus negative sense.

So it makes more copies of the negative stranded genomic full-length virus, and these are then what are made into what's called the nucleocapsid. The nucleocapsid binds to the proteins that are on the surface of the cell and the virus exits.

SPECIAL MASTER HASTINGS: The virus what did you say?

THE WITNESS: It exits. The virion leaves the cell without killing the cell.

SPECIAL MASTER HASTINGS: I didn't understand the word. Can you spell it for me?

THE WITNESS: Exit. It exits. It leaves.

SPECIAL MASTER HASTINGS: Exit. I'm sorry.

THE WITNESS: The term that most virologists use is buds. It buds off the cell. I'll show you a picture a little bit later of this process of the virus budding off.

It uses part of the host cell membrane as its own envelope, so it inserts its protein into the host cell membrane and then it packages the nucleocapsid containing the nucleic acid information, and the virus buds off and goes to be coughed out and infect the next child.

So when Dr. Kennedy specifically stated that the primers used in the Uhlmann and Unigenetics targets the positive sense, the blue strands, that was partly true. It does target those blue strands, but it also targets these red strands, the genomic negative sense RNA.

There will be a test later today.

MS. BABCOCK: Let's hope not.

BY MS. BABCOCK:

Q Now, Dr. Kinsbourne cited to an article by Bosch, which I believe is a 1948 article written in German. Were you able to review this article?

A Yes. Well, I was able to review the

translation. I took a year of college German, and it was quite humbling to try to use my 20-year-old German to actually read the article, but I was supplied with a translation of the summary and so I was able to review that.

Q Now, are you aware of any known association between wild measles infection and autism?

A No. The reference to this article came as a bit of a surprise and so I really wanted to get it. It was reported in 1948, and the summary was basically they summarized two cases of infantile dementia with onset shortly after natural measles.

I was intrigued that Dr. Kinsbourne was referencing a paper that was published in 1948, and because I often like calculating on the back of napkins I immediately sat down and said well, if this is true what's the possibility that this is the last case of wild-type measles associated with the development of autistic spectrum disorder.

I calculated that between 1948 and 1978 when measles vaccines became widely available that the birth cohort of the world was about nine billion children, so nine billion children were born in that 30-year period. The Expanded Program on Immunization came in around 1974, so the world's children started

to have access to measles vaccine around that time.

The U.S. Census Bureau says that there were 2.5 billion people in 1950, 3.7 in 1970, and so if you estimate that there were three billion children per year during this entire period and that one child is born per year for every 100 people in the population, which is roughly what happens, you get a number of nine billion children being born.

Prior to measles immunization, every single one of these children would have experienced measles virus, wild-type measles virus, probably before the age of three years. In addition, once the vaccine started to come into the world, you have between a half a million and a million children every year who still got measles right up until this year.

And I'm not aware of there being a single case report of an association between measles, natural disease, and the development of ASD between 1948 and 2007. So, I mean, if it occurs, if there is an association, it is occurring at a frequency in the neighborhood of one in nine to 10 billion, which seems remote.

Also, the authors in their own summary said that a search of the available literature to them at the time didn't reveal any additional cases. So even

prior to 1948, they couldn't identify any cases. So this seems to me to be extrapolation from the weakest of data.

Q Now, are you aware or do you agree with the use of MIBE as models for Michelle Cedillo in this case? That is the next slide, Slide 11.

A Well, SSPE and measles inclusion body encephalitis are essentially 100 percent fatal diseases. Unless in the case of measles body inclusion encephalitis unless the immuno suppression of the child or the individual is reversible it is the most likely outcome in both of these diseases that the individual will die.

Human cells can be infected, human astrocytes. In fact, almost all epithelial cells can be infected. Most immune cells can be infected by measles, and the typical pattern when wild-type or vaccine strain measles infect a cell is that that cell dies.

When replicating morbilliviruses get into the brain, whether they be measles virus, canine distemper virus, phocine distemper virus, when a replicating virus gets into the brain in this class of viruses the organism dies.

Dr. Kennedy stated that very clearly when he

was asked a series of questions. Phocine distemper virus in the brain. The seal dies. Canine distemper virus in the brain. The dog dies.

So while SSPE and MIBE are interesting, they are certainly not models for what appears to be the case in Michelle Cedillo where she has had this hypothetical measles virus in her brain since it was administered at 15 months of age and her clinical course is stable.

She's alive and in many cases receives immuno suppressive therapies that most of us would predict would make a persisting viral infection much worse, and she's still alive. In fact, she's improved after several of these therapies.

If we go on to Slide 12, SSPE is the exception that in a sense kind of proves the rule because it demonstrates that measles virus, wild-type measles virus, again being very specific, has the capacity under certain circumstances to persist, but when it does it's a dead end for both the virus and the host because the virus does not produce any -- or if it does very rarely produces any -- virions.

On the left side of the slide you can see a picture supplied to me by Dr. Fujinami of a neuron infected with wild-type measles virus, and what you

see here at the piled up nucleocapsid, the N-gene, the N-gene product, the proteins that are piled up. They have massive amounts of the N protein because they can't make virions.

On the other hand, here you have an infectious B cell with viral particles budding off quite happily because you have the appropriate proteins in the appropriate ratios.

So when this virus, wild-type virus, persists in SSPE it does so in a heavily mutated form that is clearly abnormal. It is not present in a normal replicating form, and even this form of persisting virus is inevitably fatal.

Q Now, what are the measles antibody levels in the brain of someone with SSPE?

A Well, as you might expect, if one has a persisting viral infection but has the capacity to make some of its protein in high abundance at the front end of its genome, one would expect that those proteins would elicit an immune response, and indeed that is the case.

Most children with SSPE have elevated levels of antimeasles antibodies in their brains, the CSF, cerebral spinal fluid, compared to their blood, and in fact that's one of the very useful diagnostic criteria

for SSPE is the ratio of CSF antibody level to the peripheral blood antibody level.

In normal circumstances it is higher in the blood than it is in the CSF because the immune response in a child who has measles or has been vaccinated, the immune response occurs in the periphery, in the tissues, not in the brain, and so the antibody that gets into the brain is derived from the peripheral blood, so the ratio is systemic blood predominant, lower in the CSF.

If you have a persistent virus infection in the brain that ratio is reversed, and that's one of the very useful diagnostic tests for SSPE or a persisting viral infection in the brain.

Q Now, are you aware of any testing that was done on Michelle Cedillo for antibody levels or persistent infection in her brain?

A To my knowledge, Michelle Cedillo never had a lumbar puncture so there was no CSF available for analysis.

Q And in the records did you observe any other evidence of inflammation in her brain?

A No. It is interesting though in Dr. Bradstreet's paper where he does state he isolates measles virus from the brain.

Antibody levels were measured in those cases and were in fact lower in the CSF than they were in the periphery, so the normal pattern, not an SSPE type pattern.

Q Now, I believe there are several sources Dr. Krigsman may have cited done by Dr. Gupta and Singh purporting to find elevated measles virus titers in autistic children. Do you agree with these papers?

A That's right. There's been isolated reports of an elevated peripheral blood measles antibody titer in children with autistic spectrum disorder.

I think that I have some technical problems with that paper, as have others. There have been criticisms of that paper in the literature. We attempted to replicate that work on our own study of ASD spectrum children and were unable to replicate the work. That's the D'Souza paper published just in 2006 in *Pediatrics*.

And I'm aware that Dr. Fujinami has also tried to replicated that work and has a paper that is in press which again fails to replicate the observation by Singh, et al. In fact, the autistic spectrum disorder children had lower measles antibody titers than some of the other children in Dr. Fujinami's study.

Q And that article is in press?

A That article is in press.

Q Now, Dr. Kinsbourne cites work by Carbone and Oldstone, among others, for the proposition that measles virus can persist in the brain without obvious pathology. Do you agree with this?

A Well, in the case of Borna virus it clearly can with either lower levels or very limited demonstrable pathology.

Dr. Carbone and I actually trained together in the same neurovirology unit, so I would get in trouble if I said something different from that because that's her life's work.

However, to achieve that state of immune tolerance in the Borna virus model the virus has to be injected directly into the brain of perinatal rats. That is clearly not what happens to Michelle Cedillo, so while it is a related virus that's in the same global family of mononegala viruses -- it's a negative sense RNA virus; it has the possibility of persisting -- it's not a great model for measles because we already have the model of a persisting measles virus infection in the brain, and that's called SSPE.

We have another biological model called measles inclusion body encephalitis and so the

hypothesis that there is yet a third way in which the virus and in this case the vaccine strain, which has never been demonstrated to persist in SSPE, can persist is completely new biology.

I'd also like to point out that Dr. Oldstone is one of the éminences grises of the respected authorities. It means you have gray hair. No disrespect intended. I'm heading that way rapidly myself.

One of his favorite viruses, a large part of his work, has been on measles virus and measles virus in the brain and neurovirulent measles virus. This is an area that Michael Oldstone has spent a great deal of his life studying.

If Dr. Oldstone believed the MMR autism hypothesis to be true and if Dr. Oldstone believed the persistent measles virus in the brain following MMR to be true, he would be I think enormously excited by this new biology, by this new data, and I'm not aware of Dr. Oldstone quoting any of the literature quoted by the Petitioners' experts in support of this hypothesis in any of his cutting-edge work up until papers published in 2007.

Q Now, you touched on this earlier. I believe it was Dr. Kennedy's testimony that measles virus is

part of the morbillivirus family, correct?

A Correct.

Q Now, when a morbillivirus gets into the brain of the host what usually happens?

A Well, that was what Dr. Kennedy directly testified to and is the general understanding. When a replicating morbillivirus, be it measles, canine distemper virus, phocine virus, gets into the brain it kills people or kills the organism -- dogs, seals, dolphins.

Q Now shifting specifically to measles virus, are there any instances where measles virus can be treated if it gets into the brain?

A Well, there's a debate about whether anybody has ever successfully treated someone with a life threatening measles condition, measles related condition, SSPE or measles body inclusion encephalitis, but people have certainly tried.

People have tried using high dose Ribavirin, which is an antiviral drug. People have tried using high dose immunoglobulins that are specifically tested to make sure they have high levels of antibodies against measles.

There are case reports of people who improve, and in the case of measles body inclusion

encephalitis where some people have actually recovered concomitant with recovery of some immune function in individuals who are transiently immune suppressed.

So if one has a life threatening disease like measles body inclusion encephalitis, if one has replicating measles virus in the brain one could make a pretty convincing argument that even though there is no guarantee that you will improve with these therapies that these therapies might help to control a virus that has not yet been controlled by the body's immune response.

Q Now, are you aware of whether anyone considered treating Michelle Cedillo with antivirals targeting measles virus in this case?

A Well, in my reading of the case reports there was no specific recommendation that this ever be administered so that no therapy directed against a persisting measles virus was considered in Michelle Cedillo's case.

As I've already pointed out, one could make the argument that several of the therapies she did receive for her inflammatory bowel condition would be considered by many people to be risky, if not outright unwise, to administer to somebody with a replicating measles virus infection in their body, let alone in

their brain.

Q Now, you touched on this earlier. Several of the Petitioners' experts have cited the article by Dr. Bradstreet to extrapolate that measles virus was likely in Michelle Cedillo's brain. Do you agree with the conclusions Dr. Bradstreet reached?

A Well, for a variety of reasons I don't agree with the conclusions that Dr. Bradstreet reached, for one reason, because I have little confidence in the testing that was performed to demonstrate the virus, and the manuscript that is published does not provide -- it's missing a great deal of detail that would allow one to evaluate it rigorously.

Q Where was this paper published?

A It was published in the *Journal of the American College of Physicians and Surgeons*.

Q And what type of journal is that? Is that an indexed or a non-indexed journal?

A It's a journal that's produced by the American College of Physicians and Surgeons and distributed to their members. It is a non-indexed journal, which means that it is not searchable by the public search engines. You can find it by specifically going to the American College website, but you do not find it when you use the traditional

medical scientific literature search engines like PubMed or the others.

Q What are the primary reasons, generally, for a journal not to be indexed?

A Well, there are really only two reasons; that the journal is brand new and has no track record of publishing rigorously evaluated science, or it is considered by the scientific community not to publish rigorously reviewed science, and therefore, not of any use to the medical scientific community, and therefore not indexed.

Q Now, Doctor, is this a new journal?

A No. As far as I know, the journal has been around for decades.

Q I wanted to move on to gut testing for measles virus.

A If I could?

Q Certainly.

A If a colleague were to come to me with brand new, never before described in man, observations, as Dr. Bradstreet and his colleagues are purporting to have discovered, that would shift a whole paradigm and possibly explain a mysterious illness, in this case autistic spectrum disorder, my advice to Dr. Bradstreet would not be to publish it in the *Journal*

of the American College of Physicians and Surgeons.

That kind of landmark study, if indeed it is a true study, if the results are reliable, should be targeted more at *Nature*, *Science*, *Journal of Clinical Investigations*, the high impact journals where, you know, that kind of finding should be published. So it is scientifically to me very odd that this work was published in a non-indexed journal.

Q Okay. Now we'll move on the gut testing for the measles virus. Now, nearly all of Petitioners' experts, if not all of them, rely heavily on assertions that measles virus was present, measles virus RNA was present in Michelle's gut, correct?

A That's correct.

Q And this is the testing by Unigenetics?

A That's correct.

Q And Dr. O'Leary used PCR to run the testing?

A That's correct.

Q Is this a testing method you are personally familiar with?

A Yes.

Q Now Dr. Hepner discussed PCR at some length, and Professor Bustin is certainly going to discuss it later on today, so I don't want to duplicate too much here. However, in her discussion of PCR, Dr. Hepner,

was there anything you wanted to clarify or add to Dr. Hepner's outline?

A I thought Dr. Hepner did a very good job of summarizing the mechanics of PCR and explaining in an understandable way how PCR works. I also thought she was appropriately cautious in her interpretation of her own data that she's produced with Dr. Walker, where she very specifically characterized these data as preliminary, unblinded, and uncontrolled.

Q Now, what are some of the vulnerabilities of PCR? And I believe we are moving on to slide 14.

A Well, if I were to take issue with any part of Dr. Hepner's testimony, it would be in leaving the impression that PCR is somehow a magically sensitive technology where you walk in, put the sample in the machine, press a button, and you get truth out the other end. For those of us who have struggled with PCR in our research and our diagnostic laboratories, we would all wish that that were true, but it is not.

PCR, like any other technology, is vulnerable to a number of vagaries, some of which can be dealt with and others of which are problematic even in the best of labs. So specifically, and Dr. Bustin is going to talk about this in much greater detail so I'm just going to touch on the surface, the quality of

the RNA used is really critical; where you derive the RNA from, whether you derive it from frozen tissue or fresh tissue, because RNA is remarkably labile compared to DNA.

DNA, if I were to do a PCR reaction with my machine in this room at this time and open a tube, and probably not even have to blow on it, so that if I have a PCR reaction in my tube and I blow on it, this room would be contaminated from months to years with those little pieces of DNA, because DNA is remarkably stabile. RNA is just the opposite. It is remarkably labile, and so the quality of the RNA that you use to generate your data is critically important, and so determining what the quality of your RNA is is essential to the validity of your results.

What I just mentioned about opening a PCR reaction in your lab, or your courtroom, is probably the largest Achilles' heel of PCR, because contamination is very frequent even in the most compulsive of labs. Each reaction, I said 'bazillions,' but in fact a quick calculation comes out to 2.7 trillion copies with a 38 to 40 cycle PCR reaction, starting with one copy. If you start with a thousand copies, then you're up into numbers that I don't even know the name for, and so you have my

'bazillion.'

You just have massive amounts of DNA that are produced in a single PCR reaction, and so all labs have to be completely compulsive about dealing with all aspects of contamination, and very rigorous in their standard operating procedures for dealing with that inevitable contamination. If one doesn't have the SOPs to anticipate the contamination, one cannot produce reliable results.

There are many strategies that one can use, doing amplifications in different rooms, putting in enzymes that degrade the DNA, many different things can be used, but to my reading of the Uhlmann paper, not all of these procedures were satisfactory, and Dr. Bustin will go into more detail.

The specificities of the primers and probes is critical. If you do not have probes that are absolutely specific, if you do not have primers that are absolutely specific for the target you are amplifying, then you will generate misinformation rather than information, and one has to be completely compulsive in evaluating your -- any PCR assay as you are developing it, to make sure that the results are internally consistent and do not generate false positive results. That means you have to use your

best tools possible for testing the specificity of your assay, and in PCR, that means sequence data.

The last thing is you have to maintain a healthy skepticism. This is not press button, get truth. You have to use your controls. You have to standardize your operating procedures and your analysis, because you can also jigger the results by doing things post hoc. You can get your results, you can move things and change the result from positive to negative and negative to positive, by manipulating the data on the machine after the result is produced.

And so, every available means has to be used to confirm these results.

Q Now, is it easy to interpret PCR data? Maybe this is the next slide. 15?

A Well, absolutely not. As we discovered in our work trying to optimize the primer pairs that were generated by Uhlmann et al. in their publication, the data is really simple to generate, but interpretation requires expertise and rigorous maintenance of standard operating procedures for the analyses as well. As we've mentioned, you need care in probe/primer selection, appropriate controls, assay optimization, including locks of the machine parameters -- even minor changes in machine settings

can change your result from positive to negative -- and we've already mentioned the strategies for minimizing contamination.

Here are just two examples of the kinds of data that you would get from the machine where you would get a signal. And so here you have your positive control is giving you what's called a melt curve. The PCR, in some of the real-time PCR manipulations, you can generate what's called a melt curve, and that is the temperature at which the two pieces of DNA that have been amplified will break apart.

The closer they are to being identical -- each segment of DNA has a specific temperature at which it will break apart, and so you can measure the temperature at which it breaks apart, and that is a good indication that you are looking at the sequence that you think you are looking at. And so here is the control melt curve temperature, so this is temperature on the X axis, and here you have the melt curve analysis, and here you have an unknown that is giving an identical melt curve.

So the melt curve in this specific example is yielding the expected result, and so that would tend to give you some more confidence in your result.

However, you shouldn't stop there. You should then go on to a gel, and using a DNA ladder to assess the size of the gel. So this is a ladder that is made up of different specific-sized DNA pieces, and this one here is at 200 base pairs. So anything above this would be higher than 200, and anything below this would be lower than 200.

And in this specific amplification, we were looking for a product that was approximately 150 base pairs in size, and you can see that the positive control using the Uhlmann primers, in this case I think this was for F-gene, it gives you a band. However, it also gives you this blurring, this messy band, which suggests that there is some nonspecific amplification. That's a good indication that maybe your primers aren't perfect or your assay is not fully optimized.

Here is an example of a sample where we had the correct melt curve, but when we took that amplified DNA and ran it out on a gel to look at how big it was, there was no band at the expected size. So even though it had the correct melt curve, it didn't have the correct size band, and therefore was unlikely to be the product that we thought we were amplifying.

Q Was Uhlmann blinded?

A No. To my knowledge, there was no blinding in the Uhlmann study.

Q Now, is it accurate to say that PCR can be used both as a research tool and also as a diagnostic tool?

A Yes. Almost all assays start out as research tools, and once they are optimized and carefully controlled and you have a great deal of experience with them, they can be successfully translated into a diagnostic tool. And several companies have done this quite well for HIV, chlamydia, gonorrhea, and so on. These are standard PCR-based technologies.

Q What precautions must be taken if you are using it as a diagnostic tool?

A Well, you have to be absolutely certain that you are getting what you think you've got. You have to have enough experience with it to be one hundred percent certain that when your machine, when you press the button and you have followed all of your standard operating procedures, that when you press the button, the data that will come out will in fact be true and valid as a diagnostic test.

Q Now, would you describe the Unigenetics

tests that you saw for Michelle Cedillo and that have been discussed as a research tool or a diagnostic tool?

A Well, I would have characterized it primarily as a research tool, but in the case of Michelle Cedillo, it's clearly being used as a diagnostic tool.

Q Now are you aware of published papers providing molecular information about a link between MMR and autism?

A Well, as far as I'm aware, there is only the Uhlmann paper, a very similar paper with identical authors in reverse order in the same journal, lead author Martin. It's unclear if that is the same group, the same data, the same study, as a duplicate publication. I don't know. There's much less detail in the Martin paper. And a small study by Kawashima where they used a slightly different PCR technique in a small number of children with autistic spectrum disorder.

Q This is another area I don't want to duplicate too much with Professor Bustin, but are you aware of concerns that were raised about the Uhlmann paper in particular?

A Well, yes. I think there have been a number

of concerns raised about the Uhlmann paper, and specifically, when Dr. Afzal tried to identify measles genomic sequences in peripheral blood mononuclear cells of children with autistic spectrum disorder, he was unable to find them. And our own studies, where we tried to replicate aspects of the Uhlmann and the Kawashima assays, we were unable to replicate their findings.

Q So let me be clear. Your own lab designed in an effort to replicate Uhlmann's work?

A Well, we tried to get data that would be informative to the question of whether measles virus can persist in the tissues of autistic spectrum disorder children.

Q Now, what are PBMCs and why did you target them?

A Well, our study, like that of Dr. Afzal's, targeted peripheral blood mononuclear cells. PBMCs are what's left over when you take out the red blood cells, platelets, and the neutrophils from the peripheral blood. They are composed primarily of macrophages, dendritic cells and lymphocytes. All of these cells are susceptible to in vitro and, we presume, in vivo infection with measles virus and measles vaccine strain virus.

Q Okay. So to be clear, you had reasons to think that finding measles virus in the gut was similar to finding measles virus in blood?

A Well, Kawashima actually reported that he found -- Kawashima targeted peripheral blood mononuclear cells in his study, and he reported finding measles virus genomic material in 30 percent, so three out of the nine samples he studied. So one of the two studies where this has been reported specifically used PBMCs.

In addition, Dr. Bradstreet, in his article that we just discussed a moment ago, reported that peripheral blood mononuclear cells were targeted and in at least one out of the three children, measles virus genomic information was identified, so again, that would be 33 percent, and in Dr. Kinsbourne's testimony, he said very clearly that if measles virus is replicating in the gut and it's in the brain, it moves between those two places in the blood.

There is every reason to expect that if a measles virus is replicating in the gut, and there is inflammation -- inflammation means white blood cells. It means immune cells. So if there's measles virus replicating in the gut and the immune cells are coming in to deal with the inflammation, there is really no -

- and those immune cells are fully susceptible to infection with measles, there's no way to hypothesize that the virus doesn't get from the inflamed and infected gut cells into the immune cells.

Those immune cells, by their very nature, do not stay put. That's not their job. Their job is to move and alert other tissues in the body, and so the macrophages and some of the other cells would be expected to move to other parts of the body, therefore carrying the replicating measles virus in their cytoplasm, making the peripheral blood mononuclear cells a very adequate tissue to test this hypothesis.

Q And what were the results of your study?

A Well, they were interesting in several respects. And I won't go through in any detail, but the most interesting aspect I think is that when we looked at control versus autistic spectrum disorder children, and their PBMCs, and we followed rigorous standard operating procedures for the evaluation of the positive results that we got, we found that the Uhlmann primers, and this was across the board, the H, F and N primers gave us a lot of nonspecific amplification.

They gave a signal in almost all of the cases when we looked at the melting curve, both in the

controls and the ASD children. However, many of these positives fell out when we looked at melting curve, so they were not amplifying the appropriate sequences. When we looked at melting curve, many of them fell out, but not all of them. So we had signal in the machine that yielded a good melting curve. When we took it further and took those DNA fragments and ran them out on a gel, we found that in fact, many of these actually proved to be negative.

So on the control side, we went down this far and we found none of the amplicons were of the correct size, suggesting that these results had been false positives, but on the autistic spectrum disorder side, in these PBMCs, we actually were left at this point with 3 out of 42 of the children having a signal that appeared to be good signal in real-time PCR, the correct melting curve, the correct size that we were anticipating by the gel.

And if we had stopped at this point and not completed our standard operating procedure of analyses, we would have reached conclusions that were very similar to those of Kawashima. We would have said 3 out of 42 individuals with ASD have circulating measles virus in their blood, compared to zero out of 17 in the controls.

Q Now can you briefly explain the difference between probe versus dye-based PCR? We're at slide 17.

A Right. We did not use an identical PCR strategy to either Kawashima or Uhlmann. In the case of Uhlmann, we didn't use gut biopsy tissue. In the case of Kawashima, their lab uses nested PCR; we used a modified real-time PCR. In the Uhlmann case, they used a probe-based PCR assay, and we used a PCR assay that has a different detection system.

So very briefly, and you will have seen this described by Dr. Hepner, but the difference is that in the Uhlmann assay, the primer runs along and makes a new copy, and in the running along, it hits a primer that is -- it hits a probe that is fluorescently labeled, and as the primer hits the probe, it destroys the probe, resulting in the release of the fluorescent molecule that tells you that the DNA is being amplified.

If used properly, probe-based PCR assays can be very, very specific, very powerful. We used a slightly different PCR technology where we simply put in a dye that binds to double-stranded DNA. So the more double-stranded DNA we had, the more dye that fluoresced, because this dye is not shining when it is

not bound to double-stranded DNA, and it shines when it sticks itself in or intercalates into the DNA.

We used the same primers, and we used essentially the same machine. We used slightly different technologies for detecting the end products.

Q Now, getting back to when you were working through and you got to a point where your results appeared on their face to be somewhat similar to what Kawashima found, did you then sequence?

A Yes. The standard operating procedure that we had in place prior to starting these studies was that any positive result that made it through our analysis would actually, would then be cloned and sequenced, because cloning and sequencing is the gold standard. That is the absolute guarantee that you know you are amplifying the correct piece of DNA.

And when we did that, what we discovered was that all of the gene products with the Uhlmann F, N or H primers that we used, that had correct melting curve and correct amplicon size, when we actually went through the fairly laborious process of cloning and sequencing them, were actually human genes. In no case did we find measles virus genomic material.

Q Slide 19, please?

A Obviously, as a logical question to ask, is

if -- and I assume that the Uhlmann primers were designed in good faith. If one is using a set of primer pairs that you have designed in good faith that target the measles virus genome, why would you get nonspecific results? Well, we now had the human genes amplified, and so we had the sequences. And we were able to ask a very simple question: Do the Uhlmann primers, would you predict that the Uhlmann primers might actually stick to this human genetic information?

And when we did that, we found with varying degrees of homology that in fact, for example, the F-1 primers that we used, that there was 65 percent homology if you look at the whole primer, but actually 85 percent homology if you looked at the business end of the primer, because this is where the DNA is going to be amplified from, so it's critical that this part of the primer stick to the underlying DNA sequence.

So we had 80 to 85 percent homology between these primers that were supposed to be targeting measles, but were in fact also not bad as primers for this particular human gene. This is at least one possible explanation for why we were getting those blurry bands and why we were getting the incorrect melt curves, because we believe that several of the

Uhlmann primers are in fact yielding nonspecific results.

SPECIAL MASTER HASTINGS: Can you explain what homology means?

THE WITNESS: Oh, I'm sorry. Homology means identity. So that you really want these primers to be identical to the sequence that you are targeting. So a perfectly homologous primer would have the same nucleotides at each one of these positions. So here you have a T and a T. So here's the genomic sequence you are targeting, here is the primer sequence. So you have TT, that's a perfect match.

Again here, you have AA, AA. But these are nonhomologous. You have an A versus a G, then a G versus an A. In theory, you want your primers to be perfectly homologous to a unique sequence of DNA found only, for example, in this case, in measles virus.

In the case of the Uhlmann primers, they were close in their degree of homology to several human genes, which would mean that if you didn't rigorously optimize your assay conditions, you could get the primers binding to non-measles genes, and the amplification of non-measles sequences, which is what we've demonstrated by sequencing the DNA bits that were amplified.

BY MS. BABCOCK:

Q So to summarize, if I'm understanding this correctly, when you sequenced, you discovered that what was amplifying was not measles virus?

A That's correct.

Q Okay.

A We were getting signal that looked good. The real-time PCR machine said, you've got measles virus here. The melt curve analysis said, you've got measles virus here. The band size said the same thing, but all of those are not the gold standard, because you can have exactly what we found. You can have things that look like they are correct, but when you apply the gold standard sequencing, they are erroneously amplified bits of human DNA.

Q And let me be clear. Gold standard means?

A The best.

Q In that, you're referring to sequencing?

A Sequencing. When you are developing a PCR test, you have to sequence.

Q How long did it take your lab to generate the sequencing information?

A A year and a half and a lot of tears on the part of my graduate student who did this work. It's very frustrating to try to blunt-end clone PCR

products.

Q To your knowledge, has Uhlmann ever published sequencing from his 2002 paper?

A To my knowledge, he has not.

Q And you, to be clear, you published the results of the study you just described?

A Yes, these results were published in *Pediatrics* at the end of 2006.

Q Now, Dr. Hepner discussed in her report and in her testimony that comparing PCR results from gut tissue to PCR results from PBMCs is like comparing apples to oranges. And that's slide 20.

A Okay. Well, the DNA material in the body is the same whether you're a liver cell or a brain cell. Primers that are designed to target DNA based on liver DNA will amplify brain DNA. There really is no difference between the genetic information that's available to a PCR machine, depending upon the tissue you're looking at.

Since our assays, like the Uhlmann assays, target RNA, which is the message form in the human body, and in this case also the viral RNA, there are tissue differences, there can be tissue differences between the expression of post-RNA. So the brain does not produce all the same proteins that the gut does,

and to produce those proteins, they have to make their own restrictive set of RNA, because you need to make the RNA to make the protein.

So it is possible that you could generate a set of primer pairs that was targeting RNA that would work in one tissue but would not work in another tissue. That makes sense. However, if a primer pair gives nonspecific results in any tissue, then it is suspect in all tissues.

Q Now, setting that aside, good rationale, but why didn't you just compare apples to apples, or at least according to Dr. Hepner?

A Well, because I would not have requested of my ethics committee and they would not have granted permission to perform endoscopic biopsies on most of our children with autistic spectrum disorder. They would consider them to be medically unnecessary procedures, a fact that Dr. Krigsman himself confronted in his New York practice.

Q Nevertheless, have you also used these primers --

A By the way, that was the same rationale for Dr. Afzal not targeting the gut materials of ASD children. His ethics committee also would not permit him to do what were -- he would not ask and he would

not have received permission to do that study.

Q I'll re-ask the question now.

A Sorry.

Q Nevertheless, have you also been able to use these primers on gut tissue?

A Yes, in fact, the original intent, Yasmin D'Souza's original master's thesis project was to test biopsies from children with inflammatory bowel disease and compare them to controls. And we did the ASD study first because we got access to those specimens first. Through Dr. Fombonne's funded study, we got permission to use those samples first. It took us longer to collect the biopsies from children who were undergoing diagnostic endoscopies for inflammatory bowel disease for conditions that proved not to be inflammatory bowel disease.

Q So to be clear, these children had preexisting gastroenterological issues?

A Yes. Their endoscopy was considered to be medically required.

Q Now what primer pairs were used?

A Well, the study was essentially a carbon copy of what we did with the PBMC in the autistic spectrum disorder study, where we took the control biopsies, which were children who had, essentially, a

diagnosis of inflammatory bowel disease ruled out by their diagnostic endoscopy, and biopsies from children who had their IBD diagnosis ruled in by the biopsy, and we isolated the RNA from the biopsy tissues, essentially, identically to the way that it was isolated by the Uhlmann and Kawashima investigators, and used the same primers.

We used the Uhlmann F, H and N primers, or some of the Uhlmann F, H and N primers, the Kawashima primers, and our own in-house F-gene assay to try to identify measles virus genomic material in the guts of children with IBD.

Q And did you encounter any issues?

A The data were very similar to what we observed in the PBMC study of ASD children. We found a lot of positive results in the machine. We found some that had the appropriate melt curve analysis, we had some that had the correct size on gel electrophoresis, but again, when we blunt-end cloned and expressed those sequences, we found them to be human genetic material, not viral genetic material.

Q So you did sequence that?

A We sequenced it as well, and again found, in some cases, similar human genes, and in other cases dissimilar human genes to what we found in autism.

Q And were the results published?

A The results were published a week ago in *Gut*. So they were just released in electronic form at the beginning of June.

Q And who funded this research?

A The technical aspects of the work were funded by the Crohn's & Colitis Foundation of Canada, a very small grant but very welcome nonetheless, and the collection of the ASD specimens and control specimens was funded by a grant to Dr. Fombonne from the Quebec government funding agency. It's called the FRSQ.

Q Now, Dr. Krigsman and Dr. Hepner discussed, both discussed an abstract by Walker and we were provided with additional information from that poster last week. Have you reviewed those items?

A Yes, I have.

Q Does what you reviewed allow you to have confidence in what they were presenting?

A Well, I think it should be obvious to the Court that Dr. Hepner herself characterized this study as preliminary, uncontrolled and unblinded, and so if one gives Dr. Hepner the benefit of the doubt, I think that the most that can be said about these observations is that they are potentially interesting,

but they would have to be subjected to much more rigorous scrutiny before they were accepted.

Q Now how does the scientific community view abstracts?

A As interesting ideas by and large.

Q Now, on that topic, have you ever made a presentation or prepared an abstract concerning testing on gut tissue?

A A case in point, the project for identification of measles virus genomic material in gut biopsies of children was started almost two years before Yasmin D'Souza came to my lab as a master's student. It was started by a summer student, a medical summer student, where we got a small number of gut biopsies from adult and pediatric gastroenterologists at McGill, and we designed a set of primers that were not the Uhlmann primers because they were not yet available in the literature.

What we tried to do was we took all of the sequences of wild-type measles, vaccine strain measles, and a bunch of closely related viruses, in the same family of viruses, and we said, maybe these results are not due to the amplification of measles, but they are due to the result of the amplification of a closely related but as yet undiscovered virus. And

it was the perfect study to do with a medical student in the summer, because it was potentially really cool that we could identify a brand new virus, and it was something that could be accomplished in a period of a summer.

And so we developed a set of what we called degenerate primers, which had -- we selected regions of these different genes, sorry, these different viruses, that were as close to as identical as possible, so that in those areas, each one of these viruses had very close homology. Not perfect, but they were very similar, so that we would have a reasonable hope that if we designed a primer that was close enough to all of these different viruses, we might amplify each one of these viruses, including measles, but also, an as-yet unknown virus that was closely related.

So our hypothesis was that the gut tissues of these children might contain either measles virus or a closely related virus that was either known or unknown. These types of primers are called degenerate primers, and they can amplify -- they are intended to be not perfectly specific. And so the medical student set it out to do this work, and generated some interesting results where we believed we had some

amplification.

We were using classic PCR at that time, not real-time PCR, and we took some of them to sequence. And if I remember correctly, one out of the two had a sequence that was related to measles, and the other one that we succeeded in cloning had sequences that didn't, that looked like a paramixovirus, but it did not look like any of the viruses that we had that were known. And so we were actually pretty excited that this was interesting new biology.

Right around that time, I was contacted by the American Academy of Pediatrics because they knew that we were thinking about working on this, and they said, do you have any data that might inform this debate? I was invited to present this data, which was presented as, just as what it was. It was a limited, preliminary study, and performed by a medical student, and here was our preliminary data.

And in a subsequent medical student summer job the following year, we demonstrated to our satisfaction that what the first medical student had generated was probably not true, for a variety of technical reasons, contamination, and inadequate procedures by a relatively inexperienced operator. And so that data was presented as an abstract at the

American Academy of Pediatrics in preliminary form, and has never been published because, in fact, it was wrong, as often happens with abstracts.

Q Now, did you later discover that the abstract you presented had been used by others in the scientific community?

A Well, yes. I was a little bit surprised at the Institute of Medicine meeting when I was on the review panel when Dr. Wakefield actually presented my data with his own interpretation, and actually had the courtesy of thanking me for doing this very important work, even though he knew that it hadn't been published, was preliminary, and I took pains to explain to him after that presentation that the work hadn't been replicated and therefore shouldn't be used in the fashion that he was using it.

Q Now, overall, based on your professional experience, review of the medical records, literature, reports, listening to the testimony, do you place any reliance on the Unigenetics laboratory report for Michelle Cedillo?

A I do not.

Q Do you think there is any evidence to show that the MMR vaccine more probably than not caused Michelle Cedillo's autism?

A I do not.

Q Do you think that the MMR part of this hypothesis is biologically plausible?

A At this point, I do not. At some point in the life of any hypothesis -- well, in the absence of data, you can hypothesis just about anything.

So at the initiation of a hypothesis, many things are possible, and all hypothesis have a life. Some hypotheses go on to be proved based on data generated. Some hypotheses go on to be disproved, based on the data generated; and other hypotheses can never be proved or disproved. Because for example, they are not scientifically empirically testable.

In this case, I think that the MMR ASD linked to the Petitioner's hypothesis has been disproved. Therefore, I think at this point, I would say that it is not biologically plausible.

MS. BABCOCK: I don't have any further questions.

MS. CHIN-CAPLAN: Could we just take a quick break?

SPECIAL MASTER HASTINGS: Why don't we take our morning break at this point. We'll meet back at 11:00, 15 minutes from now.

(Whereupon, a short recess was taken.)

SPECIAL MASTER HASTINGS: All right, we're back from our morning break, and Ms. Chin-Caplan, you'll be doing the cross examination.

MS. CHIN-CAPLAN: Yes, Special Master.

SPECIAL MASTER HASTINGS: Do you want to do the pin microphone? That might be a good idea.

MS. CHIN-CAPLAN: Is this on battery, because it doesn't seem to be turning on?

SPECIAL MASTER HASTINGS: It doesn't seem to be working?

MS. CHIN-CAPLAN: No.

SPECIAL MASTER HASTINGS: Okay.

MS. CHIN-CAPLAN: I'm thinking that if we left it on, the battery would be dead. But I don't know if it's battery or not.

SPECIAL MASTER HASTINGS: Okay.

THE WITNESS: There's one here. Do you want to use that one?

SPECIAL MASTER HASTINGS: Is that one working?

MS. CHIN-CAPLAN: No, I think it's the same situation. I think we left them on.

SPECIAL MASTER HASTINGS: All right, to those listening in, we're having a bit of a technical glitch here, which we hope to be done with in a

moment. All right, go ahead, Ms. Chin-Caplan.

MS. CHIN-CAPLAN: Thank you, Special Master; if I wonder over to you, remind me to take this with me.

CROSS-EXAMINATION

BY MS. CHIN-CAPLAN:

Q Doctor, you indicated that you can't compare wild measles virus to the vaccine strain. Is that true?

A No, I think some comparisons are appropriate. But when talking about one or the other, one needs to be specific.

Q Okay, but there are certain similarities between the wild strain and the measles strain, isn't there?

A Of course.

Q And one of those similarities is its effect on the immune system?

A There are aspects that are the same, yes.

Q And you, in fact, have written an article about the similarities between wild virus measles and vaccine strain, correct?

A Absolutely, yes.

Q And you've written articles on its effect on the immune system, correct?

A I've written articles on its effects on the immune system, yes, because there are phenotypic changes that are measurable in the cells, isolated from somebody with vaccination, and in the function of some of the immune cells taken out of the body of a child following vaccination.

Q So Doctor, on page five of your presentation, which is Respondent's Trial Exhibit Number 12, you're talking about immune system balance there?

A Yes.

Q And you've indicated that many populations are relatively TH2 bias; and the final sentence was that there's no evidence that measles vaccination causes TH2 deviation?

A What I said was, clinically relevant TH2 deviation.

Q Okay.

A I also said that in vitro, you can see changes and you can see changes in immune cell function that suggest a TH2 deviation. But the key there is distinguishing between what you can demonstrate in a test tube and what you can demonstrate in real life.

Q So Doctor, in Petitioner's Exhibit 79 --

would you like to give him 79?

MR. MATANOSKI: Your Exhibit 79?

MS. CHIN-CAPLAN: Yes, Petitioner's Exhibit 79.

MR. MATANOSKI: I don't know that we have your Exhibit 79.

MS. CHIN-CAPLAN: Oh, well, then let me give it to you.

BY MS. CHIN-CAPLAN:

Q Doctor, this is an article that's entitled, "Changes in Cytokine Production After Measles Virus Vaccination; Predominate Production of IL4 Suggests Induction of TH2 Response." Am I correct?

A Yes, you've read it correctly.

Q And this was published in 1993, correct?

A Yes, a long time ago.

Q Yes, it's good medicine still, isn't it?

A Excuse me?

Q It's still good medicine. Then you published it with Dr. Griffin, correct?

A That's correct.

Q And you indicated that you were a post-doc fellow; was that it?

A Yes, that's right.

Q In her laboratory?

A Yes.

Q Now you indicated that this would be an in vitro response, is that it, in the test tube?

A Well, these were PMBCs isolated from children. So yes, it's an in vitro phenomenon.

Q Okay, and Doctor, if you look in the abstract on page one, the very last sentence, "These data suggest that TH2 cells producing IL4 are preferentially activated by measles vaccine, and may contribute to the immunologic abnormalities associated with immunization for measles and possibly other viral infections." Have I read that correctly?

A You have.

Q And you agreed with that statement when you wrote it, correct?

A Yes.

Q Do you agree with that statement today?

A It has a conditional word in it.

Q Okay, "may"?

A Correct.

Q Now Doctor, if you go to the discussion, which is contained on page 174 -- are you there? It states, "Alteration of immune cell function can be detected in most individuals after vaccination or re-vaccination with live attenuated measles virus."

That's what we're dealing with here; a live attenuated measles virus?

A The question at hand is yes, regarding live attenuated measles virus vaccine. That's the only vaccine available.

Q Correct, at least in this part of the United States and perhaps Canada?

A In the world.

Q In the world -- and then you say, "In this study, we have confirmed the frequency of the defect in PHA-induced lymphoproliferation." What is PHA?

A PHA is a mitogen derived from a bean that by chance was discovered to cause a large proportion of T cells to proliferate, to divide, in response to that stimulation.

Q Is that an indication of some sort of immune function?

A Yes, mitogen responsiveness is a functional immune test.

Q Okay, a functional immune test of anything, or a particular arm of the immune system?

A It's a functional test of cellular immunity.

Q Then continuing on with that sentence, "and have shown that this defect is not associated with altered proportions of CD4 and CD8 T cells, but is

associated with alterations in cytokine synthesis." Have I read that correctly?

A Yes.

Q So Doctor, if we go to back to your slides, did you have a slide on total numbers, of CD4s and CD8?

A It's the first slide.

Q The first slide. Thank you. So, Doctor, if we go back to the first slide, which is page 3, is that it?

A Yes.

Q So Doctor, would it be fair to say that they indicate there is a known total amount of CD4s and CD8s from the sentence that I've just read to you?

A Would you please repeat the question? I'm not sure what I'm being asked.

Q Certainly. With reference to this sentence, "In this study, we have confirmed the frequency of the defect in PHA-induced lymphoproliferation, and have shown that this defect is not associated with altered proportions of CD4 and CD8 T cells, but is associated with alternations in cytokine synthesis."

So when we look your page three, they're talking about proportions between the number of CD4s and CD8s, correct -- a ratio?

A Right, in the paper, we were specifically referencing prior work that had purported to show a change in CD4/CD8 ratio after natural disease, and I believe there was one paper with vaccination. I'd have to look back and find that out. We did not find that similar change in proportion.

Q You found something different, correct?

A We found that that didn't occur.

Q Right, but you also found that there was an alteration in cytokine synthesis?

A Absolutely.

Q Yes, and when we talk about cytokine synthesis, these CD4s and CD8s actually produce cytokines. Is that true?

A Yes, they do.

Q Right, and what you found is that the ratio between CD4s, the actual cell wasn't altered. But what they were told to produce, the cytokines, were altered. Am I right?

A I'm not even sure what you're asking. But yes, we found changes in the total production of some cytokines in the peripheral blood mononuclear cells. But, in fact, some of those cytokines are not produced only by a single cell. So extrapolating to which cells produced which cytokines is inappropriate.

Q And we haven't done that here, right?
A Well, you appear to be doing that.
Q Oh, but we haven't done it, right?
A I guess once warned, you won't.
Q That's right.
A The techniques weren't available at the time to do single cell cytokine staining. If we were to replicate that study today, we would have used a flocytometer to identify which cells were producing the cytokines.
Q So the general statement that the cytokine synthesis was altered in this paper in 1992 was true then, correct?
A I'm sure it's still true today.
Q Okay, and now medicine has progressed to the point that you might even be able to determine which cytokines are affected. Is that it?
A Since 1993, which I think is when this was published, we have almost tripled the number of cytokines known. So yes, were we to do this study today, we would be able to generate more information.
Q Thank you, and Doctor, when you continue on in that paragraph, it says, "Production of IO4 was also increased after in vitro stimulation of vaccinee PBNC with PHA, suggesting that measles immunization

had prime T cells in vivo for production of IL4." Now is IL4 a pro-inflammatory cytokine?

A No.

Q Is it an anti-inflammatory cytokine?

A I don't think many people would consider it to be an anti-inflammatory cytokine. It does have affects on regulating the antibody-type responses; but also allergy and asthma.

So depending on how much of IL4 you produce, where you produce it, and in what context, it can lead to very inflammatory effects: allergy, asthma, those kinds of things. In other circumstances, it may act to decrease some cellular responses.

Q What cellular responses would it decrease?

A The answer to that simple question is impossibly complex. The overall theory, the general theory, is that aisle four and now aisle thirteen, which was not even known at that time, counter-balance gamma interferon as sort of key regulatory cytokines in an immune balance that plays out between TH1, which is often simplistically viewed as cellular immunity, and TH2, which is simplistically reviewed as antibody responses.

One can demonstrate in contrived, but very important, inbred animal models, that if you don't

have any aisle four -- or even better, if you don't have any aisle four receptor, which is what controls both aisle four and aisle thirteen signaling, for example, animals missing that receptor cannot be made to have asthma or allergic-type responses.

So if you take out that receptor pathway, you completely eliminate the possibility of a mouse that might otherwise be inclined to make an allergic response to make that response. So that would be a good thing.

On the other hand, if you do that, if you challenge that same animal with an organism, for example, that absolutely requires a strong antibody response to elicit good protection. Those animals might get sicker if they don't have that pathway.

So at the level of the inbred animal species, one can demonstrate that knocking out one of these pathways, either the aisle four receptor pathway or the gamma interferon pathway, can tip a balance in your ability to response to certain organisms in a way that can harm the animal in the event that they are challenged by a specific type of organism that requires a specific type of response.

Q So Doctor, when you have that imbalance, as you call it, and in your paper, you indicated it was

an induction of a TH2 response, what you're indicating is that the TH2 level is higher than the TH1 level? Am I correct?

A No, I was indicating that there was more TH2.

Q Okay, so it's out of balance, correct?

A Actually, probably for the response to measles, that's the perfect balance.

Q So it's out of balance. You've got a little more aisle four, less interfering gamma, and isn't interfering gamma the TH1 response that is necessary to clear infections?

A Well, even though I said that it might be the perfect response, you immediately return to imbalanced. In fact, what the body wants and needs to do is to make an appropriate response; and in some cases, the appropriate response is, in fact, a TH2 deviated response. That is the best response for that particular organism.

So demonstrating a difference does not necessarily demonstrate that it is a mal-adaptive difference. It simply demonstrates a difference.

Q Yes, you're right; and Doctor, with that imbalance, a skewing towards TH2, doesn't that also correspond with the period of maximum viremia that you

would see in a measles vaccine?

A The data that we have available shows that you initially actually produce a TH1/2 combined response which, as you clear the viremias, which is to a TH2 deviated response. But the initial response is, in fact, not TH2 predominant. It is a mixed response, and that's probably a very good adaptive response.

Q Doctor, isn't it true that the period of maximum viremia is approximately seven to fourteen days after an immunization?

A Well, there are a large number of studies that have measured viremia after vaccination. But that would be within the timeframe when you might expect to isolate virus from the blood. So starting about six or seven days after, it would be unusual to isolate the virus after two or three weeks.

Q So the molecular aspect of this corresponds to what's known about viremia, and if we go on further, it probably corresponds with the clinical course, correct?

A (No response.)

Q Let's take this one step at a time, okay? The initial response is TH1/TH2, which is what you expect to see. That's the normal response.

A Correct. But I'm not sure that we had an a

priori expectation. That's what we observed.

Q Right, right. And that's still true today, right?

A Well, I don't think it's been replicated in any great detail.

Q But it's still true. Nobody has disproven it, correct?

A Correct.

Q Okay, so it's TH1/TH2 initially, and then after a period of time, a skewing toward TH2, correct?

A That's correct.

Q Okay, and the timeframe in which there is the skewing of TH2, would that correspond with the period of maximum viremia after a measles vaccine?

A Yes, roughly.

Q Okay, and since it corresponds roughly to that, that's also the period that immuno suppression begins, isn't it?

A You're mixing wild-type virus and vaccine. There's no evidence of immuno suppression after vaccine, none.

Q Well, doesn't this article indicate that this can happen?

A It indicates that there are in vitro differences in the PBMC from before vaccination to

after vaccination. It doesn't say anything about immuno suppression.

Q Okay, but if you go further into this article, Doctor, on page 174, it says, "This observation may provide insight into the abnormalities of T cell function induced by measles virus vaccine and possible other live virus vaccines.

A Right.

Q So isn't this true, that from this article, your belief is that you can extrapolate from this in vitro experimentation to the normal, to a human?

A These were human cells.

Q Yes.

A Yes.

Q But going from a test tube into real life; you're thinking that you would be able to extrapolate this information and use it in a real life situation. Shall I read it again?

A No, I don't understand what you mean by "use it in a real life situation." It's an observation.

Q Okay.

A And what had been observed before this was that cells -- science has to be taken in context. What we knew prior to this article being published was that when you took peripheral blood mononuclear cells

out of a child with natural measles, and we demonstrated with after a measles vaccination as well, that there were a number of immunologic parameters that were different.

That doesn't say anything about a child after vaccination being immuno suppressed. It's an in vitro observation that we went then further to look at the cytokine patterns produced by these peripheral blood mononuclear populations, and we could then explain, we thought, some of the in vitro observations with the demonstration that these cells had different cytokine production patterns.

This was not driven by a clinical need to define how children, after vaccination, were immunocompromised, because they were not/are not, that we know of, immunocompromised.

So extrapolating to real life, if you mean this observation can be extrapolated to a immuno suppressive state in the child, this article says nothing about that.

Q This article doesn't. But haven't there been earlier articles that looked at measles vaccine and its effect on immune response?

A Well, yes, if you have measles vaccine, you can demonstrate again subtle changes in response to

specific antigens. There's a clinical prohibition to using other vaccines within the month after taking the measles vaccine, because the immune response to those other vaccines might be reduced -- might. But that's also true for other viral infections -- I'm sorry, other live viral vaccines.

Q Wasn't there an earlier article dating from maybe the 1960s or the 1970s by Firestone, which discovered that when you administer a measles vaccine, a person loses his reaction to tuberculin?

A Absolutely and, in fact, a better paper is Kawashima -- no, Takashira, who worked with Dr. Griffin, where they looked at loss of PPD responsiveness following natural disease.

Q Yes.

A Absolutely, there are changes in immune cells following natural measles and following the vaccine. But the PPD is used as an indication of immunologic memory to tuberculosis. There is no evidence that measles virus vaccination has any clinical impact on reactivation expression or manifestations of clinical tuberculosis. So it is an observation of a change of no apparent clinical significance.

Q So Doctor, if somebody who had previously

reacted to a tuberculosis test suddenly lost his ability to mount a reaction to a tuberculosis test after measles vaccine, there's no concern about this person's immunity?

A None whatsoever, because in clinical experience, we have vast experience with individuals who have -- well, a third of the world's population, me included, since I ran a TB ward in the refugee camp, have been exposed to tuberculosis. There would be no hesitation to administer measles vaccination to an individual who had a known prior exposure to tuberculosis and currently had latent TB.

Q Okay, and Doctor, you're familiar with the Institute of Medicine, 1994 edition, aren't you?

A I am.

Q I think you're cited in this.

A I am.

Q Doctor, I'm afraid I don't have another copy. So I'm going to wander over to you. For the Court's benefit, this is on page 64 of the 1994 edition of ION, Adverse Events Associated with Childhood Vaccines.

SPECIAL MASTER HASTINGS: I'm quite familiar with the document. I don't have my book copy here. Well, I don't know, is there a copy of that in record

of the case?

MS. CHIN-CAPLAN: I don't believe so, Special Master.

SPECIAL MASTER HASTINGS: Okay.

MS. CHIN-CAPLAN: Okay.

BY MS. CHIN-CAPLAN:

Q Now Doctor, I'm going to ask you to look at page 64 of this book, and I'm going to read over your shoulder, and please let me know if I've read incorrectly, okay?

"It is also well known that many natural viral infections, particularly measles, can temporarily suppress components of the immune system." Your cited here.

"And there have been concerns that live attenuated viral vaccines might have a similar effect. Soon after the live attenuated measles vaccine was developed, it was shown that immunization temporarily suppressed the delayed-type hypersensitivity skin test response to purified protein derivative." That's the tuberculin test, correct?

A Correct.

Q "An index of cell mediated immunity to microbacterium tuberculosis; the suppression was, however, less consistent and less prolonged in the

following natural measles infection, presumably because of the attenuation of growth of the vaccine virus at all levels." Have I read that correctly?

A Yes.

Q Then it says, "Other viral vaccines, both live attenuated and inactivated, have been shown to have similar, though often mild and inconstant effects, on skin test responses to various antigens." Have I read that correctly?

A Yes.

Q "In addition, more studies have shown that after measles immunization or re-immunization, certain lymphocytic functions such as the ability to replicate when stimulated with phytohemagglutin" --

A PHA.

Q PHA.

A Easier to say.

Q Thank you -- "or to excrete certain chemotactic factors are mildly but measurably depressed; and the number of CD8 positive lymphocytes falls slightly." Have I read that correctly?

A Yes.

Q So overall, IOM accepts that measures vaccine can cause a temporary suppression of the immune system, doesn't it?

A Can you read me the section where it says that this results in clinically relevant immune suppression? If one has clinically relevant immune suppression, one expects the facts; not isolated in vitro observations or a transient depression of a cutaneous hypersensitivity response.

The huge difference here is taking these observations and trying to link them together into a clinically relevant state of immune suppression. All of these effects are fairly transient. Natural measles is immuno suppressive. There is no evidence in the literature, that I am aware of, that despite these subtle changes in immune cell function, that there is any clinically relevant immune suppression in a child that receives measles vaccine.

Q So your interpretation is that even when you lose your response to a tuberculin test, that that's not an indication that you're more susceptible to infections?

A The proof is in the fact that these children who transiently lose their tuberculin skin tests do not release their latent tuberculosis and, therefore, the clinical facts are very clear. There is not a relevant loss of immune protection from blatant tuberculosis following measles virus vaccination.

Q So your interpretation of what I just read is that children who have immunosuppression don't have any clinical abnormalities?

A I completely disagree with the statement that these children have immunosuppression. What they have is a transient loss in their tuberculin skin test.

Q There are lots of people walking around with loss of tuberculin skin test responses following varicella, following natural infection with a wide range of other things; and none of these people, including those who receive measles virus vaccination, crash with tuberculosis.

It is a clinical test that can be performed. It does not appear to have any clinical relevance following vaccination. If an individual were, for example, to receive -- I'd be willing to be large amounts of money, although I don't have any.

Q Nor do I.

(Laughter.)

THE WITNESS: That if Michelle Cedillo were to have been exposed to tuberculosis, although she does not need more problems -- if she had been exposed to tuberculosis as a young child, I can guarantee you that Michelle Cedillo would have lost her PPD response

following steroids, Imuran, Remicade, and Humira.

BY MS. CHIN-CAPLAN:

Q I don't think there's any doubt about that.

A I don't think there is either.

Q Right, right.

A So in those conditions, that's one of the major complications of the anti-TNF therapies. You lose things like delayed-type hypersensitivity responses, and you reactivate latent tuberculosis and other diseases. You become more susceptible to the other diseases.

The proof is in the clinical phenotype. There is no clinical phenotype of immunosuppression following measles vaccination, even though one can measure a number of changes in immune cell function, transiently following vaccination. They're very different things.

Q Now Doctor, it's also understood that with the wild-type measles, that the period of immunosuppression seen with wild-type measles can lead to the development of opportunistic infections. Isn't that true?

A Yes, it's believed that the combination of the damage done by the virus, plus the immuno suppressive effects of the virus result in a period of

increased risk to a range of opportunistic infections. Well, they're opportunistic in the case of measles. They're not the classic HIV opportunistic infections.

Q Yes, understood; and in your opinion, does that include also measles vaccine? Does measles vaccine, with the known immunosuppression that it causes, lead to the development of opportunistic infections in a child?

A I have to reiterate, because you keep saying that measles vaccine causes immunosuppression, that there is no clinical evidence that measles virus vaccine causes immunosuppression; and, therefore, no, there is no clinical evidence that measles virus vaccination results in enhanced susceptibility to any organism.

Q Now Doctor, you're familiar with the VAERS system here in the United States, aren't you?

A In fact, excuse me, the only really good evidence that we have, we would argue, is in directly the opposite direction.

Because following measles vaccination, the reason that we do not vaccinate individuals with, for example, other live attenuated viruses, the other vaccines like yellow fever vaccine, after measles, is because the antiviral state initiated by the virus

might actually decrease the ability of the other vaccine strains to replicate and grow and induce an immune response. It is not because there is a fear that those live virus vaccines might actually over-grow and cause problems.

So in the only clear-cut example of one event followed by another -- two infectious events, two live viruses -- prior receipt of measles vaccine does not cause problems with the control of the subsequent live virus, because of immunosuppression. It actually causes problems because of the anti-viral state that is generated that limits the replication of the subsequent viral vaccine.

Q Okay, now we were discussing the VAERS system here in the United States. Are you familiar with it?

A I wasn't discussing it.

Q Oh, I was.

A It has been discussed before, yes.

Q Let us move into the VAERS system, just very briefly. You know that there is a passive reporting system, a surveillance system, here in the United States.

A Yes, I'm well aware of it.

Q Is there a comparable system in Canada?

A Yes, there's a comparable passive surveillance system that's run by the providences and ineffectually -- oh, I'm going to get in trouble for saying that. It is increasingly effectively administered by the Federal Government.

Q It has it's limitations.

A I really hope they're not listening.

(Laughter.)

THE WITNESS: Up until very recently, it hasn't been very effectively administered by the Federal Government.

SPECIAL MASTER HASTINGS: Speak into the mike, Doctor.

THE WITNESS: They're allowed to retract stuff; can I?

(Laughter.)

BY MS. CHIN-CAPLAN:

Q Well, it's a passive system. Is that it?

A It's a passive system, yes.

Q And there are limitations associated with passive systems, right?

A We actually have two systems. We have the only active system, that I'm aware of, in North America, called the Impact System, that covers about 80 percent of admissions to tertiary care pediatric

hospitals. It's an organization of 12 or 13 tertiary care pediatric hospitals, where there are designated nurses that seek out and evaluate a specific set of clinical presentations that could plausibly be associated with vaccine adverse events.

Q Okay.

A So most of it is passive.

Q So that's the Impact system that you're referring to; is that it?

A That's correct.

Q Now Doctor, with Impact, isn't the criteria to determine whether an adverse reaction is related to a vaccine determined by whether that reaction could occur in its natural state? Should I rephrase that?

A Would you rephrase that? I don't understand that.

Q Let's assume it's measles. Under your system, if measles can allow the development of opportunistic infections, isn't it presumed that vaccine strain can do the same thing?

A Well, first of all, I'm not a member of Impact. So I don't know. I was a reviewer of the Impact system for their funding renewal in 2001/2002, and I don't recall any statement of principle of that nature in their mandate. I mean, I fairly carefully

reviewed the entire program, and I don't recall that ever being a statement of principle.

Q Okay, so you don't know.

A I reviewed the Impact Statement of Mission. What you're stating is a general statement of causality. It is a reasonable hypothesis that if a natural disease does "x", that an attenuated virus of the same kind might do less of "x", but might still do a little bit of "x".

So absolutely, it's another one of these hypothesis, where it was a reasonable hypothesis at the time that the attenuated vaccine was introduced. We now have more than 50 years of experience with this vaccine, and it hasn't proven to be the case. So, therefore, that hypothesis has proved to be false.

Q But I'm talking about your surveillance system here. Under your surveillance system, if wild measles can allow the development of opportunistic infections, would it be presumed that measles vaccine could do the same?

A On the date of the first dose that was administered, that was a very reasonable hypothesis and, in fact, it proved to be true. Edmonston B, when it was first introduced, was two rough a virus. It wasn't attenuated enough. Too many children got sick.

Too many children got a rash. That vaccine was withdrawn and replaced with a more attenuated virus.

If you were to ask me, do I think that wild-type virus and measles vaccine virus, as it's currently used, where would I place Edmonston B virus in its virulence and for a variety of side effects, my hypothesis would be that it would fall in between. However, we don't have 50 years of experience with Edmonston B virus, because it was withdrawn because it was too much like the wild-type virus to be accepted.

We have 50 years of experience plus with the attenuated virus that we have really in current use, and it has not proved to be immuno suppressive in any clinically relevant way.

Q So to your knowledge, how would the Canadian surveillance system treat such a situation where a child who was immunized with measles vaccine appeared within seven days, with an opportunistic infection?

A You'll have to define an opportunistic infection for me. Do you mean if a child appeared with otitis media?

Q Otitis media -- what is your understanding of opportunistic infections?

A The term is primarily used to define individuals who have immunosuppression. So it's

fairly simple for HIV and for those with clearly defined immuno suppressive states, following chemotherapy, following other things.

I mean, it's impossible to answer, because we define opportunistic infections as those that occur following immuno suppressive events, and measles vaccine is not known to be immuno suppressive.

So if a child arrived with a pneumonia or an otitis, the impact nurses would not look for it, because it's not relevant, clinically.

Q Okay, so your answer would be no.

A Well, my answer was a lot longer than no.

(Laughter.)

THE WITNESS: But the general tenor of the answer is no, because opportunistic infections are not known to occur following measles vaccine.

BY MS. CHIN-CAPLAN:

Q Now Doctor, on page six of your handout, you talked about cytokines and inflammation.

A Yes.

Q This is just some general information. Do you know the effect of inflammation on the blood brain barrier?

A Well, sure, I have a reasonable understanding. It is not my area of expertise. I do

not study the blood brain barrier. But as a scientist interested in viral infections and infectious diseases in general, the general statement would be that local inflammation damages the blood brain barrier badly, and peripheral inflammation can influence the blood brain barrier in more subtle ways.

Q Okay, so infection in general can influence the blood brain barrier?

A Well, that's an area of very active investigation right now. How important is it? To what extent can peripheral inflammation influence the integrity of the blood brain barrier? There are many investigations ongoing right now that are trying to address that question.

Q So would it be fair to state that normally the blood brain barrier is in tact, and doesn't permit large molecules to pass through?

A Yes, I think that would be a reasonable, general statement.

Q Okay, and when there's infection or inflammation present, does the blood brain barrier become more porous?

A If there's an infection of the area of the brain, absolutely. The blood brain barrier becomes a sieve. It absolutely does not apply to the same

extent for peripheral inflammation.

But it's a reasonable hypothesis that extensive peripheral inflammation might influence the blood brain barrier to some extent. But as I said, that's an area of active investigation, where I have limited expertise.

Clearly, for example, interleukin-1, elaborated at a distant site, gets into the brain, because it has its effect on the brain in raising body temperature. That's why it was called endogenous pyrogen. You have an abscess in the periphery. The white blood cells produce interleukin-1. It goes to the brain, and the brain then increases your thermal regulatory set point, and you have fever.

So there's clear evidence that molecule cytokines can get into the brain and have some effects. But the general effect of peripheral inflammation on the integrity of the blood brain barrier, I think at this point, is a very complex subject.

Q Okay, now could we just generally turn to the D'Souza articles, of which you are co-author; am I correct?

A I guess I can claim to be senior author.

Q Doctor, let's look at the first D'Souza

article, which is contained at Respondent's Exhibit BB, Attachment 30.

A I cannot resist. But when I offered this project to Yasmin D'Souza several years ago, her initial response was, "But this problem is resolved; no one will be interested in this work." So I say publicly, "I told you so."

(Laughter.)

MS. CHIN-CAPLAN: You can tell her that her reputation is skyrocketing.

BY MS. CHIN-CAPLAN:

Q Doctor, this is the first D'Souza article, correct?

A Yes.

Q And the title of it is, "No Evidence of Persisting Measles Virus in Peripheral Blood Mononuclear Cells From Children with Autism Spectrum Disorder."

Now Doctor, you touched on this very briefly, and I just wanted to explore this a little bit further. You decided to do the blood work of children, as opposed to the gut tissue of autistic children in this situation, correct?

A That's correct.

Q And your reason for not doing the gut tissue

was because you believe that it was unethical to do it?

A Actually, I had a discussion with the head of our IRB, the Institutional Review Board; and I asked the question whether it would be acceptable to enroll autistic children in a study where a part of the study protocol included endoscopy and a gut biopsy.

The simple question was, in the pediatric gastroenterologic community, would an endoscopy be considered to be a medically-necessary procedure? So I spoke to the pediatric gastroenterologists in the area, and they indicated that it would not be.

So it was a non-issue. We could have had access to biopsies from autistic spectrum disorder children, who were having endoscopies performed because of the severity of their gastrointestinal symptoms; but not from other ASD children.

Q Okay, for ASD children who had GI symptoms, they thought it would be appropriate for them to have an endoscopy performed?

A Only if the GI symptoms met a certain level of severity, which is not my area of expertise. But the opinion of the pediatric gastroenterologist, most particularly Ernie Seidman at the time, who was the

head of Hôpital St. Justin, St. Justin's Hospital, at the University of Montreal, his opinion was that only a rare autistic child would meet the criteria of a medically-necessary endoscopy.

Q And did he tell you the symptoms that this rare autistic child would need to have before we had an endoscopy?

A I didn't pursue beyond that point to get specific details of what he would consider to be necessary or unnecessary. The identical study replicating the Uhlmann work was impossible at that point. I suppose we could have done it, but it would have taken 10 years to collect the specimens.

Q Doctor, the children that you had on page 1673 of this article, indicate that almost 80 percent of the children with ASD had gastrointestinal complaints; versus 32 percent of control population.

A That's correct.

Q That's a very high number.

A It was a very low bar. A gastrointestinal complaint could be a constipation, a modest constipation, abdominal discomfort. These were not the gastrointestinal complaints that would serve as the criteria for an endoscopic procedure. So the bar was set very low.

Q And you've already told me though that you don't know what the criteria was for a child to receive an endoscopic procedure.

A None of these children, that I'm aware of, had endoscopic procedures. So I am making, I think, a reasonable assumption that they didn't meet the criteria that were placed at the Montreal Children's Hospital at the time.

Q So 80 percent of them had GI symptoms. They were considered to be minor symptoms; was that it?

A That's correct.

Q Not deserving of an endoscopy.

A I wouldn't use the word "deserving." I would use that an endoscopy was not appropriate, given the degree of their symptoms.

Q Doctor, did you have an opportunity to read the "Dear Doctor" letter that was sent out by Autism Speaks at all?

A No, I did not.

Q Okay.

A I should also say that I was not involved in the clinical care of these autistic children.

Q So just to be certain, you only drew their bloodwork to test their blood.

A I didn't even draw their bloodwork. That's

what you do when you're a senior author. You ask other people to do those things.

Q That's the good part about it. So Doctor, when you drew this bloodwork, did you know what the correlation was between gut suppositive, gut biopsies, and positive peripheral blood mononucleus sites were?

A Could you repeat the question, please?

Q Sure, when you drew this bloodwork to do this testing, did you have any idea what the correlation would be between positive gut biopsies and bloodwork?

A No, in fact, all we knew was that there were two published articles; one by Uhlmann and one by Kawashima. The one suggesting that there was detectable measles, nucleic acids in gut biopsies; and the other just demonstrating detectable measles, nucleic acids, and peripheral blood mononuclear cells.

Q Okay.

A To my knowledge, at the time, Doctor Kawashima had not tested gut biopsies, and Dr. Uhlmann had not tested peripheral blood mononuclear cells.

Q So as you're sitting here, you still don't know what the correlation would be?

A I know that in many cases, Dr. Uhlmann's laboratories received both gut biopsies and peripheral

blood mononuclear cells; and I know that Doctor O'Leary has never published any data correlating what would have been a very logical study to perform.

Q So as you're sitting here today, you still don't know what the correlation is between positive gut biopsies and findings in the blood?

A I do not, because those in a position to publish that information have not done so.

Q Okay, now Doctor, there are a lot of citations to the Afzal article, as well. You have to give me a moment.

That would be under Respondent's Exhibit BB, Attachment 4, and this is the Afzal article that everybody else cites, Doctor?

A Yes.

Q The title of this is, "Absence of Detectable Measles Virus Genome Sequence in Blood of Autistic Children Who Have Had Their MMR Vaccination During the Routine Childhood Immunization Schedule of UK." Have I read that title correctly?

A I assume so.

Q Okay, now Doctor, when you reviewed this article, it makes that same statement that you had made earlier, about endoscopies and ethical considerations in autistic children. Am I correct?

A Yes, somewhere in this article, they make a statement to that effect.

Q Okay, I'm going to refer you to page 629 of this article, under the discussion, the very last full paragraph. Doctor Afzal states, "It was difficult to obtain ethical permission for the collection of gut biopsies and CSF from the patients studied here, as collection would involve highly invasive procedures. It was, therefore, necessary to examine leukocytes in the study, in contrast to previous published work, where gut biopsies and CSF preparations had been the main tissues examined." Have I read that correctly?

A Yes.

Q So Doctor Afzal recognized that this could be a potential drawback of the results in his study. Isn't that true?

A Absolutely.

Q And Doctor, to your knowledge, when you look at his subjects, did any of these autistic children have gut symptoms?

A Well, actually, I'm not sure if that was reported in the article. I don't know.

Q Okay, so we don't know whether this result here correlates to any of the children at all who have gut symptoms?

A Because I don't know, if I can have a moment to look to see if that was detailed -- but since this study also did not test CSF or gut biopsies, then by definition, they would not have a correlate with GI symptoms.

Q Okay, but actually, this article contains even less information than yours did about the clinical status of these children's gut symptoms, correct?

A I don't know if it contains more or less.

Q If you look through this article, there doesn't seem --

A I'm willing to trust you that it contains less information than ours.

Q Okay, so this one doesn't even tell us how many of these children had gut symptoms. Your article tells us that 80 percent of them had gut symptoms. Neither one of them can correlate the findings in the blood with positive gut biopsies. Am I correct?

A You can't actually do a correlate with a negative finding.

Q Okay.

A That's an irrelevant statistical analysis. If you find nothing, then there is nothing to correlate it with. Neither the Afzal study nor our

study found any positive results. Therefore, we could not have done any correlative studies with any hypothetical, in the case of Afzal; or in our case, documented gut symptoms.

Q But you didn't look in the gut, correct?

A You were asking me about correlations, and I was simply telling you that that can't be done.

Q Okay.

A But you don't find anything.

Q Because you didn't look in the gut.

A Well, we did look in the gut in inflammatory bowel disease individuals. We shouldn't forget that Dr. Wakefield and his colleagues spent a decade trying to convince people that measles virus vaccine strain was the cause of inflammatory bowel disease. We also failed to find any evidence of measles virus, nucleic acid in inflammatory bowel tissues.

So the question of whether we did any correlative study, I answer that by saying that was an impossible statistical analysis to do, because we found no virus.

Q And you're talking about your most recent article that was published on gut?

A Correct.

Q Doctor, that Respondent's Exhibit BB, Tab 29

-- Doctor, the subjects in this case were patients with inflammatory bowel disease, correct?

A That's correct.

SPECIAL MASTER HASTINGS: Can you help me, again?

MS. CHIN-CAPLAN: It's Respondent's Exhibit BB, Tab 29.

SPECIAL MASTER HASTINGS: "B" as in boy.

MS. CHIN-CAPLAN: Double B.

SPECIAL MASTER HASTINGS: And Tab 29?

MS. CHIN-CAPLAN: Correct.

SPECIAL MASTER HASTINGS: Thank you.

BY MS. CHIN-CAPLAN:

Q So Doctor, this looked at patients with inflammatory bowel disease.

A That's correct.

Q And inflammatory bowel disease is what? Can you just describe the categories of inflammatory bowel disease for me?

A Well, again, I'm not an inflammatory bowel disease expert. But my general understanding is that it's divided into Crohn's Disease, ulcerative colitis, and other inflammatory bowel disease, that cannot be characterized as one or the other definitively.

Q Okay, and Doctor, is there a distinction

between ulcerative colitis and Crohn's Disease?

A Yes, there are clinical distinctions and pathological distinctions between the different types of inflammatory bowel disease.

Q And would it be fair to state that ulcerative colitis involves the lower GI tract?

A Yes, as a general statement -- I think there was testimony to that effect that it can occasionally involve the distal ileum as well. But it is generally restricted to the large bowel.

Q Okay. And Crohn's involves both the upper and lower GI tract?

A It can. It has the potential to do that.

Q Now, Doctor, in this study, how many were children?

A Oh, I would have to look. I think the mean age was in the young adult or late adolescent, nut I'd have to actually look to remember.

Q I see in Table 1, there's a range of 5.4 to 47.5?

A Yes, that's right. And the mean age was, yes, young adults.

Q Okay. That was for Crohn's disease, correct?

A Yes. We had a lot more detail in the full

manuscript, but the *Journal* told us to cut it down to 1,000 words so there's a lot less detail than there should have been in this paper. It was a lot longer as originally submitted.

Q Editors. So, Doctor, in Crohn's disease the age range was 5.4 to 47.5?

A That's correct.

Q For ulcerative colitis it was 13 to 77?

A Yes. A much broader range.

Q And for noninflammatory, 2.5 to 69?

A Correct.

Q Out of that group of numbers, how many of them were actually children? Do you know?

A I can't tell you that off the top of my head. I'm sorry.

Q Okay.

A They were mostly derived from -- well, you can get an idea. Most of the noninflammatory ones, because the mean age is 16 for the noninflammatory condition, so I would say that most of them were adolescents or younger.

Q Okay.

A And I would say that probably most of the Crohn's were adolescents as well, so if you define children as under 12 I would be able to say from the

ranges and the means that not very many of these children were young children in the five to six year and below range.

Q Any of them autistic?

A No, none were autistic.

Q Okay.

A They were not specifically tested as autistic or not, but they were not flagged as ASD subjects.

Q Okay. So, Doctor, isn't that a little drawback to this study that you didn't study any autistic children?

A This was not a study directed specifically to ASD MMR. This was directed to the earlier Wakefield hypothesis of IBD and MMR.

Q Okay. So no ASD GI interpretation in this article?

A Only insofar as it addresses the criticism by Dr. Hepner that the testing that we did by PCR using the Uhlmann primers is inappropriate because we used the wrong tissue and we amplified nonspecific sequences only in PBMC.

This paper demonstrates quite clearly that those same primers also amplified nonspecific host genes in gut and so that is directly relevant to Dr.

Hepner's concern regarding our PCR testing.

Q But, Doctor, her other consideration was also that you tested the bloodwork of autistic children. You didn't test the gut tissue of autistic children.

So wouldn't that be a similar criticism here; that you did not test the gut tissue of autistic children?

A Correct. This deals with the earlier Wakefield hypothesis, but it also by chance also deals I think quite effectively with criticism that you can't say anything about the nonspecificity of the primers because we only tested PBMCs.

In fact, what we demonstrate quite clearly is that the Uhlmann primers amplify human genes in the gut, and that means that application of those primers in gut tissue would be expected to amplify human genes. Therefore, sequencing is critical. Therefore, the lack of sequencing by the O'Leary Lab is a fatal flaw.

Q Doctor, I just had a question on this. I'm looking at page 15 of your slide presentation. You were indicating the vulnerabilities of PCR testing.

A Yes.

Q We're talking about amplicon. Amplicon

size.

A Yes. Amplicon just means the segment of DNA that's amplified by the PCR reaction.

Q But, Doctor, you indicated in the amplicon size that you found a band at roughly 150.

A Yes. That was the anticipated size.

Q All right. But didn't you also say that the findings were kind of smudgy? You really couldn't tell whether it was positive or negative?

A Oh, no. You can tell there's a band there, but there's also a smear, which is an indication that the assay -- that something about the assay is not optimal. It could be that the primers are bad. It could be that the conditions are bad.

All I can tell you is that we spent months trying to optimize these assays and were unable to get prettier pictures than these, suggesting that there was nonspecific amplification, which was proved by the sequencing.

Q Doctor, when you get a smudgy picture like this though wouldn't some molecular biology people say that this is not a positive; it's a negative?

A Well, you can ask Dr. Bustin that because he knows more about PCR than I ever will.

Q All right.

A I think that he would interpret this as a positive result in a suboptimal assay, a suboptimized assay, despite our best efforts to optimize it.

Q Doctor, I want you to assume something. I want you to assume that you have a child who has a positive gut biopsy for measles, a positive result in the peripheral blood for measles, positive CSF findings, and the child has a neurological condition.

Would you assume that that neurological condition was related to the positive CSF result?

A If I had confidence in the results of the testing, I think that that would be the only logical conclusion unless there was some other pathogen identified as a co-pathogen.

MS. CHIN-CAPLAN: Thank you. I have no further questions.

SPECIAL MASTER HASTINGS: All right. Let me check my notes, Dr. Ward.

THE WITNESS: May I add one caveat to that last answer?

SPECIAL MASTER HASTINGS: All right.

THE WITNESS: And it's not in any way to change the answer because I think my first response is absolutely that would be the conclusion.

However, I would expect an individual with

measles virus in the brain to have a clinical presentation that was compatible with what is currently known about the persistence of measles virus in the brain.

At the current time, the only two situations where we know that to occur are in SSPE and measles body inclusion encephalitis, so if the neurologic condition that Ms. Chin-Caplan was referring to was compatible with SSPE or measles inclusion body encephalitis, then my answer is absolutely true.

If the neurologic condition that she was referring to is anything other than those well known clinical entities then I would have to reserve judgment until I learned more because I would clearly be witnessing new biology. If there was a true association that individual would be the first person with that syndrome that I would ever have seen and would ever have been seen in the medical literature.

So I still think I would be inclined to think that there was a link, but the answer would not be as definitive if the neurologic condition was other than SSPE or measles inclusion body encephalitis.

SPECIAL MASTER HASTINGS: All right. Most of my questions have been asked. Let me ask this. You've looked at Michelle Cedillo's records?

THE WITNESS: I have, yes.

SPECIAL MASTER HASTINGS: Are you familiar with her course as stated in the medical records at that first visit after the MMR vaccine where she had the onset of fever about seven days after vaccination?

THE WITNESS: Yes.

SPECIAL MASTER HASTINGS: Given what you know from those medical records, was that fever likely a result of the measles vaccine?

THE WITNESS: I think there's every reason to believe that the fever with onset seven days after the vaccination is associated with measles.

I mean, it's a temporal association, and just my personal experience with three kids tells me that they get a lot of fevers for a lot of reasons, but the temporal association certainly suggests that that fever was a result of the live attenuated virus circulating in her blood. I think that's a very reasonable assumption.

SPECIAL MASTER HASTINGS: What about the recurrent fever several days later?

THE WITNESS: I think that's less likely. Certainly when she presented at that subsequent time she had a purulent nasal discharge and was treated with antibiotics because somebody thought she had a

bacterial infection.

You don't treat measles vaccine reaction with antibiotics, and measles vaccination has, to my knowledge, never been reported to result in a purulent nasal discharge so I think that it's entirely plausible that Michelle was incubating a second infection around the time that she was having her mild measles rash.

I don't know what the rash was. I didn't see it, but when she was in her viremic stage of her vaccination.

SPECIAL MASTER HASTINGS: All right.

THE WITNESS: The temperature of 105 is unusual, but it is not unprecedented as reaction to measles virus vaccination. Kids can get fevers that high following measles virus vaccination.

Different children react in different ways to identical stimuli, and some generate very, very high fevers and some of them generate those fevers quite consistently no matter what the stimulus.

SPECIAL MASTER HASTINGS: Thank you, Doctor.

Any redirect for this witness, Ms. Babcock?

MS. BABCOCK: I have one question.

SPECIAL MASTER HASTINGS: Please go ahead.

REDIRECT EXAMINATION

BY MS. BABCOCK:

Q Dr. Ward, you were questioned at length about an article which I believe was in Petitioners' Exhibit 79. It looks to be an article -- they've done some work -- that was published in 1993 that you did.

A Yes.

Q Is there anything else you wanted to comment on that beyond what Ms. Chin-Caplan asked you?

A No, not really. I think we went back and forth and back and forth a number of times.

That's not the only paper. There are many papers that demonstrate that the immune system after measles vaccination, as after many other vaccinations -- rubella, varicella -- the immune system has changed. There are things you can measure. That would be expected. I mean, you give the vaccine to elicit a response and so seeing the immune cells responding is not unexpected.

I think that the only point that I would make is that these in vitro, these transient in vitro phenomena that we can identify, are interesting because they tell us about how the vaccine is working, and they may in the future help us to develop better vaccines that work in slightly different ways, but

they don't say anything about the clinical status, immune status, of the child who's received this vaccine.

There's more than a half a century's experience saying that children are not immuno suppressed after they receive measles virus vaccination. We give this vaccine to children with HIV. If this vaccine virus was known to be immuno suppressive that would actually be a fairly stupid thing to do, but that is the current recommendation.

That's not saying that the WHO has never made recommendations that are always perfect, but we actually have a fair number of years of experience with that recommendation. When we give this vaccine even to children with HIV they don't do worse, so it seems unlikely that there's any clinically relevant immunosuppression.

MS. BABCOCK: I don't have anything further.

SPECIAL MASTER HASTINGS: Okay.

SPECIAL MASTER VOWELL: Just a clarification there, Doctor.

You said this vaccine is given to HIV positive children. Are you referring to the monovalent measles vaccine or the MMR vaccine or either?

THE WITNESS: In the developing world in many countries it's measles that is administered. In some emerging countries, emerging economy countries where they have high HIV prevalence rates, they give either MR or MMR, and in the wealthier industrialized countries, we typically give MMR.

The recommendation for measles-rubella or measles-mumps-rubella vaccination in all of those situations is the same; that it should be administered even in the setting of HIV so long as there is no clinically evidenced immune suppression from the HIV.

SPECIAL MASTER VOWELL: Thank you.

THE WITNESS: And in fact the monovalent vaccine contains exactly the same number of viral particles -- it's 1,000 viral particles -- as the trivalent MMR, so precisely the same amount of vaccine virus is going into the body of a vaccinee whether they're getting measles alone, measles-rubella or measles-mumps-rubella vaccine.

SPECIAL MASTER HASTINGS: All right. Anything further for this witness, Ms. Chin-Caplan?

MS. CHIN-CAPLAN: Just one last thing.

SPECIAL MASTER HASTINGS: Go ahead.

MS. CHIN-CAPLAN: Thank you.

RECROSS-EXAMINATION

BY MS. CHIN-CAPLAN:

Q Doctor, you would agree that measles can persist?

A It's documented that measles does persist, SSPE and measles inclusion body encephalitis.

Q So we know that it's a persistent virus in some people?

A Absolutely. It can persist.

Q And is there any knowledge about why it persists in some people and not others?

A That's one of the holy grails of measles virologists. We would love to know why it persists in some individuals in SSPE. In measles inclusion body encephalitis we have a pretty good idea why it persists.

Q Okay. You mentioned Oldstone earlier in your testimony, so I'm sure you've read Dr. Oldstone's articles, correct?

A He's written a huge number of them, so I doubt I've read all of them.

Q He has, hasn't he. This one that I'm referring to is contained under Dr. Kinsbourne opinion at Tab VV.

A This is the LCMV study in mice?

Q No. The title of this is *Viral Persistence, Parameters, Mechanisms and Future Predictions*.

A This is his review article.

Q This is his review article. Exhibit 61VV.

SPECIAL MASTER HASTINGS: What's the tab number again?

MS. CHIN-CAPLAN: VV.

SPECIAL MASTER HASTINGS: VV. Okay. Thank you.

MR. MATANOSKI: I'm sorry. We don't have copies of your exhibit.

SPECIAL MASTER HASTINGS: Do you have a copy of that? We're going to get him a copy.

Okay. What was your question?

BY MS. CHIN-CAPLAN:

Q Doctor, you would agree that Dr. Oldstone is a well-known virologist in his field?

A Yes. He's one of the most respected virologists in North America.

Q And his work has involved virtually his entire career about looking at persistent viral infections? Is that true?

A Yes.

Q Doctor, he's written many articles on viral persistence, hasn't he?

A Yes.

Q And you indicated that this was a review article, correct?

A Yes.

Q And a review article involves looking at the literature and summarizing what's present out there in the literature? Is that it?

A Yes.

Q Okay. Doctor, if you look at the introduction of this article it says:

"One of the remarkable advances in modern virology is the realization that persistent viral infections exist and are common. Hence, understanding the principles by which persistence is initiated and maintained, as well as the pathologic consequences of continued viral replication in a host over its life in terms of causing disease provides research areas of high significance, as well as opportunities for challenging investigation."

I've read that correctly, haven't I?

A I assume so.

Q It states further in the next paragraph, "The three foundations upon which the understanding of persistent infection rests are: First, that the host immune response fails to form or fails to purge virus

from the infected host. Thus, viral persistence is synonymous with evasion of the host's immunologic surveillance system."

Doctor, when you look at that sentence does that indicate that there has to be some sort of immune dysfunction for a virus to persist?

A Absolutely not.

Q It doesn't?

A HIV persists and infects entirely immunocompetent individuals.

Q So that statement doesn't mean that there has to be some sort of immune dysfunction for a virus to persist?

A It depends on the virus. It really is dependent upon the viral biology.

Viral persistence is a dance between virus and host, and there are clearly some viruses that can persist in entirely normal hosts.

Q It says further going on that topic --

A This has been read into the record before, has it not?

Q I don't know. Has it?

A I thought so, but go ahead.

Q Okay. "Recent advances have shed light on the cellular and molecular players involved.

"Second, viruses can acquire unique components or strategies of replication; that is, viruses can regulate expression of both their own genes and host genes to achieve residence in a nonlytic state within the cells they infect."

So, Doctor, when you hear that statement is that an indication that a virus can exist in a host and not kill the host?

A Absolutely. We all, to our credit or shame, carry a large number of herpes viruses, and they don't kill us for the most part.

You know, Epstein-Barr virus, cytomegalovirus, HSB1 and 2. We all have our fair share of those viruses, and they don't kill us. These are general statements of established virologic fact.

Q And the third criteria was, "The type of diseases that persisting viruses cause are often novel and unexpected."

Would you consider this type of situation of MMR, persistent MMR, a measles infection causing autism, novel and unexpected?

A Well, if it were true it would be novel because it has not been described.

As I said, if it were true it would clearly be novel, and therefore my comment about Dr.

Bradstreet's choice of journals to publish his purportedly novel findings. It just seems wildly illogical.

Q But novel?

A Well, if it's true then it's novel. If it's true and novel, it should not be in the *Journal of the American College of Physicians and Surgeons*, a nonindexed journal.

If it's also true and novel it should be in Dr. Oldstone's review written in 2006, and it is not. The obligation of an academic in writing a review article is to include all relevant information, and the MMR hypothesis and its relation to autism is not in this review article.

Q This review article speaks just of viral persistence, doesn't it?

A Is that not the hypothesis?

Q Yes, but that's what I'm saying. It just speaks of viral persistence. It doesn't speak specifically about measles causing autism, does it?

A It does not.

Q Thank you. Doctor, when you continue on in that paragraph it says, "The result is a disturbance in the host's biologic equilibrium. That's one important direct effect of persistent virus

replication is to disorder the normal homeostasis of the host and thereby cause disease without destroying the infected cell."

That's happened, correct?

A In a variety of viral infections it happens, yes.

Q Okay. So nothing new there?

A It would be brand new for measles because it does not happen with measles that we know of.

Q Okay. That we know of.

A When measles infects a neuron it causes abnormality as we saw on the slide. When measles infects most other cells it kills them through syncytia formation. That is the known biology.

You cannot extrapolate from observations with herpes viruses and HIV and go to measles without implying new biology, which is possible. New biology is always possible. It's just that the evidence to date doesn't support that new biology.

Q Okay. One last sentence. Dr. Oldstone gives an example: "A virally caused neurotransmitter defect of neurons altering cognitive learning and yielding behavioral disorders."

That's a little like what autism is, isn't it?

A I am not an autism expert, but as a general description of a category into which autism would fall, yes, I guess that would describe some of the children with ASD.

Q Okay.

A But he does not talk about measles.

MS. CHIN-CAPLAN: Okay. Thank you, Doctor.

SPECIAL MASTER HASTINGS: Anything further for this witness?

MS. BABCOCK: No, Special Master.

(Witness excused.)

SPECIAL MASTER HASTINGS: I suggest we take our lunch break at this time.

I guess we have Dr. Bustin coming up this afternoon?

MR. MATANOSKI: That's correct, sir.

SPECIAL MASTER HASTINGS: Which I'm guessing may be a while.

Do we still want to take the full hour lunch? Do you both want to cut it short, or would you rather take the full? You want the full break? Okay.

It is 12:27. We will be back here in one hour to start the afternoon.

Thank you.

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(Whereupon, at 12:27 p.m., the hearing in the above-entitled matter was recessed, to reconvene this same day, Wednesday, June 20, 2007, at 1:30 p.m.)

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A F T E R N O O N S E S S I O N

(1:35 p.m.)

SPECIAL MASTER HASTINGS: We're back from our lunch break, and next we're going to take the testimony of Dr. Bustin, who is in the witness chair.

Ms. Babcock, you're going to do the honors?

MS. CHIN-CAPLAN: Special Master?

SPECIAL MASTER HASTINGS: Yes.

MS. CHIN-CAPLAN: Before Ms. Babcock starts questioning Dr. Bustin I'd like to make a motion to preclude the government from utilizing the reports of Dr. Bustin that were performed in preparation for the U.K. litigation.

They're untimely, and as a result of their untimeliness I have been severely hampered in my ability to cross-examine Dr. Bustin, and I have not been able to confer with my expert regarding the highly technical nature of this matter.

SPECIAL MASTER HASTINGS: Well, that's the same motion you made at our status conference on Friday, June 8, isn't it?

MS. CHIN-CAPLAN: It's virtually the same. It's just that now I've had the opportunity to review these reports and find that they are indeed highly technical and would require the input of somebody who

is also skilled as a molecular biologist to assist me in any preparation of this material.

MR. MATANOSKI: Your Honor, if I may briefly?

SPECIAL MASTER HASTINGS: Is that all you wanted to add?

MS. CHIN-CAPLAN: Yes.

SPECIAL MASTER HASTINGS: All right. Mr. Matanoski?

MR. MATANOSKI: To clarify one point, when Petitioners' counsel made the oral motion that you decided on June 8, she had the copy of the report with her at the time.

The other thing that's come to light since then is that one of the testifying experts for the Petitioners, Dr. Ronald Kennedy, had not only knowledge of the Unigenetics procedures, but in fact discussed the Unigenetics procedures with the personnel from that lab by his testimony in late 2001 or 2002, so he had access to information about Unigenetics far earlier than any of this came to light for the government.

SPECIAL MASTER HASTINGS: All right. Well, I believe that I stayed at the office until 9 p.m. on Friday, June 8, to do a written ruling on your motion,

which I thought about and discussed with my colleagues. That written ruling is filed into the record.

As I said, I'm sure I articulate it better in my written ruling than I can here, but I have sympathy to the argument raised by the Petitioners that these were being brought into the proceedings at a late time. My ruling was that I would allow the two reports in question of Dr. Bustin to be discussed at this hearing, which now I'm going to stick to that ruling.

I also noted though that to the extent the Petitioners' counsel was prejudiced for lack of opportunity to adequately study and respond to those reports at this hearing, if at the close of the hearing they felt that there was prejudice to their case arising out of the lateness of the reports I would hear a motion if they wanted to file additional evidence in response at a later time. I would be amenable to such a request.

I'm going to stick by that written ruling, which I filed into the record on June 8. We can discuss those reports. I will decide at a later date whether to give those reports any weight and entertain any efforts by the Petitioners to respond to those

reports.

With that, Ms. Babcock, if you wanted to step up here for the examination of Dr. Bustin?

Dr. Bustin, I'm going to ask you to raise your right hand for me.

Whereupon,

STEPHEN A. BUSTIN

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

SPECIAL MASTER HASTINGS: All right. Go ahead, Ms. Babcock.

DIRECT EXAMINATION

BY MS. BABCOCK:

Q Could you please state your name for the record?

A My name is Stephen Bustin.

Q And what is your profession?

A I'm a molecular biologist.

Q And could you briefly describe your collegiate and graduate education?

A I did my first degree, a B.A. in Genetics, at Trinity College-Dublin and my Ph.D. in Molecular Genetics also at Trinity College-Dublin.

I then did a postdoc at the Animal Virus Research Institute in Pirbright on positive-strand RNA

viruses, spent a few years at a biotechnology company looking at basic molecular biology techniques and then decided I wanted to get back into academic science and joined the London Hospital Medical College as a senior research fellow.

Over the next few years then I was promoted to senior lecturer and reader until in 2004 -- 2003 or 2004; I can't remember -- I was awarded the chair of Molecular Science at our institution.

Q And that institution is Barts and the London?

A Yes. We are the medical school of Queen Mary's, which is part of the University of London.

Q And do you also teach at that institution?

A I do, yes.

Q Do you teach undergrads? Medical students?

A We teach medical students, medical postgraduate students. I don't do an awful lot of teaching, but I do teach.

Q Okay. Now, during your career have you developed any particular expertise on laboratory technique or practice?

A Yes. I think the one I'm most familiar with is PCR, RT-PCR and real-time PCR. It's called RT-PCR.

Q And to be clear, PCR is a technique that you

use in your every day research and practice?

A I do, yes.

Q And are you familiar with TaqMan PCR in particular?

A Yes. We were one of the very first labs in the U.K., academic labs in the U.K., to use TaqMan PCR.

Q Now, have you published in the field of molecular science and in particular concerning PCR?

A Yes. We publish continuously on PCR.

Q About how many times in the past five years have your labs published articles on PCR?

A Well, in the peer reviewed literature we have 14 articles, and then we've published eight or nine book chapters, and of course in 2004 I wrote and edited the *A to Z of Quantitative PCR*.

Q We'll get to that in a minute. Have you also written papers that are considered authoritative in the field of PCR?

A Yes. In 2000 I wrote a review of quantitative RT-PCR, which has been cited in the peer reviewed literature over 1,000 times.

Q One thousand? I'm sorry.

A One thousand times.

Q Okay.

A The follow-up paper in 2002 is at the 500 citation level, and at the end of last year we published a definitive protocol for real time RT-PCR in *Nature Protocols*.

Q And what is *Nature Protocols*?

A *Nature Protocols* is one of the premiere methods journals. Like any nature journal, it is very highly regarded.

Q And you mentioned earlier you had also written a book entitled *A to Z of PCR*?

A Yes. Together with a colleague I wrote most -- well, all -- of the technical bit, and then we had some additional contributions from various labs around the world for specific applications.

Q And how is that book viewed in the field of molecular science?

A Well, some people refer to it as the Bible of QPCR.

Q Okay. And you've written book chapters and other publications on the topic of PCR particularly?

A Yes.

Q Do you also review scientific papers for journals?

A I do all the time, yes.

Q Do you have learned society memberships?

A Yes. I'm a fellow of the Royal Society of Medicine.

Q Do you also play a major role in organizing conferences or symposiums on an annual basis for PCR?

A Yes. I organized three national meetings in London, and we had about 400 people attending, and I am a co-organizer of the annual meeting in Germany.

In fact, next week I'm a co-organizer and organizer of a workshop at EMBO in Heidelberg, which is on QPCR, and I'm an advisor to CHI on their annual QPCR meeting here in the United States.

Q Just for the sake of the record, what is CHI?

A Cambridge Health Institute. They organize all kinds of scientific meetings.

Q Okay. And you speak frequently on the topic of PCR?

A Yes. I travel around the world giving talks on QPCR.

Q Okay. Now, what material from the Cedillo case did you review in preparation for your testimony today?

A Well, everything you provided me with, which was Dr. Hepner's report, the parts of Dr. Kennedy's report that were relevant to QPCR and the one-page

Unigenetics report, and I've read the transcripts as far as they're related to PCR.

Q Okay. Were you also able to review the Walker abstract and the subsequent material presented last week?

A Yes. I was able to do that, yes.

Q Okay. Now I'd like to start with having you comment on Dr. Hepner's report and testimony.

Obviously she focuses on Dr. Uhlmann's 2002 paper. Just so it's clear, how does Uhlmann relate to Professor O'Leary?

A Dr. Uhlmann was a postdoc in Professor O'Leary's laboratory at the Coombe Hospital in Dublin.

Q So is the methodology that was used in Uhlmann's 2002 paper the same methodology that would have been used on the testing carried out in Michelle Cedillo's samples at Unigenetics?

A It was, yes.

Q Now, Dr. Hepner goes into some depth on the methods used for Uhlmann, starting with a discussion of experimental controls and cross-contamination.

She stated in her testimony last week that she was relying only on what is contained in the paper itself to make her conclusions and her testimony.

What is a positive control?

A Well, a positive control is an essential control that tells you whether your assay is working, so what you would do is you would take the target that you're interested in detecting and put it into a test tube and use your assay to detect it.

If you don't detect it, you know there's a problem with your assay because it's a positive control. If you do detect it, you know your assay is working. If you do this consistently each time, you know how efficient your assay is from day to day.

The positive control is simply something that tells you that your assay is okay.

Q And a negative control?

A A negative control is something very crucial. There you leave out your target, so if you don't detect it then that means that there's no amplification, which is what you want.

If you do detect a positive in a negative control then you know there's a problem with your assay because it should not be there, and you always get suspicious of any assay that gives you a positive result in a negative control.

Q And what is template?

A Template refers to in this case the measles virus targeting, for example, the F gene or the H gene

that it might be amplifying. In this case, template means the same as target or measles virus RNA.

Q Were no-template controls claimed to be in use in the Uhlmann paper?

A Yes. They have a list of controls in the materials and methods that they list as having been used. Yes.

Q And did Uhlmann provide the information necessary to establish whether these controls were working as expected?

A No. One of the surprising aspects of this paper is they give you very little information about how the assay was performed, about what the results actually were, and it really does not let you evaluate at all how reliable and consistent the results are.

SPECIAL MASTER HASTINGS: Now, Doctor, you mentioned the no-template control, or you were asked about that. That's a negative control?

THE WITNESS: Yes.

SPECIAL MASTER HASTINGS: Okay. Go ahead.

BY MS. BABCOCK:

Q Is there any discussion in Uhlmann about contamination?

A No.

Q Is this important?

A It is essential because obviously if you are trying to detect a very low copy number target and there is contamination around, and if you do not know whether there's contamination around, then you can't rely on your assay.

So it is crucial whenever you do any biochemical assays, including PCR, that you have all your controls lined up properly and report the results of your controls. If you don't report the results of controls, then you can't as a reader evaluate the validity of your data. So controls are always essential, and contamination controls in particular are always essential.

Q So to distill the steps, you need the positives to be positive, the negatives to be negative?

A That's right. You always need the positives to be positive and the negatives to be negative.

Q If the negatives test positive, there's a problem and vice versa?

A Yes.

Q Okay. What's the difference between conventional and TaqMan PCR?

A Conventional PCR and TaqMan PCR are very closely related. Conventional PCR relies on two

primers, two DNA oligonucleotides that define the extent of the amplification product.

So for example, if you enter Washington there's a sign that says Washington, D.C., and if you leave Washington there may be a sign You Are Now Leaving Washington, D.C. That's what primers do. They delineate the beginning and the end of your target, in this case measles virus RNA.

For conventional PCR you conduct 20, 30, 40 cycles of the preliminary chain reaction and run these reactions out in gel as we've seen in various presentations, and you look for bands on gels. The band is either or it's not there ideally. Sometimes other than being seen you're getting smears.

However, all you need to know is that there's a band. You don't know what that band is, so you have to do additional techniques to confirm the identity of that band because it could be a spurious band so there's various ways of confirming that the band you're seeing is your target.

The best way of doing this is sequencing is getting a DNA sequence. However, of course, that is all very always time consuming and labor intensive, and it doesn't allow you to get a fast result and a reliable result, so real time PCR improves on

conventional PCR.

Now, the analogy I would use is that we've got Washington, D.C., and we've got Washington in England. If I'm talking about Washington you don't know what I'm talking about, but if I say the White House in Washington there is an additional, very specific pointer towards the specificity of the location we're talking about. If I say the White House in Washington you know I'm not talking about the Washington in the United Kingdom.

What TaqMan PCR does is it provides the White House. It provides an additional probe that would be very specific for the target that you're amplifying, so the specificity that you get from your primers which we have in conventional PCR is augmented by the specificity of a probe that binds to the amplified DNA only if the correct target is being amplified. So really what TaqMan PCR does, it gives you an additional level of confidence in the result that you're obtaining when you do your PCR reaction.

Q Okay. So, in this instance, for the White House, Uhlmann's White House was supposed to be measles virus?

A That's right. In this case, the White House, the probe would detect measles virus.

Q Now can you have complete confidence that measles virus is actually there when you test for it?

A Well, that's a difficult question to answer because it's a complex question you've asked me, not a simple question. The problem of course is that you're still only looking at the appearance of fluorescence. The actual way you're detecting your template is by degradation of the probe as Dr. Ward explained this morning. You're talking about fluorescence.

So in principle if you're detecting hybridization of the probe, the White House, if you're detecting the White House it should be measles virus, but there are additional possibilities. The most obvious one is that you detect a contaminant. It is less likely that you're detecting irrelevant sequence because the probe wouldn't bind to that, although it could do that as well.

The contaminants are always a problem. In addition, you could have problems with the actual assay itself, that they're are artifacts. As you know biology is not always clear cut, and sometimes you'll have problems that appear, and so you might have a problem with a contaminant and you might have a problem with the actual assay itself that would give you a positive result.

Q So in order to make sure that you're not having these problems what do you need to do?

A Well, there's a lot of different things you can do to look at the quality of a real-time PCR assay. I can go into that at a later stage. The obvious thing you would do when you design an assay is you sequence the amplicon that's being generated because if you find concordance between the sequence, which should be measles virus, and the appearance of a positive result when you attack the preliminary assay, then you can be reasonably confident that you're amplifying the right thing.

But if you don't sequence your target at least when you're designing and validating and optimizing the assay, you can never be certain that what you're getting is the right sequence.

Q Now, did Uhlmann discuss how the RNA was handled?

A No. As I think I said one of the things about this paper is that it's fairly unique in my experience, and it's given no information at all about what actually was done. It actually tells you in outline what they did, where they got their samples from and that they prepared RNA, but it gives you no information whatsoever about, for example, the quality

of the RNA, the quantity of the RNA and how the different RNAs were extracted from different samples which they refer to.

Q Now, from a PCR perspective what's the difference between fresh-frozen biopsies and a formalin-fixed, paraffin-embedded biopsies? I think it's FFPE.

A Right. Dr. Hepner used the term apples and oranges as referred to something, and I would use the term apples and oranges to refer to formalin-fixed and fresh-frozen materials. Let me go through both and explain it to you very carefully. Fresh-frozen as the name implies is a biopsy that is taken say following surgery or following a colonoscopy, and it is put into liquid nitrogen and frozen, so it is a fresh piece of tissue.

The quality of RNA that you get from that kind of tissue as long as you extract the RNA carefully and handle it carefully is usually very good so that if you're looking for a very low copy number target you will tend to detect it in a fresh-frozen sample. We all love to work with fresh-frozen material.

However, a lot of medical research is carried out on sick people, for example, in my

particular specialty in cancer patients, and so every hospital has banks of archival material that's been stored for the last 120 years perhaps and has been formalin-fixed and paraffin-embedded because this is used by pathologists to, for example, collect for cancer stage for cancer.

So this is a very unique source of material for any medical research, so we like to also use that kind of material. The problem is that formalin-fixated and paraffin embedding destroys nuclear gases. As Dr. Ward said this morning RNA is particularly susceptible to degradation. So what formalin-fixation does is it cross-links the RNA, it degrades it and it basically makes it less available to reverse transcription.

So if you have the formalin-fixed sample and the fresh-frozen sample, and again, there's several papers in the literature that suggest this, you should never compare the results you're getting from those two because they will be different.

Q Now, did -- I'm sorry.

A Sorry.

Q Did Uhlmann use both fresh-frozen and FFPE?

A Yes. Again, this brings me back to the lack of information. According to the materials and

methods they used both fresh-frozen and formalin-fixed samples, but they don't tell us which samples were fresh, which were frozen, whether the same percentage fresh and frozen were used for controls and samples.

All of these things of course are crucial to be able to evaluate the validity of the assay because to take an example if he had used all fresh-frozen samples for his cases and all formalin-fixed materials with controls you would not be surprised if he never detected his target in the controls because he's using formalin-fixed material.

So in order to be able to evaluate the reliability and validity of any data that you could use if you're using mixed templates such as this you must very clearly state what you're using, and he hasn't done that.

Q And did he distinguish between the two in reporting his results?

A Not in the paper, no.

Q Did you also identify a mismatch between the measles virus sequences listed in the paper and the probes?

A Yes. This is, again, well, it suggests that there's a problem with the probe design. If you look in his paper the paper actually lists the probe they

used for the F-gene measles virus and lists the sequences from Genbank, which is the database that collects all nuclear gases sequences they use to design the probe.

If you then go back and look up these sequences then you'd find there's a single mismatch between the consensus sequence of all of those sequenced and the probe that they have used to generate the TaqMan probe. Now, what this means is that, again, as Dr. Ward explained this morning that there's a lack of sequence identity in one base between the probe they use and the actual measles virus sequence.

Now, I suppose it's likely too much of an analogy, but if you had a black house then you wouldn't necessarily be in Washington. So you have a mismatch in your probe then you can't be quite certain that it's going to detect your measles virus particularly if you're looking at very, very low copy number targets.

So having a single mismatch is not fatal to the assay, but it certainly raises questions about the validity or the reliability of the assay that is being produced for the F-gene target.

Q Now, what genes did Uhlmann target in his

research for the 2002 paper?

A Well, again, it brings me back to this problem of not knowing from the paper. He lists two F-gene primers and, is it two, yes, two H-gene primers but doesn't distinguish between the use of these primers in the TaqMan PCR, so we just don't know which primers he has used for the TaqMan PCR assay.

Q Okay. Did he also design primers for the N-gene?

A Yes. He designed primers for all three potential targets. Yes.

Q Okay. And I think you might have just said this, but in the result section did he distinguish between *F, H* or *N*?

A No. In the abstract he refers to having targeted the *F* and the H-gene for TaqMan PCR, but in the result section he doesn't distinguish what results he got.

Q Now, regarding consistency and reproducibility did Uhlmann provide any data regarding amplification sensitivity or efficiency?

A No. I need to come back to what I've been saying several times now. There's this lack of information that doesn't allow you to evaluate this paper properly in terms of its validity. There's no

mention whatsoever about any parameters that you would be looking for as an independent or a disinterested reader to be able to evaluate whether his results are correct or not.

He gives no information at all about any of his parameters.

Q And did you identify any other items you would like to have seen in order to evaluate his findings?

A Well, one of the things I've been doing for a long time is trying to get standardization into the reporting of real-time PCR data. In fact, in my 2002 review I had a section at the end of the paper that suggested certain parameters that need to be consistent, and in the latest nature of protocols we expanded on that.

When you do microrate, which is another biological technique, there are so-called minimum information required to give you information that you require to be able to evaluate a microrate experiment. We're trying to do the same thing for real-time PCR. So what I would look for today and would have looked for in 2002 is at the very least the amplification efficiency of the PCR should be known.

That's the first thing. I certainly would

expect to have a statement that suggests that the negative controls were negative and the positive controls were positive. I might have wanted to see a standard curve. Certainly, if I'm looking at quantification I'd want to see a standard curve.

I would want to see certainly some suggestion that the RNA they were using was of high-quality or at least I would like to know what the quality of the RNA was. And I'd like to certainly have known which samples were obtained from fresh-frozen material and which samples were obtained from formalin-fixed material for the reasons I outlined. It is crucial in order to be able to evaluate the validity of this assay.

Q So these items should be included in a paper discussing PCR?

A Yes. Nowadays you'd have even more rigorous standards, but in those days as a minimum you would have included those. Yes.

Q Now, I'm going to move on to Walker. Actually, Dr. Hepner and Dr. Krigsman discussed the abstract presented by Dr. Walker at an autism research meeting, and again, there was some additional information provided with us during the hearing last week. Have you been able to review those materials?

A I have. Yes.

Q And did you look at the PCR results listed in the Walker paper on page 4?

A I did. Yes.

Q Keeping in mind that this is a poster, it's an abstract presented at a conference, nevertheless, if you were looking these PCR results would you have any concerns?

A Yes. I would like to say first of all that it is preliminary. I think Dr. Hepner, as again Dr. Ward said this morning, made it very clear that she would not come to any major conclusions from this work. So bearing that in mind if I take us through the slides then I will explain what my concerns would be, although of course it is a poster.

Q We started with Slide 1. I believe now we're going to switch to Slide 2.

A Right. Slide 1 is, actually, it's taken straight from the poster. So that's correct, isn't it? That's what it's supposed to look like. Now, what I've done here is I've separated the two figures, the left-hand figure and the right-hand figure, and I'm going to leave the first figure first. Now, what we have on the left here is the size standard that they have used according to the handout which is from

Invitrogen and is a 100 base pair ladder.

What this means is that there are DNA fragments here that differ by 100 base pairs each. The other thing Invitrogen tells us is that the 600 base pair of fragments here runs up normally, so you're getting two, so it looks like a doublet. You can see it's somewhat more intense. Can you see that? If you look at the figures from the Hepner poster you can see that it's a slightly more intense band here. Can you see that?

This band here is slightly more intense than all the others.

SPECIAL MASTER HASTINGS: Yes.

THE WITNESS: Yes? Okay. So that locates the 600 base pair fragments here. Now, what she then tells us is that if the PCR has worked okay you expect to get a yield, a band of 726 base pairs. So we can count quite easily 600, so a 700 base pair band is just above the doublet, so it makes it very easy to locate.

So if you look at the band just above the doublet here and then go across here then albeit this being a very poor representation we certainly can't really see a band at that location.

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BY MS. BABCOCK:

Q So this is a reference to the height at which a band should be evidenced?

A Yes. What we can see is in every well a band at roughly 654, 320 base pairs because if you count down from the 600 doublet 500, 400, 300, this band roughly co-migrates with this band. So the first thing to note is that this gel produces a band of 300 base pairs, which you don't refer to, and this of course immediately tells you there's nonspecificity there.

You're expecting something at 700, you're getting something at 300, there's a problem. In addition, you can see the very same band here, again, at about 400 probably and then up here at about 900. So there are bands on this gel, but they are nonspecific. Now, even more importantly I think or just as importantly she refers to 12 patient samples.

If you count along here you've got one, two, three, four, five, six, seven, eight, nine, 10, 11, 12. Now, as I tried to point out today I think virtually every expert in this case has referred to controls are essential. You always want controls of your samples. There's no controls on this. So even though this is a poster presentation at the very least

there should be a negative control on there to show that the PCR in the negative control hasn't worked.

We don't have that information. So this immediately invalidates these results because we can't now say whether these are genuine or not because there's no negative control there. So that's a real problem with this figure.

Q This was the conventional PCR, now we're moving on to network PCR --

A Right. So this is the second picture in our second panel of Figure 4.

SPECIAL MASTER HASTINGS: Before we go on here let's do housekeeping. Again, Dr. Bustin is showing some slides here, and we have a paper copy of those slides. Let's mark that as Respondent's Trial Exhibit 13, and note that now you're turning to Slide No. 4. Is that correct?

THE WITNESS: Three. No, this is Slide No. 3.

SPECIAL MASTER HASTINGS: Slide No. 3. Okay. I see. It's slightly different. Now, you said something here about did I hear you say Figure 4?

THE WITNESS: Yes. Figure 4 is the actual Figure 4 from the poster.

SPECIAL MASTER HASTINGS: Okay. Figure 4

from the poster is on the right on your Slide No. 3?

THE WITNESS: Yes.

SPECIAL MASTER HASTINGS: Okay. Go ahead.

THE WITNESS: This is the second panel from the figure from the poster. Someone loaded the side standard there for me again. So, again, to locate the doublet here and the size ladder here. Now, according to the legend again we've got 12 samples loaded, and we're expecting a nested PCR or a PCR fragment at 407 base pairs. This is present in Lanes 3, 4, 6 through 9 and 10.

Now, again, it is immediately obvious that you've got based on bands down here again suggesting nonspecificity. In this case they're very small bands, and they're most likely to be the primers coming together and being amplified across each other. We call that primer dimer. You may have heard that term before in this Court. No?

MS. BABCOCK: No. I know primer dimer is a bad thing?

THE WITNESS: I'll explain it in a second.

MS. BABCOCK: Okay.

THE WITNESS: What it basically means for the purpose of what I'm discussing now is nonspecificity. Now, we've got these smears, and we

referred to these early on, and these are typical for conventional PCRs, RT-PCRs, because you're getting some nonspecificity and some specificity.

So it's certain you would believe that we're getting amplification products here, and probably here, probably here as well, not so certain here or here, but without doing further analysis here, for example, a southern blot, which has been discussed, or DNA sequencing, you don't really know what this is. And this is a nice example actually where real-time PCR would be far better to use than conventional PCR, but that's another matter.

Again, notice, and again, it's crucial here, what we're talking about is nested PCR. Now, nested PCR is where you take a PCR reaction and do a second PCR reaction on top of the first one, so you basically double, or triple, or quadruple your chances of getting contamination. So it's even more essential to have a negative control here.

Of course, if you look at this there's no negative control here. So whatever these bands are you can't tell because there's no control. So as Dr. Hepner says it's preliminary, but technically this is a flaw because she should have had negative controls on this gel.

Q Now, Dr. Hepner mentioned during her testimony that they were running 35 to 40 cycles. Is this an appropriate mask given the type of PCR being used?

A Well, I think for you to ask me whether it's appropriate or not presumes that, you know, I have a God given right to say what is appropriate or not. I think it is high. I would certainly not be happy using a nested PCR using a 35 cycle PCR and then another 35 cycle PCR. In the olden days when we used to do nested PCR we would have run 15 or 20 cycles followed by 30, 35 cycles because then you minimize the likelihood of contamination.

Q Unless these issues are resolved would you have confidence at least in what's been presented from the Walker lab?

A I can't have any confidence because there's actually no results I can evaluate without referring to a negative or a positive control, and these don't give them to me. So I would have to ask to repeat the assay with a negative control.

Q Is there any indication that this abstract was ever published?

A I've never seen it published. This was the first time I've seen this or heard about it.

Q Okay. I wanted to talk briefly about Michelle Cedillo's results in the O'Leary lab.

A Right.

Q You mentioned earlier that you've reviewed it. Look at Petitioners' Exhibit 28 at 179.

A That's Slide No. 4.

Q 1.67 x 10(5) copy the measles virus per nanogram RNA.

SPECIAL MASTER HASTINGS: Ms. Babcock, can you give me a minute to get to this?

MS. BABCOCK: Absolutely.

SPECIAL MASTER HASTINGS: Okay. Exhibit 28?

MS. BABCOCK: At 179.

SPECIAL MASTER HASTINGS: All right.

MS. BABCOCK: It's up on the monitor as well.

SPECIAL MASTER HASTINGS: Okay. Very good. Thank you.

BY MS. BABCOCK:

Q Okay. 1.67 x 10(5) copies the measles virus per nanogram RNA present in the biopsy. Taken at face value on a scale of low to high where does this fall?

A It seems a high number.

Q And what would the significance be?

A Well, it means that A) this is not an assay

that is at its limits so this should be easily detectible, and it also means that if you've got that much measles virus in a gut sample it probably is in other cells as well and you should be able to detect it, for example, in blood.

Q Now what gene was tested for according to the report?

A The F-gene.

Q Would it have been helpful if they had also tested for *N* or *H*?

A Well, it is surprising because the Uhlmann paper suggests that they have used at least two viral genes, so yes, I think in order to have confidence in your results you should have more than one target particularly when you're looking at the virus, which makes it quite easy.

A virus is a foreign object, so it shouldn't be present in the body. So if you're going to detect a virus you want to be certain it is there. So the obvious thing to do is not to decide with one viral gene, but two or more. So you would have probably targeted the F-gene and the H-gene, for example, and look for concordance between the results and assume that if you got both *F* and H-genes positive that you really are detecting measles by its target.

If only one of the two is positive then you'd have severe doubts about your result. So from that point of view I think one should have used two markers, yes.

Q Was there any information included in the report on assay repetition or control?

A No. There's nothing. Again, no information provided at all.

Q Was a blood sample submitted?

A It was submitted, yes.

Q Were the results of the blood testing provided in this report?

A No.

Q Now, moving on to Unigenetics specifically, as part of your prior work, have you had an opportunity to examine the testing methods used by Dr. O'Leary while at Unigenetics?

A Yes. As part of the U.K. litigation, I was given access to all of the raw data that underlies the Uhlmann paper and all the other assays that were carried out for the U.K. litigation.

Q Now, who approached you about this project?

A I was vying my April 2003 Q-PCR meeting in London, and it's hard to remember, but it must have been attended by scientists working for

GlaxoSmithKline because at the end of June, or a month or so later, or a couple of months later I was contacted by the solicitors for GSK who asked me whether I had heard of the MMR trial, which I vaguely had, but it wasn't any concern of mine, whether I had been approached by anyone to give any opinion on any of the results that had been underlying the results of the analyzer test kit and whether I would be willing to come and look at a paper that had been published in 2002, the Uhlmann paper, and some of the documentation they had been given by Unigenetics and Professor O'Leary as part of the disclosure for the litigation.

Q And what was your understanding of your task in this project?

A Well, it was made clear to me by both the solicitors and the barristers acting for the three companies that my main overriding duty was to help the Court, so I'm a Court witness. So this is overriding the duty I would have to anyone employing or paying me to do the work, and this was stressed to me again and again, and that my opinions have to be independent and that independence is measured by whether if I had been given the instructions by the opposite side I would come to the same conclusion.

And so I have seen and always have seen my

involvement in this case as simply someone coming along, looking at the data and to the best of my ability coming to a very fair and unbiased conclusion.

Q Now, to be clear was this the first time you had offered an opinion for purposes of a legal proceeding?

A Yes. I've never been involved in any legal work before.

Q And is today the first time you've ever testified?

A Yes.

Q Welcome. Now, you were granted physical access to the Unigenetics laboratory?

A I was, yes.

Q When?

A In January 2004, and then again in May 2004.

Q And what did you do during your visits to the actual laboratory?

A By the time of the first visit Unigenetics had agreed to supply us with the raw data from the life of the TaqMan assay. So one of my reasons for going to the Unigenetics was I wanted to reanalyze the data on the computer that was attached to the actual instrument to see whether the analysis I was doing at home on my computer would give identical results to

the computer that was being used by Unigenetics so then I could conclude that my analysis was equivalent.

That was my main reason for going. The second reason for going was to actually look around the lab to see how it was set up. The third reason was to actually get as many of the files as possible.

Q Okay. To be clear you used their own equipment as well as your own?

A That was the aim for the first visit. However, a couple of days before we got there I think either the ABI or the computer, I can't remember which, broke down and couldn't be repaired and was physically removed from the lab, so I didn't have access to the computer that was connected to the ABI instrument, but I did have access to another MacIntosh that was in the lab.

So I was able to analyze some of the data on another computer they also used for analysis.

Q Okay. And just as an explanation for the non-PCR people, the ABI instrument is an instrument used that's essential in?

A That is the TaqMan instruments that they used and we used at the time.

Q But were you able to use their equipment on the second visit?

A On the second visit the computer was there and I was able to analyze the data on both their MacIntoshs, yes.

Q Okay. Now, when you say analyze data what is the scope of what you did for this project?

A I did a lot of work for this. First of all, like any instrument, for example, if you're looking at a website you see a very pretty website, and it's all easy to understand. You click on something, something happens. Underlying that website is the HGML code, which is incredibly complicated, and you and I normally don't see and wouldn't want to see.

So similarly, with any software there is the pretty front and the much more difficult back end. An instrument of course, an instrument puts out raw data that needs to be interpreted by the software and then presented in a user-friendly form. So the data that I'm showing you today, yes, all of those data are the output from the instrument is in user-friendly form.

That is why we wanted the access to be the raw data files. What I was able to do is was to analyze the raw data output from the instrument which is something one normally never does because it is, you know, incredibly time-consuming, there's lots and lots of data points occur well, and it is a bit of a

nightmare and it certainly is terribly time-consuming.

Q So it's fair to say you spent a substantial amount of time on this project?

A A very substantial amount of time on this, yes.

Q About how many hours?

A Approximately 1,500 hours.

Q Okay. What was your hourly rate? What were you paid?

A 150 pounds.

Q So $1,500 times 150 pounds is roughly the nature of 200,000 pounds?

A 250,000 pounds. An awful lot of money, yes.

Q Okay. Now, did you also analyze samples from a gentleman named Professor Cotter?

A Yes, I did.

Q We'll talk about this a bit more later, but who is he and how is he related --

A Sorry. Professor Cotter is the professor for exponential hematology. He works in an institute a couple of doors down the road from me. It's like we work for the same institution. He's a PCI, but he has lots of routine PCRs. DNA-based PCRs mainly, but also RT-PCRs. He had the same instrument as both Professor O'Leary and I have.

Q In addition to the data what else were you able to review?

A I looked up all the disclosed notebooks that the solicitors sent, I looked at all the witness statements from all the workers at Unigenetics lab, I looked at the expert witness reports from Professor O'Leary and from Dr. Shields, and all the expert witness' reports as they related to PCR, I looked at all the operator sheets that were produced, all the experimental reports that were produced. I've probably forgotten something. I looked at everything that I --

Q The standard operating procedure?

A There was a standard operating procedure that was disclosed with Dr. Shields' testimony, yes.

Q And you put your conclusions and reports that were filed with the litigation --

A I did, yes.

Q And these are the reports filed here as well?

A Yes.

Q Now, your reports are very technical to say the least, and we could certainly be here for hours discussing it. I think for the sake of some brevity we will attempt to just hit the highlights. As a

manner of setting the table I will apologize if we ask some slightly repetitious questions of PCR, but I think that some of these questions need to be asked here so we understand the context in which they're important.

So with that said I wanted to start by touching briefly on the standard operating procedure or SOP. What is this, and why is it important?

A Well, a standard operating procedure is like a recipe. If you boil an egg for three and a half minutes you get a soft egg, if you boil it for five minutes it's a hard egg, so if you want a soft egg you have to boil it for three and a half minutes, and if you don't do that you get a different end product.

Similarly, a standard operating procedure is a recipe that allows an investigator to reliably repeat any results he obtained last week, last month, and within a year's time because what he does is lay down very specifically how he obtains his samples, how he obtains his RNA, how he goes about preparing his RNA, how he quality assesses it. All of those things are laid down in a standard operating procedure as the name implies.

Q So it's a fairly detailed document?

A It depends from lab to lab, but yes.

Certainly the Unigenetics one was actually fairly detailed, yes.

Q Now, if we wanted a tissue collection in preparation phase why is this important?

A Again, consistency. As. Dr. Ward mentioned this morning RNA is labile, so if you leave your tissue out on a bench, for example, the RNA will degrade. So it is essential that you have some quality control program in place from the moment the tissue leaves the body until it reaches your test tube and you start extracting RNA.

So sample preparation is one of the essential steps in a Q-PCR assay and needs to be controlled properly particularly if you are using different kinds of sample, for example, fresh-frozen and formalin-fixed samples. You need to know which is which.

Q Now, did Unigenetics use both fresh-frozen and FFPE samples?

A They did. They used both.

Q What was the percentage between fresh-frozen and FFPE?

A Okay. What is really interesting about the analysis that I did was that because I looked at so many data points and so many different samples there

are lots of internal controls provided by the work that Unigenetics' Professor O'Leary did that gives me confidence in my analysis because I've got controls provided by O'Leary himself.

Therefore, I don't have to refer to the outside literature to be able to come up with conclusions. Now, if you look up the difference in formalin-fixed and fresh-frozen materials as I said to you already one is much better quality than the other. So what you would want to show is that if you extract RNA from one sample you get the certain amount of RNA, and if you take the RNA from a formalin-fixed sample you get less RNA.

This is in the literature, but this is what Professor O'Leary's own results show. So what you've got here on the left is samples extracted from fresh material.

Q Slide 5.

A Slide 5. Sorry. Yes. And tested for a reference gene. Now, the purpose of this reference gene is to show that there's amplifiable RNA in your sample. That's all that does here. As you can see you get an average Ct, special cycle, which I'll explain in a second, but an average amount of roughly 25 Cts here. Now, the formalin-fixed material you can

clearly see is shifted upwards, i.e., the average amount of GAPDH target is significantly less.

In fact, it shifted by eight or nine Cts here or is about 200 to 300 fold. There's 200 to 300 fold less apparent target in the formalin-fixed sample, and this is because the RNA has been scrambled up and is unavailable for the reverse transcription, okay? So importantly these are Professor O'Leary's own control experiments.

So if he takes his reference gene and amplifies from fresh material he gets this, if he amplifies from formalin-fixed material he gets that, as expected from the mutation.

Q Now, did you see other findings by comparing SSPE and fresh-frozen materials? Slide 6.

A Well, this is rather interesting. Yes, I did. The next slide is Slide 6. So just for comparison again these are the controls here showing the expected shift from the literature in the formalin-fixed samples. Now, this is what is supposed to be the virus F-gene, and if you now look at the average amount of F-gene target in your positive frozen and your formalin-fixed samples there's no difference.

Now, Professor O'Leary's own controls tell

us that this should have been shifted upwards because this is much poorer quality RNA. The evidence from his own data is completely clear. There's no such shift. This must mean that whatever this is is a contaminant that has been introduced after the sample has been formalin-fixed.

So by definition this cannot be part of the original biopsy because if it had been it will have shifted upwards.

Q Now, moving on to the RNA extraction phase, why is this important?

A The RNA extraction phase, why is it important? RNA extraction is important because the quality of the RNA determines the kind of result you're getting as you can see very clearly here. Poor quality RNA, low copy number, high-quality RNA, higher copy number. So it is essential A) that you have a standard operating procedure; and B) that you follow it to the letter.

Q Did Unigenetics check for quality of RNA?

SPECIAL MASTER HASTINGS: Did it check for what?

MS. BABCOCK: The quality of RNA.

SPECIAL MASTER HASTINGS: Quality of RNA. Okay.

THE WITNESS: Well, first of all they used different procedures from the SOP for their RNA extractions, but that's another matter, and they used two different ways to check for their quality of the RNA, one of which is unacceptable and the other doesn't give you terribly much information, but it's very useful for me from the point of analyzing the data.

They used a method which looks at the ratio of two optical densities, which identifies the presence of contaminants in your RNA sample, but they did that few samples. Most samples were not produced under standard quality controlled. What they did do is they used GAPDH, and in the SOP what they say is that we make an RNA preparation and we will use a reference cellular gene to look for the presence of RNA.

If we can't detect that RNA then the RNA is no good because obviously if your target cellular gene can't be detected there has to be something wrong with the RNA prep. He is completely correct. Are you following me?

SPECIAL MASTER HASTINGS: Why don't you say that one again?

THE WITNESS: If you make an RNA preparation

and it is of good quality then you expect to detect RNA in that sample.

SPECIAL MASTER HASTINGS: All right.

THE WITNESS: So if you have a reference gene in that sample that is a cellular reference gene you should detect it if the RNA is of good quality. If you don't detect it there's something wrong with the RNA. As Professor O'Leary's SOP states, if we can't detect the GAPDH we shouldn't use the sample for analysis, which makes perfect sense.

Now, it happens that Professor O'Leary did use those samples for his analysis, and that's why I was able to then hopefully identify what the contaminant is. This is the next slide.

BY MS. BABCOCK:

Q Okay. Well, I wanted to move on to the reverse transcription phase, and talk about it a bit in general first. Sorry. Reverse transcription.

A Okay. Reverse transcription. We have been talking about real-time PCR all this time, but I think it is important after this point to say that the real-time PCR really only refers to the DNA amplification side of things. In order to be able to amplify an RNA molecule you have to convert the RNA to DNA. I think that's been said several times over.

Now, this is crucial here because measles virus does not exist as a DNA molecule in nature, so you must identify the RNA. If you ever identify DNA then it has to be a contaminant. This is a very crucial point. Because measles virus does not exist as a DNA molecule you can't detect DNA. If you do it's a contaminant.

Q So you have to use the RT step to amplify?

A So you must use an RT step to detect the measles virus RNA. If you detect a target that is apparently measles virus in the absence of an RT step by definition it can't be measles virus because it has to be DNA. It's a very simple concept. At least it is to me. It's not to everyone else.

Q Perhaps not so much the rest of us, so be patient.

A So this is why I was talking about reverse transcription PCR, okay? So, again, the standard operating procedure of Unigenetics requests the use of specific primers. In a reverse transcription step there's a combined RT-PCR step. So to make life simpler they have used a combined RT-PCR step. You can do it different ways. It is okay to do it that way, not ideal, but it's okay to do it that way, the crucial point being you must have an RT step.

Q Now, are temperature and time important?

A I'm sure as you have gathered by now timing and temperature are crucial for PCR because you're relying on small bits of DNA binding to other bits of DNA in a vast gamish of other molecules, and in order to find their partners or their targets they have to be at the right temperature.

If the temperature is too low you get nonspecificity, if the temperature is too high you don't get any binding at all, if you give them too long they will, again, be nonspecific, if you don't give them enough time they won't, again, bind and be less efficient.

So it is crucial that once you have got your primers you optimize your assay and then stick by that assay in temperature in terms of 20.

Q Now, what was happening in the Unigenetics lab with respect to time and temperature?

A There was quite considerable variation between runs in both the temperature of the reverse transcription and the time they reversed the runs for.

Q And just can you clarify what a run is?

A A run. A run is a single assay which involves a 96 well state, and it typically involves 90 odd samples they have been looking at. A Q-PCR assay

we call a run because it's called a run. I don't know why we call it that.

Q Now, were there instances where they forgot to use the RT step?

A Well, again, as I said in a run my results are -- we've missed that on the slide, haven't we?

Q That was probably the questioner error. Forgive me.

A Can we go back to the previous slide, please, Slide 7?

Q You'd like to briefly describe Slide 7?

A Yes. Is it okay to go back to that?

Q Of course.

A Let me remind you we were talking about GAPDH and how the Cts were much higher in the formalin-fixed samples as compared to the fresh-frozen whereas the F-gene was no different. Then I mentioned to you that the GAPDH can be used as a control to look for the presence of RNA in a test tube. In the absence of GAPDH there's clearly no RNA present, so we have to discard it.

Unigenetics still continues to use that. This gave me a handle on things to analyze F-gene results from RNAs that were completely degraded, okay? This is what this shows. So on the right here we

have another sample where the GAPDH result was positive, i.e., there was RNA there, and the F-gene results give you a Ct of just below 35 here.

Now, these are from samples that should have been discarded according to the SOP from Unigenetics because there was no GAPDH present, i.e., the RNA is degraded. If you look at the Cts for the F-gene which they reported as positive you can see they're the same. Now, if this is degraded RNA yet I'm getting the same Cts for my F-gene target this can't be RNA because it would have been degraded.

That's what the GAPDH showed me. Now, if it isn't RNA it has to be DNA. If it is DNA it can't be measles virus it has to be a contaminant.

Q All right. Now we'll skip back to where we were before.

SPECIAL MASTER HASTINGS: So that was Slide 7.

MS. BABCOCK: Yes. Now we're on Slide 8.

SPECIAL MASTER HASTINGS: Now we're going back to eight.

THE WITNESS: Now we're going toward Slide 8. So throughout this stage I had an inkling that I was looking at contamination, and I had evidence that it was actually DNA that was the contaminant.

Now, again, fortuitously, and again, I stress this is their own data so I do not need to interpret it, I just look at the run and can tell from the run from their own information, and we all make mistakes, in two runs they actually forgot to include --

SPECIAL MASTER HASTINGS: Just a moment. In the audience, please. Sir, no talking while the witness is talking, please. Thank you.

Would you ask the last question again, Ms. Babcock?

BY MS. BABCOCK:

Q You were discussing when the RT steps have been forgotten and then your investigation of contamination.

A Now, if you remember I had an inkling that it was DNA that I was looking at so it was a contaminant. So the next set of data I'll show you again are Unigenetics' Professor O'Leary's data. Did not require interpretation. I just analyzed them on the instruments without any further input on my part. What I immediately observed was that they had forgotten to do the RT step for those two runs.

Now, we all make mistakes so, you know, that does happen, but it was very, very informative for me

because as I tried to explain earlier on in the absence of an RT step you cannot efficiently get a result for an RNA molecule, RNA virus. This is very nicely illustrated here. The run of April 11, which is this one here, the F-gene --

SPECIAL MASTER HASTINGS: The lower left is the April 11 run?

THE WITNESS: Yes.

SPECIAL MASTER HASTINGS: Okay.

THE WITNESS: On Slide 8 the lower balance or the lower standard curve, this is what it is, is done in the presence of reverse transcription. You can see for different amounts of target you're getting certain Cts, and this is what a standard curve looks like. Now, as you can see on June 19 you're getting a dramatic shift upwards of the F-gene standard.

Now, this is an RNA molecule that they used to generate the standard curve. So what this tells us is, again, it's their internal control, which is so nice because I don't need to interpret here, in the absence of an RT step dramatically shifts upward exactly as you would expect because you haven't got a reverse transcription step there.

All you're doing is a PCR. The PCR enzyme can inefficiently -- it does do some polymerization of

RNA, but it's very inefficient, and that's why you're getting this dramatic upward shift which is 200 fold plus. It depends on how you do it, but you get a roughly 200 fold decrease in the apparent amount of RNA for their control.

So, again, as before with the GAPDH the control they use here or the control that I have used tells me exactly what I expect to see. If I use F-gene RNA for the standard I get an increase in the Ct because there's no reverse transcription. This is what happens with RNA. So we go now on to Slide 9. Again, fortuitously on this run where they forgot to add the RT step there were four lead cases from the U.K.

Now, if you look and compare the F-gene Cts for the four lead cases to the F-gene Cts from the majority of the samples where they did the reverse transcription step there is no difference. The Cts are roughly the same. If this had been RNA this should have been shifted upward beyond the 40, which is by definition the absence of any target source.

You wouldn't have detected it. So in the absence of an RNA RT-PCR step, an RT step, they are detecting roughly the same amount of F-gene target as in the presence of reverse transcription. So by

definition this cannot be RNA, it has to be DNA. So we have two independent lines of evidence that show very clearly that the target that is being amplified it is F-gene, but it's F-gene derived from DNA, but because measles virus doesn't exist as a DNA molecule it can't be derived from measles virus RNA.

BY MS. BABCOCK:

Q Now, moving on to the actual PCR step for accurate results do you need to validate the primers and probes?

A Books have been written on how to validate primers and probes, and, yes, this is one of the most important aspects of any RT-PCR aspect.

Q Now Dr. Ward talked about this a little bit this morning, but can you just briefly summarize what primers and probes are and why this is important?

A As I tried to explain earlier on, primers delineate the extent of the amplification that you get. It is equivalent of the sign saying Washington, D.C., welcome to and you're now leaving Washington, D.C. That's what the primers do. They signify, delineate your target.

Now the important points about these primers are obviously that they recognize their target, in this case a virus RNA sequence, so you want to be

certain that you've got the right DNA sequence to amplify your target.

You want to also be certain that you get as few primer dimers as possible. What this means is if you imagine you've got two primers, roughly 20, 22 base pair individual nucleotide long, if they're similar to each other you'd imagine they could bind and stick together and then it gets amplification of those primers.

Because there's two primers you call them primer dimers.

SPECIAL MASTER HASTINGS: I saw that term in your report. Primer dimers, D-I-M-E-R-S.

THE WITNESS: Dimer, D-I-M-E-R.

SPECIAL MASTER HASTINGS: All right. Go ahead.

THE WITNESS: The effect it has is it reduces the efficiency of the assay because obviously the preliminary spends time amplifying a nonspecific primer dimer. If you have an assay that is less than robust you then can tip the assay altogether into making it very unreliable, so you always aim to have as few primer dimers as possible.

BY MS. BABCOCK:

Q Now, had Unigenetics designed primers and

probes for *F*, *H* and N-genes?

A Yes. Well, I can't remember the *N* to be honest with you, but I remember the design primers were *F* and H-genes, yes.

Q And did you see instances where one gene would test positive and another gene would test negative?

A Yes. It was interesting. They did attempt to optimize the assays for both the F-gene and the H-gene. The obvious reason for doing this was to get concordance between the different results. So when they got a positive F-gene they would have been a positive H-gene result, or a negative F-gene, negative H-gene. What in fact they got was they found the H-gene assay was much more sensitive on their standards -- the H-gene assay was more sensitive than the F-gene assay.

But then there are instances in accord in the lab book where the F-gene gives them a positive result and the H-gene gives them a negative result. They comment on this in the lab book and say the H-gene isn't concordant. They then looked at a sensitivity and found that the H-gene was more sensitive.

So the obvious conclusion is that there's a

problem with the F-gene assay, and we should redesign the F-gene assay because we're looking for concordance. What they in fact did was they ignored the H-gene results and went with the F-gene results. That would give them positive results.

Q Okay. So in the instance that you would have a positive *F* and a negative *H* Unigenetics' practice was to?

A There's only a few runs where that happened because they then gave up on the H-gene, but there are instances where the *H* was negative, the *F* was positive and they went with the F-gene.

Q So they reported the F-gene as positive?

A Yes.

Q They reported the test result as positive?

A Yes.

Q Now, as we discussed in Uhlmann and regarding the Walker paper you also establish controls?

A Do you?

Q No. The person running the lab.

A I think they establish controls. Yes.

Q Yes. As a general part of part of the PCR staff. I apologize.

A I was lost for a second. Yes, of course,

and they did run controls of every assay.

Q Okay. And what controls are necessary? Again, we've touched on this already.

A Okay. We mentioned the positive control, and the positive control in this case tends to be the standard curve which is a nice positive control because the standard curve is generated by taking the target *F*, or *H*, or whatever gene you're trying to amplify and cloning it into a DNA plasmid.

You put that into a bug and make lots, and lots of copies of that so you've got a DNA molecule that contains your target. You then make an RNA copy of your target from the DNA and destroy the DNA so you're left with the RNA only. Now you've got an RNA molecule that because you know the size of the insert that you're looking at and the amount of DNA you've got you can calculate the copy numbers of your target gene.

If you then do field dilutions, and this is a standard biochemical practice, you generate a standard curve. If you do this properly you get a very nice linear response, which were these straight lines you saw in, for example, here.

Q Slide 10.

A Sorry?

Q Slide 10.

A Thank you. What you have here is a standard curve for one of the F-genes, and you can see you've got tenfold dilutions here. Now, this is what you always use to quantitate your copy numbers, and this is how, for example, we've come and got the copy number in Michelle's F-gene result. Now, standard or best practice of course is to have the standard curve span the range of the unknown samples you're going to quantitate because bear in mind the standard curve is produced using optimal conditions.

You have a very clean RNA that you dilute into water, and so that all you've got present in that test tube is your target. So obviously under those conditions if you do it properly you will get a very sensitive and very linear response across a wide range. Your target of course is present and there's lots of other competing molecules, so it will not be as clean and you may expect a different result if you're quantitating from the target that is present or contaminated with cellular RNA.

So at the very least you must make sure that the linear range of the assay includes all of the possible concentrations of your unknown samples. Do I make myself clear? Yeah. Sorry.

BY MS. BABCOCK:

Q Let me be sure of that. So basically ideally we see the red dots on the charts. The red dots should be within the black dots?

A Yes. They should extend through the whole curve. Now, this is a typical Unigenetics result, and as you can see in red you've got all the unknown samples, and they're all way below the linear range of your standard curve.

SPECIAL MASTER HASTINGS: Go back over that last part. I didn't understand.

THE WITNESS: Okay. The standard curve is generated by taking tenfold dilutions of your control. So this would be the highest concentration and tenfold dilutions. Now what you should -- okay.

SPECIAL MASTER HASTINGS: No, please go back again. Each time you turn around I'm missing some words.

THE WITNESS: Okay. I'm sorry. What you've got here is the highest concentration of your standard.

SPECIAL MASTER HASTINGS: The highest concentration. Highest concentration of what?

SPECIAL MASTER VOWELL: The standard.

THE WITNESS: The standard. The copy

numbers of your target. Now, this is a standard for F-gene.

SPECIAL MASTER HASTINGS: A standard curve?

THE WITNESS: A standard curve for the F-gene.

SPECIAL MASTER HASTINGS: All right.

THE WITNESS: You generate that standard curve by taking a high amount of F-gene RNA and running it through a PCR assay and you get a certain Ct. In this case you're getting a Ct of 15.

SPECIAL MASTER HASTINGS: In what case? I'm not sure in what case you're getting it.

THE WITNESS: The first dot.

SPECIAL MASTER HASTINGS: I see. Okay.

THE WITNESS: The first dot on the right-hand side.

SPECIAL MASTER HASTINGS: All right.

THE WITNESS: The second dot is a 110 dilution of this, and you can see the standard is roughly at 18 Cts. Can you see that?

SPECIAL MASTER HASTINGS: I see that.

THE WITNESS: The next one is another tenfold dilution and is roughly at 21 Cts.

SPECIAL MASTER HASTINGS: All right.

THE WITNESS: The following one is another

tenfold dilution at roughly 25 Cts.

SPECIAL MASTER HASTINGS: Right.

THE WITNESS: And the last black dot is roughly at 28 Cts or 29 Cts. So this constitutes your standard curve. What in this case they have done is they have extrapolated the blue line to quantitate all the red dots, which are all the unknowns.

SPECIAL MASTER HASTINGS: The red is not a standard curve?

THE WITNESS: The red dots are actually samples.

SPECIAL MASTER HASTINGS: The red dots are samples.

THE WITNESS: The red dots are the samples. What they should have done is all of the black dots should surround the red dots because, otherwise, you don't know whether the last red dot is really in the linear range of the assay.

SPECIAL MASTER VOWELL: I think I have it, Doctor. Let me try this. You've got a quantity down at the bottom --

THE WITNESS: I'm sorry?

SPECIAL MASTER VOWELL: -- and each time you're moving to the left on your slide, you are diluting the quantity even more.

THE WITNESS: That's correct.

SPECIAL MASTER VOWELL: On the left-hand side of your slide, you've got "CT." So these are the number of cycles that you are running this sample through the machine to amplify it.

THE WITNESS: In principle. That's when you detect that sample, yes.

SPECIAL MASTER VOWELL: And what you are saying is that if you are using a known F curve, a known sample of the F gene, you would have a point for the highest concentration of the gene, and then it would require more amplifications at a lower concentration of the gene.

THE WITNESS: That's correct.

SPECIAL MASTER VOWELL: And that would generate either a straight line or could conceivably be a curve.

THE WITNESS: It should be a straight line.

SPECIAL MASTER VOWELL: It should be a straight line. All right. What you have had happen here is that when you plug unknowns into this curve, and you run them through the number of cycles that are demonstrated here, what you get is something that's outside the limits of the F gene standard curve.

THE WITNESS: That's correct.

SPECIAL MASTER VOWELL: So what you are finding is probably not the F gene. Is that the conclusion?

THE WITNESS: No. You can't quantitate it.

SPECIAL MASTER VOWELL: Okay.

THE WITNESS: It may well be the F gene, but you can't put a number on it because the standard doesn't go past that.

SPECIAL MASTER VOWELL: You're not saying that it's not the F gene; you're saying that you don't know what the quantity of F gene you have because you don't get the results until you run it through more cycles.

THE WITNESS: That's right.

SPECIAL MASTER VOWELL: So you have a known quantity of the F gene that you are putting in, in the black dot on the right-hand side of your chart, and you have another known quantity that's the black dot that is in the center of the chart.

THE WITNESS: That's right.

SPECIAL MASTER VOWELL: But you can't tell, because none of the red dots are between the black dots, how much gene is present.

THE WITNESS: You've got it.

SPECIAL MASTER VOWELL: Thanks.

BY MS. BABCOCK:

Q So what would be much better here, if all of those red dots were farther down and to the right?

A Standard practice and best practice would have been to have all of the black dots shifted to the left so that they would all include the red dots. The red dots should be in between the black dots. That's what's accepted practice.

What this tells you is that they are unable to amplify below roughly 20,000 copies. So this assay is not terribly sensitive on this day. Now, this is a typical result from Unigenetics.

Q We have another slide on that.

A The next slide actually expands on this a little bit. I think maybe you'll understand it now. In this case, they ran 45 cycles of the assay. So, by definition, if something hasn't come up by 45 cycles, it's not there.

So if you look at the very left-hand black dot here, you can see it's at cycle 45. Can you see that? So, by definition, even though they have put some sample in there, and, in fact, they have got 200 copies of their target in there, the assay is not picking it up.

So it isn't sensitive enough to pick up 200

copies in the standard, which, if you remember, is optimal conditions.

You'll also notice that the second set of black dots from the left are diverging. Now this is typical because the less material you have, the less accuracy you get. As, again, you can notice, there are unknowns all coming up here, even though the standard has not come up.

So this tells you that the assay is working very poorly and that you can place no reliance whatsoever on any quantification of any of these samples. It is meaningless to quantitate these samples. They should not be quantitated. You can say they are there, or they are not there, but you can't quantitate.

Q Now, was Unigenetics also using negative controls?

A Unigenetics always use negative controls.

Q Were they getting proper results from those controls?

A Approximately one-third of their runs had positive results in the negative controls. So this means that, in one-third of their runs, the negatives were positive, suggesting there was some contamination, and this is not just the negative

control that it contained no target and was set up to contain no target.

But in at least one case, they had a negative, what they call "environmental control," where they take a tube and leave it open on the desk, and if there is DNA floating around, it might set in the tube, and you amplify that. On at least one occasion, that was positive.

So the presence of contamination in one-third of your assays suggests they have a significant contamination problem in your lab, as Dr. Ward suggested this morning as well, and that is accepted. It's nothing unusual; that's just what happens.

Q Now, is the quality and amount of RNA important?

A Well, the quality of RNA is critical because if you have poor quality RNA, you have much less sensitivity than if you have good quality RNA.

The quantity of the RNA is also important because if you know how much RNA you're putting into your sample, you can then calculate copy numbers, and you can make sure that you standardize your results according to the amount of RNA you're adding.

So it is important to know how much RNA you have and what the quality is, yes.

Q What was Unigenetics' practice with respect to this?

A Variable. They were not consistent. In terms of quality assessment, there was very little quality assessment. What I've taken as quality assessment, the GAPDH assay I've already shown you, that, in a number of samples, they, by their own criteria, which should have discarded the RNA because the internal control didn't come up, but, nevertheless, they continued with that sample.

So if you take the Uhlmann paper as an example, they started off with 91 cases that they refer to in the paper, and in their summary table of the QPCR, they refer to 70 out of 91 patients that are positive by RT-PCR. This is the Uhlmann paper on page 87, Table 2.

Now, of these 91, nine were degraded and reported as degraded, 18 were not assessed for quality at all, and eight were degraded but were, nevertheless, reported as positive for the F gene. Now, nine plus 19 plus eight really should not have been reported at all, which leaves 55 samples out of the 91, two samples. Of those, 35 came up positive with the F gene test. It doesn't mean there was F gene there, but it means there was an F gene target

that was amplified.

So what this means is that now that I was able to start looking at the data underlying this paper, I was able to, for example, say very clearly that the RNA quality in roughly one-third of the samples that they used was unacceptable for RT-PCR.

Q Now the equipment you're using, is that important?

A The equipment is important, less important than a lot of other things, but why it is important, and particularly in the case of this paper and the work that was carried out, and again, that's not Unigenetics' fault -- this is a first-generation instrument. It's a so-called "ABI 7700." You may have seen reference to it. We've had one in our lab. Actually, we just got rid of ours last year.

The problem with that instrument, as with all old instruments is that the technology has moved on. In the olden days, we were happy to have something that worked. Nowadays, we have more demands.

A well-known problem with these instruments is that they rely on a heating block that contains 96 wells. So they can do 96 assays in a heating block. In order, as we established, to get a good PCR result,

you have to have very accurate temperature control and time controls.

The problem with these older instruments is that heating/cooling is not uniform across the block, and, again, this is well known. So, very often, people don't use the outside wells because they already know there's problems with these wells.

Now, I was able to look at the instruments that they have used, and this is not a specific criticism about Uhlmann because they could never have known about this. In fact, they had the instrument services, according to Dr. Sheils, and ABI should have picked this up, but the problem was that there was a problem with one of the instruments they were using.

Because I was doing all of the basic looking into the innards of the instrument, I was able to finger print the results and identify which run was done on which instrument, and what I found was that the instrument that was used on most of their runs had a huge variation in the heating and cooling characteristics across the block. What this means is that there is variability of your results, depending on where you place your tube on the instrument.

So, regardless of any problems you have with the assay, this contributed to the variability that

we're seeing in the results produced by Unigenetics.

Q And you discussed this earlier, but is RT sensitive to temperature?

A It is very sensitive to temperature, yes.

Q And PCR?

A And PCR even more so because there is an exponential amplification, yes.

Q And was Unigenetics aware of this problem?

A I suspect they were not, of this particular problem because, as I said, Dr. Sheils told me that ABI serviced the instrument. We had ours serviced once a year. I was surprised to see this, but certainly their first runs were in 2000 and 2001 from this machine, and the last ones, I think, were in 2003.

So it was three years' worth of runs that I was able to fingerprint, and the problem persisted from the very first run to the very last run, a very characteristic pattern of problems with the heating block. So if they serviced it, they didn't do a good job.

Q Now, moving on to the data-interpretation phase, and you have referenced this several times, what is a "threshold cycle"?

SPECIAL MASTER HASTINGS: What is a what?

MS. BABCOCK: A "threshold cycle."

SPECIAL MASTER HASTINGS: Threshold. Okay.

THE WITNESS: Can we show the next slide?

Now, these are some data from my own lab. They are terrible data; that's why I'm showing them. You've heard the term "amplification plot."

SPECIAL MASTER HASTINGS: Amplification what?

THE WITNESS: Amplification plot.

SPECIAL MASTER HASTINGS: Blot.

THE WITNESS: Plot.

SPECIAL MASTER HASTINGS: Plot.

THE WITNESS: Have you heard that term before? Yes. And you've heard the terms "threshold cycle" and "baseline." So let me explain to you. I want to make sure you can hear me.

You can see a blue line. This is what we call the "threshold," and you can see that there are kind of waves coming up from below, and they cross the blue line at a certain point, for example, here, here, and here. Do you see that?

SPECIAL MASTER HASTINGS: Yes.

THE WITNESS: That is the CT, the threshold cycle. So this is the very first time the instrument can reliably detect fluorescence from its target, and

the more targets you have, the earlier the machine detects it, and the lower the CT.

So, as you can see, the CTs start at one here and go to 40 there. So the more targets you have, the more to the left these curves are. So these contain lots of target; these contain very little target.

Now, there are certain things you can notice about this, and this is a typical result from a real-time PCR instrument. The first few cycles are very noisy. You can see there's all sorts of funny things going on here, and this is one reason that the ABI recommendations for analysis of your sample are to exclude the first three cycles, at least, from your analysis.

BY MS. BABCOCK:

Q ABI is a manufacturer of PCR equipment?

A ABI is the manufacturer of the instrument that both Unigenetics and myself and Professor Cotter used.

So, clearly, you can see this noise here, and it's not surprising that they suggest you don't use that free analysis because it can give you spurious results.

SPECIAL MASTER HASTINGS: There you were

indicating the data on the left-hand side.

THE WITNESS: Yes, this kind of -- whatever you want to call it.

So this is the first amplification plot, and it's a duplicate. So it shows you get very nice reproducibility, and you get a characteristic slope here, which I've indicated in red. Now, this slope indicates how efficient the reaction is. So what you can see for the first four amplification plots, the slopes are virtually parallel. Can you see that?

SPECIAL MASTER HASTINGS: Yes.

THE WITNESS: So the first four --

SPECIAL MASTER HASTINGS: The first four beginning about 17.

THE WITNESS: Yes. So for those four reactions, the amplification efficiency is equivalent, and you can compare the quantification. It makes sense to compare the copy numbers from these four runs. Note that these are the ones that have more target in them, so, typically, the more target you have, the better your assay works.

Now, a very well-designed assay would be linear over a wide range, but, typically, as you get into the higher CTs, the assay becomes less efficient. You can see this here now because suddenly the slopes

become flatter. So, clearly, these reactions here are less efficient than these reactions here.

So you can now compare the copies you're getting from this amplification plot to copies you're getting from these amplification plots. That's the first thing I would like you to notice here.

The second thing I would like you to notice is that, clearly, we're getting curves coming up here that look like small versions of these plots, but they are below the threshold. So they will be recorded as negatives.

BY MS. BABCOCK:

Q And to be clear, those are curves farther to the right.

A These contain the least amount of target. Now, there can be two problems here. If these are my target, and I'm recording them as negatives, then I've got a false negative here because they really are amplifying, but the way I've analyzed the data doesn't allow me to detect them. Do I make myself clear?

The opposite problem is, if these are no-template controls, then they are coming up, but I'm not detecting them because my threshold is wrong. So it is essential that when I'm looking at my data, I look at amplification plots and analyze each reaction

on its own merit and decide whether something is positive or not.

This is why the reaction is actually -- the assay is a subjective assay, and, again, if you remember Dr. Ward this morning saying, You don't just push a button and get a result. You push a button, but then you have to decide, once you've got the result, does it make sense or not? Is it real or not? This is where the interpretation comes in.

Q Now, how was Unigenetics setting the threshold cycle?

A In a very peculiar way. They sometimes followed the ABI guidelines, and sometimes they didn't. Now, this is, again, a typical result from Unigenetics.

SPECIAL MASTER HASTINGS: We're looking at Slide 13 now.

THE WITNESS: Slide 13, and this is the output you get from the ABI 7700. So this is the software I was referring to that looks pretty and gives you a result. So I rely on this, but then I also, obviously, looked at the underlying data.

You can see very nicely here they have used the ABI standards, so the baseline starts at number three and goes to 15. So they excluded all of the

initial noise and get a CT for one of their samples of 33, the red one, and a 37 is the green one.

MS. BABCOCK: For the record, the witness is indicating --

THE WITNESS: This one here.

MS. BABCOCK: -- the green line.

THE WITNESS: So you've got two lines: a red line and a green line.

Now, you can immediately see that these are two different quality of lines. Clearly, something is happening here. There is a take-off. There is PCR going on, but, clearly, nothing is happening here. This is a spurious result, yet it's crossing your threshold. So this is an example where a TaqMan assay gives you a positive result, even though there is no amplification going on.

BY MS. BABCOCK:

Q Okay. So let me make sure I understand this. So, in this assay, Unigenetics would have reported both the red line as positive and the green line --

A Both of these were reported as positive by Unigenetics, yes.

Q -- even though there is clearly no amplification with the green line.

A That's correct.

Q Did you find that this was happening frequently with Unigenetics?

A Yes. Now, just go on to the next slide. What I want to say is that can happen if you don't look at the amplification plot. If you rely on your experimental report, which is the printout the machine gives you, and you don't use your judgment, then this kind of thing can happen. It doesn't mean that -- doing it deliberately; it just can happen. Okay?

What you should do is you take your threshold, and this, again, is where subjectivity comes in, and just move it up slightly, and now you've excluded this green line, which you've decided, as an investigator who is qualified to do so, is not real, and now your CT is 40. It's negative. So this result is recorded as one positive, one negative. But unless you have the experience and the time and inclination to go through each individual result, you can miss this kind of a positive that is a false positive, even though using a TaqMan assay.

Q Is there a third slide?

A There is this one. This demonstrates the point in a little bit more detail and shows you something else.

SPECIAL MASTER HASTINGS: Now what slide did you have up now?

THE WITNESS: This is Slide 15. If you look at the bottom half of Slide 15 --

SPECIAL MASTER HASTINGS: Wait a minute. Right now, your slides are not corresponding to what I have the paper in front of.

THE WITNESS: It's labeled 14 on this one.

SPECIAL MASTER HASTINGS: Fourteen?

THE WITNESS: It's 14 on your handout, and it's 15 on the screen.

SPECIAL MASTER HASTINGS: That's right.

MS. BABCOCK: We'll go by yours because mine are incorrect.

THE WITNESS: Which one?

MS. BABCOCK: So we're on 14.

THE WITNESS: Slide 14 of the handout.

SPECIAL MASTER HASTINGS: Slide 14 of the handout. Okay.

THE WITNESS: Now, you're getting familiar with these outputs from the ABI instrument. The first thing to look at is the baseline, and you'll notice it starts at two. So rather than using three to 15, for some reason, Unigenetics has used two to 15. Do you see this?

SPECIAL MASTER HASTINGS: I'm reading it as two to 13.

THE WITNESS: I'm sorry. Two to 13. They used two to 13 rather than two to 15. Do you see that?

SPECIAL MASTER HASTINGS: Yes.

THE WITNESS: Now, this is a negative control, and the effect of using two to 15 is to give you a negative CT because the amplification is below the threshold.

BY MS. BABCOCK:

Q So, again, the threshold is at the very top of this picture.

A The threshold is the black line at the very top.

Q Okay.

A So this is a no-template, negative control that, if positive, would suggest contamination and make you doubt the run. This has been analyzed using an inappropriate setting. If you change the setting to the ABI-recommended setting, you get this result, a positive CT, a very high positive CT, but, nevertheless, you're getting a positive CT.

Q And by "ABI-recommended," you mean starting at Cycle 3 or greater.

A Three. If you use the recommended settings, your no-template control appears as a positive. If you change the baseline setting, it appears as a negative. This was reported as a negative, even though, clearly, without meaning anything to the analysis, it's positive.

SPECIAL MASTER HASTINGS: On Slide 14, you're showing the same data.

THE WITNESS: Yes.

SPECIAL MASTER HASTINGS: I'm not sure if I'm using the proper term.

THE WITNESS: You are.

SPECIAL MASTER HASTINGS: But, at the bottom, it's run with the baseline, two to 13.

THE WITNESS: Yes.

SPECIAL MASTER HASTINGS: At the top, you're showing the same data here --

THE WITNESS: Yes.

SPECIAL MASTER HASTINGS: -- but with what you call the proper baseline, three to 15 --

THE WITNESS: Yes.

SPECIAL MASTER HASTINGS: -- which would produce an improperly -- well, the one at the top shows a positive result when it shouldn't.

THE WITNESS: That's correct.

SPECIAL MASTER HASTINGS: Are you suggesting they did this on purpose? I mean, --

THE WITNESS: I'm not suggesting anything. All I'm reporting is what I've seen. What I noticed when I looked at the experimental report, it struck me as very odd that they very often did not use the three-to-something setting; they used a one or two setting, and it's something that we have never done, and we know, from ABI literature, you shouldn't do.

So I was always curious why they did that, and until I got access to the raw data, I couldn't explain it. But this explains the result of doing that kind of change. You can clearly see these are the same curves. They look exactly the same. The only difference is how the instrument analyzes the data.

SPECIAL MASTER HASTINGS: The proper way to do this would be for every run to use the same baseline.

THE WITNESS: Not necessarily at the upper level but certainly not go down beyond three at the lower level, yes.

SPECIAL MASTER HASTINGS: Okay. Go ahead.

SPECIAL MASTER VOWELL: I'm not sure I heard you correctly, Dr. Bustin. This was a no-template,

negative control --

THE WITNESS: Yes.

SPECIAL MASTER VOWELL: -- with a positive result, if you ran it in accordance with the machine standards.

THE WITNESS: Yes. This is one example of the one-in-three runs that are positive.

BY MS. BABCOCK:

Q Could we go back to Slide 13 for a moment? Now, I wanted to have you sort of expand upon this. You mentioned that, although the red is the only one that's amplifying, both red and green were reported as positive.

A This is a common thing. This happens again and again and again. Virtually every time I can remember -- I haven't looked at these in a very long time -- to my recollection, whenever there is a hint of a positive result from the F gene in a sample, it is recorded as positive, regardless of whether it is genuine amplification or not.

If it is a no-template control, it appears as a negative. Either it is completely omitted from the experimental report, or the baselines are altered to generate the impression of a negative-negative control.

Q Now, did you also observe problems with discordant replicates? You should start by explaining what that is.

A There are various ways of carrying out a real-time PCR assay. In general, you will do it at least twice and do each assay twice at the same time, a duplicate or sometimes even a triplicate assay.

Now, the reason for doing this is, if you have a genuine result, if you do the experiment twice at the same time, you should get the same result. If there's any major discrepancies, you get worried about it and repeat it. In the old days, we used to say, we'll have to do triplicates, but I think duplicates are fine.

Certainly, one would expect an assay to be repeated, not just done once but done twice. Now, typically, that didn't happen with Unigenetics. They did their assays once only and duplicate. So it's not ideal, but at least they had duplicates.

The problem was that, in most instances, in many instances, they had discordant replicates for the F gene. That means that one was positive; one was negative. One gave a very high CT; one gave a very low CT. Now, this immediately suggests there is a problem because if you've got a genuine result, it

should be repeatable, and this shows one of the very few instances when they actually repeated an assay on two separate occasions.

What you see here in the open bars is an assay done on the 21st of March, and the black bars on the 26th of March. All I'm doing here is I'm comparing the CTs obtained at the different days or dates, and you can see there is massive variability. Intriguingly, on the first day, some are positive, and others which are negative on the second day and vice versa.

Now, what Unigenetics did here was, the ones that are positive on the first day, they were reported as positive, and if they were negative on the second day, they were still reported as positive from the first experiment, and if they were negative on the first day and positive on the second day, they were still reported as positive.

So we've got two sets of positive results from one set of experiments, and they are discordant.

Q Now, you also discuss in your reports repeatability and reproducibility. Why are these important?

A Clearly, if something is real, then I can repeat it. If I repeat my experiment, and I don't get

the same result, I, at the very least, question the result of the first time and do it again.

Q Now, you mentioned Professor Cotter earlier. Did he undertake an effort to reproduce some of the results, positive results, from Unigenetics?

A This is very crucial because Professor Cotter was instructed by the Claimants to try and reproduce the earlier results. Professor Cotter, I think I said, works in our institution and has an ABI instrument. So what happened was that he was sent samples by Unigenetics, and Unigenetics extracted RNA, and Professor Cotter extracted RNA from those samples. In every instance where Professor Cotter extracted RNA, the results were negative, even though Unigenetics got positive results.

So Professor Cotter extracts his own RNA and never sees a positive CT, clearly suggesting there is no F gene target in any of these samples. Unigenetics then sent Professor Cotter their RNA, and then he assayed their RNAs, and these are the results shown on Slide 17. I think it's 17, isn't it?

Q I believe so.

A Yes. They are concordant. What you can see here is, first of all, it looks as though Cotter's assay is more sensitive because the CTs are lower for

Cotter in general than they are for Unigenetics, and that explains why he is getting more positives than Unigenetics.

SPECIAL MASTER HASTINGS: The red ones are the positives.

THE WITNESS: The red ones are positive; the green ones are negative.

But you can immediately see that there is discordance already between Cotter and Unigenetics: the same RNA, the same assay, done in different locations. Now, bear in mind that all of these were negative when Cotter extracted the RNA, so all of these samples must be false positives, or they are most certainly contaminants because Cotter, who is the benchmark, as instructed by the Claimants themselves, and, in fact, Professor Cotter reported this in his report for the Claimants, that he was unable to show any sign of F gene target in any of the RNAs that he prepared and was able to show discordant results with Unigenetics' results.

So what we're seeing really is further confirmation of the fact that what Unigenetics is amplifying are contaminants, and if you extract your RNA properly and include the proper controls in your assays, you get a negative result. The same assay

carried out at Unigenetics gives you a positive result.

BY MS. BABCOCK:

Q Now, is final data interpretation an objective process?

A No. Again, as I tried to intimate, data analysis for real-time PRC is very subjective, and this illustrates the point very nicely. For some reason -- I can't remember why -- four control samples were substituted with four other control samples, and the student who did this experiment prepared the RNA, ran the RT-PCR run, and then reported that all four samples were negative. So, by her interpretation, these four samples were negative.

When I analyzed these results, this is what I saw, and you can see, I can get at least three of these three positive.

SPECIAL MASTER HASTINGS: Now, I can't see that, Doctor.

THE WITNESS: I'm sorry.

SPECIAL MASTER HASTINGS: We're on Slide 18.

THE WITNESS: We're on Slide 18.

SPECIAL MASTER HASTINGS: I wasn't clear. What medical student at what lab did this? I didn't understand anything.

THE WITNESS: I'm sorry. That's my fault.

SPECIAL MASTER HASTINGS: Okay.

THE WITNESS: If you look at the Uhlmann paper, you notice there's a lot of authors on that paper.

SPECIAL MASTER HASTINGS: A lot of authors. Okay.

THE WITNESS: So a lot of different individuals have gone through the Unigenetics laboratory and carried out the different experiments. This is the work of lots of different individuals.

Now, this particular run was carried out by a student who was there and got the samples, prepared the RNA, and did a run looking for F gene in those control samples.

SPECIAL MASTER HASTINGS: All right.

THE WITNESS: And what you see here is the black line, the thresholds, and you can see at least a purple, a red, and a blue line that you can get to cross the threshold --

SPECIAL MASTER HASTINGS: Yes.

THE WITNESS: -- and I recorded here as positive.

SPECIAL MASTER HASTINGS: Okay.

THE WITNESS: Note, I do that by keeping the

baseline on the left at three but now move the baseline on the right to 34. Okay? Now, this is the subjective interpretation of this run. That's all I'm trying to demonstrate here. The reason I've done that is, as you can see, this noise here moves downwards, and this masks your results. So you have to compensate for this by changing the right-hand baseline.

Do you remember, you asked me earlier on whether you change both baselines, and I said, You don't change the left one; you can change the right-hand one. This is an example of where you can change the right-hand one. Then these four controls now, which they report as negative, are positive in my hands.

Now, I think this is a genuine application from a contaminant, but they record this as negative, simply based on a different interpretation of the settings of the instrument. So the demonstration here is that I can get whatever result I like by varying the analysis conditions.

SPECIAL MASTER CAMPBELL-SMITH: Let me ask you, Dr. Bustin, when you said that you're making the adjustment to the right-hand side of the baseline, the stop part, that correlates to the 34 that is at the

bottom --

THE WITNESS: That's right.

SPECIAL MASTER CAMPBELL-SMITH: -- and that's the end of what looks like that sort of noise area where it looks like there begins to be some activity.

THE WITNESS: That's right.

SPECIAL MASTER CAMPBELL-SMITH: So you're subjectivity there, the subject of your interpretation, is really getting some sense of where it appears that noise has stopped --

THE WITNESS: That's right.

SPECIAL MASTER CAMPBELL-SMITH: -- and activity is taking place.

THE WITNESS: That's right.

SPECIAL MASTER CAMPBELL-SMITH: Okay. Thank you.

THE WITNESS: Because if I hadn't taken that noise into account, this threshold cycle would be further up here and make these negative. So this is where the subjectivity comes in. Now, it can go either way. As long as you're consistent, it's probably okay, but you would always be concerned about this kind of a result, and, at the very least, you're going to repeat it. This is something that has never

happened, or very rarely happens, with Unigenetics.

BY MS. BABCOCK:

Q Now, in talking generally about good laboratory procedure, you have mentioned numerous times today your concerns about contamination at Unigenetics.

A Uh-huh.

Q Do you have any idea as to how that might have occurred?

A Well, I think Dr. Ward made a very good point this morning. He suggested that every lab has problems with contamination, and that is true. Typically, the main problem with PCR is contamination. So all of us have these problems from time to time, so this not something that is peculiar to Unigenetics. That's where I would like to start off, and I'm not berating them for that.

It is important, therefore, that you always have the correct controls and the correct procedures in place, and this is where one would fault them.

Now, one of the peculiar things we noticed when we went to their setup or their laboratory was that next to the PCR handling of an instrument laboratory was a room which was labeled "Plasmid Room."

Now, if you remember, I talked about plasmids earlier on. A plasmid is a DNA molecule that you can use to replicate lots and lots of DNA molecules in a bacterium, and, typically, in this case, it was used to clone the target F or H gene into a bacterium, prepare lots of DNA, and then make RNA for the standard curves.

So, obviously, if you have hundreds of millions, or thousands of millions, of bacteria, each containing tens of hundreds of copies of DNA, you've got a massive potential for DNA contamination. So you never want to have any plasmid DNA anywhere near your laboratory where you're doing the PCR.

So it struck us as peculiar that they had a room labeled "Plasmid Room" next to the laboratory, and that plasmid room contained a shaker for growing up bugs. I asked Dr. Sheils several times, and she assured me that they did not use that plasmid room for growing up F gene target for their standards.

So this may or may not be the source of contamination, but the DNA, again, as Dr. Ward mentioned this morning, is all-pervasive. Once you've got DNA contamination, it persists for years, and it gets in everything.

If you're handling bacteria, if you're

handling plasmids, it gets into your hair, on your hands, on your clothes, and you will carry it around with you, and that is the problem.

So I could speculate all day, and I really don't want to speculate. It doesn't actually matter. The fact is that I'm showing that they are getting DNA contamination. Where it comes from is another matter. What matters is we're getting DNA contamination, and, by definition, therefore, we're not detecting measles virus.

SPECIAL MASTER HASTINGS: Ms. Babcock, I'm trying to decide when to take our afternoon break. You still have a ways to go on direct?

MS. BABCOCK: Maybe 15 to 20 minutes.

SPECIAL MASTER HASTINGS: All right. Why don't we take a 15-minute break right now?

(Whereupon, a short recess was taken.)

SPECIAL MASTER HASTINGS: We're back from our afternoon break. At this point, we're going to continue with the direct exam of Dr. Bustin. Ms. Babcock, please go ahead.

BY MS. BABCOCK:

Q Now, Dr. Bustin, we touched briefly on standard operating procedure earlier in your testimony. Why is it important, particularly with

respect to Unigenetics?

A As I tried to explain, the standard operating procedure is like a recipe so that if you follow this recipe, you expect to end up with an acceptable result, like if you're baking a cake, and you follow the recipe, you get a nice cake; if you don't, you don't.

It is particularly important for Unigenetics because there were several individuals that carried out the assays that they then reported. So reliable results means following your SOP, and I've got a nice example of where they did not follow the SOP.

What we have here is, if you remember --

SPECIAL MASTER HASTINGS: I'm on Slide 19.

THE WITNESS: So it's Slide 19. The TaqMan probe, if you remember, contains a fluorescent label that when you shine light on it, it gives off light. In addition to this fluorescent label which is on the TaqMan probe, you add a reference dye to your tube to make sure your pipeting is correct, and if it isn't correct --

SPECIAL MASTER HASTINGS: To make sure your pipette?

THE WITNESS: -- your pipeting is okay. If the pipeting isn't okay, then the standard dye you've

added will obviously vary. The instrument recognizes that and compensates for that, and this dye is called ROX.

SPECIAL MASTER HASTINGS: Called what?

THE WITNESS: ROX, R-O-X. It's just a chemical name.

SPECIAL MASTER HASTINGS: All right.

THE WITNESS: So this kind of data you can only get when you drill into the innards of the instrument. What I've done here is very simple. For one run, I looked at the ratio of the fluorescent probe to the ROX dye in the reaction tube.

The question asked is, did they have the same amount of probe into each tube, and if they have, then the ratio of FAM, which is the label on the probe, the reference dye should be the same, or more or less the same, across the plates.

Now, just one look at Slide 19 shows you that there is at least 10 or 12 different samples where they have added twice the amount of probe to the wells because the FAM-ROX dye ratio has gone up from roughly .65 to roughly 1.3.

So this is an example of, if the standard operating procedure asked them to add one micrometer of probe, then they have done that in 80 or 50 of

these samples, but for a certain number, they haven't. They have deviated from the SOP, and this is something that happens again and again and again at every level. At the level of RNA preparation, at the level of reverse transcription, at the level of PCR, at the level of analysis, at the level of interpretation, they vary from the SOP that they have generated themselves.

The effect of all of this is to give you variability. So if you have different individuals doing different things using different samples at different times, it is not surprising that your results become somewhat unreliable.

BY MS. BABCOCK:

Q Now, how should laboratory books be used during PCR testing?

A Well, laboratory notebooks are used to record what an individual does. So, typically, you date it, and you write down everything you've done with respect to an experiment. You would write down, for example, where you got your sample from, what lot of kit you used to extract your RNA, where your enzymes came from to do your reverse transcription, how much you used.

It contains this kind of housekeeping

record, which means that if you go to write up your paper, which might be a year later, you know what you've done. It means that if someone came to inspect your lab books, they could have a clear record of what you've done. It just helps you and everybody else to maintain confidence in whatever results you've got, and if there is a problem, you can go back and see what the problem might have been.

For example, you might find that a particular lot of enzyme hasn't worked, and then you can go back to your lab book and say, I used this lot on that day, and that's why this experiment failed. That is the reason you would write up a lab book, and they tend to be sacrosanct, in terms that people don't change them.

Q Now, when you reviewed the Unigenetics lab notebooks, did you observe any discrepancies or have concerns?

A Yes. At least one set of lab books was disclosed at two separate occasions, and this is Slide No. 20, and at the top you see the lab notebook page that was disclosed at the earlier time point, and, at the bottom, you see the same page from the lab notebook disclosed at the second time point.

What is highlighted in yellow are additions

that have been made to the lab book between the first and the second disclosure, which is highly unusual, and, really, from what I told you, to begin with, is something that you shouldn't do because why would you change an entry in your lab book after a certain amount of time? This makes you worried about the reliability of any results obtained from this particular experiment.

What is interesting is that, if you look at the very bottom line of the second lab book, it mentions A-10 tipped. Now, "tipping" refers to taking the end of your pipette tip and brushing it against a reaction vessel and perhaps getting a false positive because of that, because you put a little bit of your reaction mix into the wrong tubes; you've tipped the tube.

So why they have done this, I don't know, but all I can do is report the results of, in this particular case, this particular entry from two different disclosures. There are other examples of this.

Q That was going to be my follow-up question: This was not the only example of discrepancies.

A No. I would like to take you through a rather peculiar alteration that I observed. So this

is typical of the kind of experiment report I was referring to earlier on. The instrument tells you the day --

SPECIAL MASTER HASTINGS: We're looking at Slide 21.

THE WITNESS: Slide 21. The instrument tells you the day of the run, the experimental conditions of the run, and then lists the wells and what's in the wells. So this is the record that you'll analyze, and, in fact, all of these were disclosed to us, and this is the kind of thing where there were discrepancies where I became worried about the quality of the assays and went on to drill deeper into the actual runs, the raw data, basically. Could we blow up the next slide?

So let me explain this to you. The operator sheet, which is the lab book, told me that every single one of these samples was run as a triplicate. So they used three identical tubes per sample. If you look at this Slide 22, you can see that a well, B-1, B-2, and B-3 have a one, one, one next to it. Wells B-4, B-5, and B-6 have a two, two, two. So B-7, B-8, and B-9 have three, three, three, and so on, down to the no-template control, which, as you can see, is negative here and is also run in triplicate.

So there is no problem with this. If you now look at this column here --

BY MS. BABCOCK:

Q So let's be clear. That's the one, two, three, four -- fifth column from the left.

A Yes. It starts at 18.16. If you go down the first three, you see 18.16, 18.38, 17.90. They are very close together. So this confirms the triplicates in the first tube. If you go down the column, again, you can see that they tend to go in triplicates. They all look very similar.

Next to the 18.16, you see something, 6.0_e + 05. This is scientific notation and means six there are times 10-to-the-five copies of whatever target there was. That's what the instruments calculated.

SPECIAL MASTER HASTINGS: Where are you, again, Doctor?

THE WITNESS: Here, 6.0.

SPECIAL MASTER HASTINGS: Okay. The next column.

THE WITNESS: Yes. If you go down the columns, you can see that the copy numbers are calculated out very close together, confirming that, in fact, there are triplicates.

The column next to that, two from the right,

shows the standard deviations that the machine calculates, and, again, if you look at the data, they always go in triplicates. So the first three are the same, the second three are the same, the third three are the same, and so on.

So have I made myself clear?

SPECIAL MASTER HASTINGS: Yes.

THE WITNESS: So, from the operator sheet, from the lab notebook, and from the experimental report that the instrument has put out, there is no doubt that we're looking at a run that has triplicates.

Now, the way Unigenetics submitted their data to the Court was that they had individual cases, they submitted the experimental report, which is this, and, say, for Patient 8, they would box in the results that are showing, the data they got for Patient 8.

So what I've written onto this is this squiggly arrow here, a question mark, and these brackets here. What Unigenetics have written: They have drawn this box here.

SPECIAL MASTER HASTINGS: They have drawn the horizontal line.

THE WITNESS: They have drawn the two horizontal lines and labeled it number eight -- that's

their writing -- suggesting that this is the sample submitted for Patient No. 8.

They have also drawn viral cells, one-over-100 here. Okay?

SPECIAL MASTER HASTINGS: All right.

THE WITNESS: Viral cells, if you look back to the Uhlmann paper, are the cells that they infect with measles virus to get a positive control, so that's a positive control.

So what Unigenetics have submitted here is something that, by all measures that I've been able to ascertain, are triplicates, but they have boxed in a duplicate, labeled it number eight, and called this the "patient sample." But what they have left on this experimental report is highlighted in yellow: "Posit C."

Now, I have to interpret here, and I may be wrong, but I would call that "positive control." Now, what I think this represents is a test run for the positive control, which has been run in triplicate, and five, five, five is one sample, and six, six, six is the other sample. I don't believe that this is Patient Sample 8. I believe this is a control sample.

So this is the kind of strange results that were submitted.

SPECIAL MASTER HASTINGS: When you say "submitted," did you say "submitted to the Court"?

THE WITNESS: I'm very unclear about legal terms.

SPECIAL MASTER HASTINGS: Okay.

THE WITNESS: This is what they sent as part of the evidence to support their results, and I got this from the solicitors in a folder as their submissions for Patient No. 8. I don't know what the right legal term for this is.

SPECIAL MASTER HASTINGS: So you understand that this is what they submitted in the U.K. litigation.

THE WITNESS: Yes.

SPECIAL MASTER HASTINGS: All right.

THE WITNESS: Yes. This is in my report that you have in front of you.

So let me just repeat: The horizontal lines on the number eight and the viral cells, one-over-100, are marked by Unigenetics when they submitted this as evidence. I don't know what you call it.

But, in my opinion, from all of the evidence I have, the underlying evidence suggests that this is not Patient No. 8. There is a positive control.

//

BY MS. BABCOCK:

Q Now, is this the only instance of discrepancies or, I guess, concerns with the operator sheets that you observed when you reviewed them?

A This is the only one that I put into my report.

Q Now, to your knowledge, was Unigenetics ever accredited?

A No. To my knowledge, they were not.

Q Could this be part of the reason some of these problems weren't detected earlier?

A Yes. I'm sure that is true. I also believe that Dr. Afzal tried to recruit Unigenetics into a quality-control program, which involved various laboratories in Europe; the United States, I don't know; and Unigenetics refused to take part in this.

So there was never any independent quality assessment made of any of the work that was carried out by Unigenetics. Because Ireland is not part of the U.K., so I think that the legal requirements are different there. So there's various reasons why this has never been looked at before.

Q Now, we've outlined quite a few concerns, some more significant than others, I'm sure. Based on your expertise in PCR, if you had to pick a top two or

name your top three, what are the most substantial concerns here, based on your analysis?

A I think that there is clear evidence that what Unigenetics are detecting is DNA, a DNA contaminant, and, by definition, this cannot be measles virus RNA. The evidence -- this is Slide 23, which repeats one of the previous slides. If you compare the F gene results from frozen tissue to formalin-fixed tissue, you get the same result in terms of CT, whereas Professor O'Leary's own control results show that he gets a higher CT for his control GAPDH.

So if GAPDH, which is RNA and which no one disputes is RNA, gives you a higher result, and the F gene doesn't give you a higher result, this cannot be RNA. So this is the first thing I would like to point out.

This is reinforced by the fortuitous result where they look at the RNA, identify a problem with the RNA because it doesn't amplify the internal gene, GAPDH, but, nevertheless, go on to use the F gene results. So the degraded RNA again gives the same quantity of F gene target as the high-quality RNA. Since this, by definition, is degraded RNA, the amplification target must be DNA.

And the third point here is the next slide, which, if you remember, compares the results obtained in the F gene run -- this is Slide 25 -- where they forgot to add an RT step to all of the other runs. In the absence of an RT step, you get very inefficient amplification of RNA. You would not expect to get, more or less, the same CTs.

So all of this evidence suggests very, very strongly that what they are detecting is DNA and not RNA. Because measles virus doesn't exist as a DNA molecule in nature, they cannot be detecting measles virus RNA. They are detecting a contaminant. All of the additional evidence, from the nonreproducibility by Professor Cotter of the same samples that Unigenetics analyzed to the analysis of the data where there are discordant positives, where the negatives came up positive, suggests very, very strongly to me that there is a lot of contamination in the laboratory, which is not unusual, but they have not handled it very well in how they have troubleshot their problems.

So I have very little doubt that what they are detecting is a DNA contaminant and not measles virus, and I do not believe there is any measles virus in any of the cases they have looked at.

Q Now, we know that cerebral spinal fluid samples were sent from Dr. Bradstreet to Unigenetics for testing. Do the same concerns you've outlined here apply to that testing?

A Yes. Exactly the same concerns would apply to that.

Q So, overall, based on your professional experience and expertise in PCR, your personal examination of the methods and testing used by Dr. O'Leary in the Unigenetics laboratory, do you feel that that laboratory was able to reliably identify measles virus RNA?

A No, I don't.

Q Does this opinion extend specifically to the Unigenetics result from Michelle Cedillo?

A It does.

Q And you hold this opinion to a reasonable degree of medical certainty, scientific certainty.

A I do.

MS. BABCOCK: No further questions.

SPECIAL MASTER HASTINGS: Thank you, Ms. Babcock.

Ms. Chin-Caplan, do you have some questions for this witness? Please go ahead.

//

CROSS-EXAMINATION

BY MS. CHIN-CAPLAN:

Q Hello, Doctor.

A Hello.

Q Doctor, do you have a Web site?

A I do, yes.

(Pause.)

MS. CHIN-CAPLAN: We'll just move on until we get our technical difficulties resolved.

(Discussion held off the record.)

BY MS. CHIN-CAPLAN:

Q So, Doctor, just to be sure that I understand what you're saying, on page 10 of your presentation of your slides, you indicated that the red on the slide --

A I'm sorry?

Q -- the red on the slide --

A Yes.

Q -- was outside of the expected -- I'm going to say "curve," but that's probably not right.

SPECIAL MASTER HASTINGS: Which slide are we looking at?

MS. CHIN-CAPLAN: We're looking at page 10.

SPECIAL MASTER HASTINGS: Number 10.

MS. CHIN-CAPLAN: Yes.

BY MS. CHIN-CAPLAN:

Q Now, Doctor, is this the Y axis?

A Yes.

Q Okay. And this is the X axis along the horizontal plane. Correct?

A That's right.

Q Okay. And, Doctor, on the Y axis, it talks about threshold cycles. Is that true?

A That's right.

Q So is that an indication of how many times the experiment is run, how many cycles it goes through?

A How many PCR cycles there are, yes.

Q Is there a standard number of PCR cycles that's acceptable within the profession?

A By convention, most people tend to use 40 cycles, but some people go up to 45 cycles, but convention, 40 is kind of what is used, yes.

Q Forty?

A Yes.

Q So, Doctor, convention is 40. They ran it at approximately 40 -- correct? --

A Yes.

Q -- that very first number here.

A Yes.

Q Yes. And, Doctor, underneath it, which would be the X axis, it has 10-to -- is it 10 to the one, 10 to the two?

A That's 10 copies there, yes.

Q So that's an indication of the copy numbers. Is that it?

A Yes.

Q Doctor, they had to run this experiment 40 times to get 100 copies. Is that it?

A No. You've put your RNA into a tube, and you have done your PCR run, and you've done 40 cycles.

Q Okay.

A The CT that has come up is roughly 39, say.

Q Okay.

A The software based on the standard curve has now placed the 39 CTs in a position of roughly two copies, suggesting there's two copies there, yes.

Q Okay.

A That's how it works.

Q Okay. So, Doctor, it seems like there is a problem with the low copy numbers and the higher cycle numbers.

A That's correct.

Q And that would make sense, wouldn't it?

A Yes.

Q That's what you would expect to see.
A Yes.
SPECIAL MASTER HASTINGS: You need to state your answer rather than --
THE WITNESS: Sorry. Yes, yes.
BY MS. CHIN-CAPLAN:
Q So, Doctor, could we just go to Slides -- I'm going to look at 13, 14, 15. Doctor, on these slides here --
A Could we take them one by one so I --
Q Okay. Why don't we take them one by one? On Slide 13, there is this black line across -- it looks like about one. Is this black line the threshold?
A Yes.
Q And the threshold is where you would detect the presence of measles RNA.
A These two are not equivalent. These are completely different scales.
Q Yes, I know, yes, yes.
A Yes. This is the line where the instrument, by definition, first detects reliably, above background, the amplification product, yes.
Q Doctor, again, your dispute here --
A I'm sorry. My what?

Q Your dispute here with Unigenetics involves the numbers that are in the green area. Is that it?
A Not the numbers; the shape of the curve.
Q The shape of the curve.
A Uh-huh.
Q Okay. Doctor, the shape of the curve, again, seems to involve low copy numbers. Is that it?
A Yes.
Q And they are the copy numbers that hover at or above the level of detection.
A Uh-huh.
Q Correct?
A Yes.
Q So, Doctor, if we go to page 14 --
A This one?
Q -- yes, 14 -- again, the black line represents the threshold.
A That's correct.
Q And, on B, the threshold is actually at the top of the graph. Correct?
A Uh-huh.
Q And the shape of the curve here is, again, what you're disputing.
A No.
Q No?

A I think you can see the clear difference between going up and nothing happening.

Q Okay.

A That's what I'm saying. This suggests amplification --

Q Okay.

A -- because it's going up in a straight line continuously. This suggests spurious background because it's coming on straight.;

If you remember what PCR is, it's exponential amplification, so one, two, four, eight.

Q Yes.

A So if that doesn't happen, you get a straight line.

Q Okay. But, again, Doctor, your dispute seems to be with the copy numbers that appear just around threshold.

A My dispute appears to be with very high CT positives, yes.

Q High CT positives. Does that mean the high number of cycles?

A Yes.

Q And that would make sense.

A Uh-huh.

Q So if you have a high number of cycles, it's

like sending something through a Xerox machine 20 times to enlarge it, and every single time you send it through, it gets bigger and bigger, but it becomes less clear and less clear. Is that correct?

A I can accept that, yes.

Q Yes. Okay. So that's a proper analogy.

A Yes.

Q So if there was a positive at a lower cycle --

A A lower cycle, yes.

Q -- would that support your opinion that that's probably a reliable result?

A In general, what people believe is that CTs below 35 are acceptable. Anything above 35 makes them slightly concerned, if you haven't added the proper controls.

If you have proper controls, and this is where the standard curve comes in, and that's why it's essential that the standard curve dynamic range encompasses all of your unknowns.

If your standard curve encompasses something that's 39, and your standard curve ends at 39 reliably, then you can believe that result. If your standard curve ends at 3,000, you can't believe that result.

Q Okay. So the concern really is with low copy numbers and high cycles, high number of runs.

A Yes.

Q And these are the graphs that you've shown us.

A Yes.

Q What about a high copy number?

A Uh-huh.

Q Is there anything wrong with a high copy number?

A There can be. Can I?

Q Certainly.

A If you look at the figure I showed you from my lab, which is Slide 12 -- have you got that? If you look at the first slope that is slightly horizontal, you can see there is a green line just next to it. Can you see that?]

Q Are you referring to this line?

A My eyesight is terrible. There is this -- I can point to the --

SPECIAL MASTER HASTINGS: Can you point up there, Doctor?

THE WITNESS: You can see this green line here. Can you see it?

MS. BABCOCK: I do.

THE WITNESS: Now, if you look at the CT here, that's below 35.

MS. BABCOCK: Okay.

THE WITNESS: It's about 33.

MS. BABCOCK: Okay.

THE WITNESS: So that is not a low copy number. That's medium-to-low, but it's acceptable, and you can see, that's looking odd. It is quite possible, and I haven't got it here, but it is possible to get funny results here as well.

It is not necessarily a condition of an odd run, an odd amplification plot, to have very low copy numbers, but, in general, lower copy numbers give you poor results.

MS. BABCOCK: Okay.

THE WITNESS: I can point to this one here, which actually is quite a nice plot, and you can see, it doesn't even come up here. It's a very, very little amount of target there, but, nevertheless, it's an acceptable amplification plot.

You can't generalize. I think you're right to say, if you have high CTs, you tend to be concerned, and you're right there, yes.

BY MS. CHIN-CAPLAN:

Q Okay. What if you have high copy numbers?

Would they be considered accurate?

A By "high copy numbers," I mean low CTs.

Q Yes.

A In general, what you find is, because you run duplicates, when you have low CTs, they are very concordant, yes.

Q Very concordant, meaning they are accurate?

A Yes.

Q Okay. Doctor, I'm going to try this one more time. Is this you?

A Yep.

SPECIAL MASTER HASTINGS: You're showing from his Web site?

MS. BABCOCK: This is Dr. Bustin's Web site, yes.

SPECIAL MASTER HASTINGS: Okay.

BY MS. CHIN-CAPLAN:

Q Doctor, is your Web site sponsored by somebody?

A Well, by "sponsoring," what happens is this is a British company that pays 4,000 pounds to my Ph.D. student, who is a Chinese person and has no support. He pays for himself. So they have kindly agreed to pay 4,000 pounds. In return, I've put that on my Web site, yes.

Q It's Quantace. Am I pronouncing that correctly?

A Quantace, yes.

Q What is Quantace's business? What do they do?

A They produce kits for PCR.

Q Kits for PCR?

A -- PCR, yes.

Q The things that we're talking about right now?

A Yeah.

Q Doctor, are they primers? Is that it?

A Are they what?

Q Are they primers?

A You mean, do they produce primers?

Q Yes.

A No. They produce kits: restriction enzyme, reverse transcriptase, master mixes, or nucleotides, that kind of thing.

Q So some of the experiments that we've been talking about, they produce kits for.

A In principle, yes, but not for the ABI, obviously, uh-huh.

Q Not for? I'm sorry?

A Not for the TaqMan instrument that we were

talking about for the --

Q Okay. Now, Doctor, if we look under Quantace, it says: "Our ever-expanding product range. Our SensiMix reagent is unique, as it can be used both for probe- and for cyber-based assays."

A Uh-huh.

Q "Each product from the SensiMix range comes from a separate viral ausiry green one, which can be added to the mix when this type of chemistry is desirable."

A Right.

Q And, Doctor, does Quantace provide a free sample to a lab who requests it?

A I think they have special sample packs, yes, but I think all companies do that, or most companies do that, yes.

Q Okay. It says: "We strongly believe in a philosophy of try before you buy and are happy to offer free samples of our SensiMix reagent for testing."

A Uh-huh.

Q So they do offer a free kit if you want a free kit.

Doctor, is this a private company, or is it a public company? Is it on the stock exchange?

A No. It's a very small company.
Q A very small company?
A I presume it's a private company. I don't actually know, but, yes, I presume it's private. It's not stock market listed.
Q Do you have any financial interest in this company?
A No.
Q So what they do is they --
SPECIAL MASTER HASTINGS: He shook --
THE WITNESS: I'm sorry. No, I do not. I do not know.
BY MS. CHIN-CAPLAN:
Q So you don't get any profits from every single kit that's sold.
A I wish I would.
Q You would be a rich man.
A No, I don't.
Q Now, Doctor, there was just some brief discussion about your work in the U.K.
A Uh-huh.
Q I think you indicated that you spent roughly 1,500 hours at 150 pound sterling.
A That's correct.
Q What was the exchange rate at that time? Do

you know?

A I don't know. It's 190 now. It was less then. At the moment, it would be roughly $400,000, wouldn't it?

Q So if I remember correctly, you said you received 225 pounds sterling.

A It was roughly 220,000. I can't remember the exact number, but it's that ball park figure, within 10,000, I'm sure.

Q Today's exchange rate, right now, that would be the equivalent of $450,000.

A Yes.

Q We know it was a little less back then, wasn't it?

A It makes no difference to me because I don't deal in dollars. I got 225,000 pounds.

Q Frightfully expensive.

A I agree. I feel uncomfortable about getting these types of sums. However, I feel that I was asked to do a job, and I think I've done the job very well, in my own opinion, and I cannot put an explanation for the problems associated with the Unigenetics results. So I think that's not unreasonable. I would have done it for less.

Q May I ask you what you're getting paid right

now for this testimony?

A Yes, sure. It's about 60,000 pounds.

Q Sixty thousand pounds. At today's exchange rate, that would be $120,000.

A Sorry? Yeah, that's right, but I get taxed at 40 percent plus --

Q For me, in the U.S., that's roughly $120,000. Is that it?

MR. MATANOSKI: I don't think that that's accurate.

THE WITNESS: What isn't? It doesn't matter.

(Laughter.)

MR. MATANOSKI: For the record, Professor Bustin will be paid at the same rate that our other experts are paid.

THE WITNESS: Sorry. I misunderstood the question. I thought you said, what I'm being paid at work. I misunderstood your question. I'm sorry. I misunderstood the question. I thought you asked me what my salary was.

(Laughter.)

THE WITNESS: Could you repeat the question, please? Sorry. I didn't mean to give you a heart attack.

SPECIAL MASTER HASTINGS: Mr. Matanoski, any excess will come out of your salary.

(Laughter.)

SPECIAL MASTER HASTINGS: Go ahead, Ms. Chin-Caplan. Why don't you ask it again?

THE WITNESS: I'm sorry.

BY MS. CHIN-CAPLAN:

Q I guess, for the record, we should be clear. What are you being paid for this particular hearing?

A It's $250 an hour while I'm here and $125 while I'm traveling, and nothing while I'm sleeping, I think. And also my airfare and hotel is being paid for.

Q The U.K. litigation; was that paid for by the government or by the pharmaceuticals?

A I don't know. What happened was the instructions came from the solicitors, and I got the checks from the solicitors. I assume it was the companies that paid for me, but I actually never -- I didn't ask. I was happy to get the check.

Q When you say "company," do you mean the pharmaceutical companies?

A Yes. There's Merck, Aventis, and GSK.

Q Thank you. Now, Doctor, I just want to be clear. You talked about GAPDH?

A Yes.

Q Is that a control?

A Yes. Do you want me to expand or you just ask me?

Q Certainly.

A Which way do you want me to do it?

Q You should expand.

A GAPDH is a cellular gene. So a measles virus is a virus, an extra-cellular organism. GAPDH is a cellular gene. So every cell in our body expresses this gene. So it is a very useful thing to use as a measure of whether an RNA sample, if extracted, contains RNA.

Because, as I tried to explain, but obviously I didn't quite get it right: If there is no GAPDH in your RNA, you would conclude that there is a problem with the RNA sample, because there should be GAPDH there. So you'd be concerned with any result you obtained if you had not got a GAPDH positive result.

Q Okay. But you consider GAPDH to be an adequate control, is that it?

A It depends what you're using it for. In this situation, yes. If you're using it to normalize it against a cellular gene, no. But these are two

separate issues.

I think in this very specific situation, if I understand you correctly, you're asking me: Do I consider GAPDH as an adequate control to test for the presence or absence of RNA, I would say: Yes.

Q Thank you.

A Can I add to this?

Q Sure.

A And that's, of course, while the SOP from Professor O'Leary's lab has used this to say: If a result is GAP negative, that sample should be discarded, so that's the reason behind that.

Unfortunately, of course, in certain segments, we continue to use those samples.

Q Okay. Now, Doctor, I'm not sure I've read this properly, so I'd like you to look at it for me. This is your June 30, 2003 report.

A Okay.

Q On page 41, paragraph 6.11, it says: The reported concordance between GAPDH replicates is generally good.

A Yes.

Q This is true even for those samples where cycles are high.

A Hmm.

Q So am I understanding that when you use GAPDH as a control, when you evaluate the O'Leary lab, it's concordance with the samples, even when the cycle number was high, was good?

A Yes --

Q Have I interpreted that correctly?

A Do you want an yes/no answer, or an explanatory answer, I'm not sure.

Q I want you to explain. I need to understand.

A I'm sorry. I'm just not familiar with the procedure.

SPECIAL MASTER HASTINGS: Can you ask the question again?

MS. CHIN-CAPLAN: Certainly, I think.

SPECIAL MASTER HASTINGS: I've read the quote, but --

BY MS. CHIN-CAPLAN:

Q Doctor, are you saying in this paragraph that the concordance between the control in the sample, when the cycle number was high, was good, even though the cycle number was high?

A If you have a good assay, even if your Ct's are very high, you will get good concordance at those high Ct levels.

So this suggests that the assay to test for GAPDH was a reasonably good assay because you were getting concordance at the high Ct levels, unlike the F gene where you weren't getting the concordance. So one assay good, one assay not so good.

Q So this refers to the GAPDH?

A That's right.

Q When the cycle number is high, would that also include low-copy numbers?

A By definition, a high-cycle number means high-copy number, so a low-copy number it means low-copy number.

Q Doctor, you spoke about the international collaborative study that was done with Dr. Afzal.

A Yes, I just -- all I said was that I believe that he initially when invited by Dr. Afzal to participate, and he did not. That's all I know.

Q And there were a number of other labs that didn't participate as well, correct?

A I really don't know.

Q Okay. I'm going to ask you to take a look at Petitioners' Exhibit 63, Tab B, 63 Tab B? You don't have it?

MR. MATANOSKI: It's one of your exhibits?

MS. CHIN-CAPLAN: Right.

MR. MATANOSKI: I didn't bring it.

MS. CHIN-CAPLAN: You mean you didn't bring my exhibits.

SPECIAL MASTER HASTINGS: 63, Tab B, did you say?

MS. CHIN-CAPLAN: Tab B.

SPECIAL MASTER HASTINGS: Tab B.

MS. CHIN-CAPLAN: As in boy.

SPECIAL MASTER HASTINGS: All right.

MR. MATANOSKI: Actually, we do have a copy of that.

MS. CHIN-CAPLAN: Great.

BY MS. CHIN-CAPLAN:

Q I'm looking at the results on page 173. Doctor, it says that originally thirteen laboratories had an established a record of measles virus --

SPECIAL MASTER HASTINGS: Can you go to the beginning of the results section?

MS. CHIN-CAPLAN: Yes, the beginning of the results. Let me begin again. I've lost my place.

BY MS. CHIN-CAPLAN:

Q Originally, thirteen laboratories that had an established record of measles virus RT-PCR wee invited to participate in the study. Of these thirteen laboratories, four did not show interest for

the study; one received the samples, but did not complete the study; and one responded positively in the beginning but failed to cooperate later.

So, of the 13, six, almost half, chose not to cooperate.

A Yes.

Q So, Doctor, further on in that paragraph, it says: "In total, seven laboratories completed the study successfully. The results of RT-PCR or nested PCR assays reported by various laboratories are summarized in Table 3. Despite technical differences between the assays, the findings were mostly in agreement. Of the samples supplied, no laboratory detected measles virus sequence in any of the Crohn's disease patients with the exception of Laboratory 5, which reported an ambiguous result for Sample A."

Doctor, when you go back to page 172, does it identify who Laboratory 5 is?

A Yes.

Q Who is it?

A Judy Beeler, Laboratory of Pediatric & Respiratory Virus Diseases.

SPECIAL MASTER HASTINGS: Can you say that again? I didn't hear it.

THE WITNESS: Sorry. Laboratory of

Pediatric & Respiratory Virus Diseases in Bethesda, Maryland.

BY MS. CHIN-CAPLAN:

Q And the establishment was known as CBER/FDA, wasn't it?

A Yes.

MS. CHIN-CAPLAN: I don't think I have any further questions, Special Master.

SPECIAL MASTER HASTINGS: All right. Give me a moment here, Doctor. No, I think all my questions have been asked as well.

Any redirect for this witness?

MS. BABCOCK: Just briefly, Special Master.

SPECIAL MASTER HASTINGS: Okay. Go ahead.

REDIRECT EXAMINATION

BY MS. BABCOCK:

Q Now, Dr. Bustin, for your work in the UK, we know you were compensated for the substantial amount of time you spent. Again, this is a bit of repetition. Was it expected that you were going to reach a certain conclusion, or were you told you should be independent?

A No. I think I've already said that. Everyone made it very clear to me that my prime duty was to establish truth for the court, and I've always

assumed well, I know that it was genuine and I've always followed that instruction.

I've given my opinions and my conclusions based on the actual data that I have seen and analyzed. And I think I've shown you in the genetic's own data, so these aren't my data, my interpretation of the data. These are actually their data. So that's the conclusion any reasonable person, who looks at that data, would come to.

Q Now, Michelle Cedillo's biopsy sample results, if taken at face value, has a high-copy number?

A Yes.

Q Have you outlined reasons today why you might doubt the validity of that result?

A Well, to explain, I can't. The way you obtain a copy number, as you see, is by running a standard curve. So you make an RNA, and then you work out how much RNA you've got. You know the length of the RNA molecule you've got; and knowing those two parameters, you can work out that you've got 15,000 copies of RNA in your tube.

So you say: My first tube contains 15,000. Then my second one would have contained 1,500, 150, 50 and then so on. So the copy number you obtain is

determined entirely by your calculations of copy numbers based on your standard curve.

Did I make that clear?

SPECIAL MASTER HASTINGS: No.

THE WITNESS: I'll try again.

SPECIAL MASTER HASTINGS: No, not to me.

THE WITNESS: Okay.

RECROSS-EXAMINATION

BY MS. CHIN-CAPLAN:

Q You're saying this is a very subjective process?

A No, I think it's important that I get this right. A standard curve is used to qualitate copy numbers. This is one of the powerful aspects of real-time PCR.

The way you prepare the standard curve is: You make RNA, then you qualitate the RNA. Let's say that you've got ten nanograms of RNA. You know the length of the RNA that you're trying to qualitate because that's your target.

Because you know the length and the weight of the RNA, you can work out how many molecules there are plus or minus, you know, 200 percent probably in your test tube.

So let's assume we've made RNA. In our

concentrated first tube, we've got 100,000 copies of RNA. There are 100,000 copies of RNA not because there are 100,000 copies of RNA, but because I've calculated that there is 100,000 copies based on what I know about it, but I could be out by 100 percent. There could be only 50,000 copies, or there could be 150,000 copies.

I now take that very first tube and dilute it one-in-ten, which gives me the second point on my standard curve. I dilute it again one-in-ten, and so on.

So I end up with a standard curve that started the 100,000 and ends at ten copies. I now run my unknown, and because I've defined myself what the standard curve quantification is, I can then work out the copy number of my unknown. But if I hadn't called it 100,000, if I've called it a million, I would have ten times more of my unknown.

If I've called it 10,000, I would have ten times less. So it is a very subjective measure of the actual copy number that is there unless I know how I have calculated it, unless you know how I have calculated my copy numbers.

So, simply saying: And Gene X is expressed at 150,000 copies, without any further information, is

meaningless because I do not know whether I can file new calculations.

And from the evidence I've shown you in terms of the Ct's, that unit genetics obtaining for their samples, you can see because we've just discussed this with Ms. Chin-Caplan, they're always very, very high. They're always borderline.

A copy number of 1.5 times ten-to-the-three would suggest a fair number of copies I have a fairly lowish Ct, although still in the 30s, but, nevertheless, not a borderline CT.

Is that clear?

Q Let me ask the question this way, Dr. Bustin. If you don't have copies of that standard curve that's set up, can you have confidence in the copy number, or the results --

A No.

Q -- for a particular run?

A No, you can't, not in the quantification.

Q Okay. So that's dealing strictly with quantification --

A Yes.

Q -- of the amount of RNA --

A That's correct.

Q -- in your sample? It is not whether the

sample is positive or negative?

A That's a different question.

Q So we're dealing with two separate analyses?

A Yes, yes.

Q Quantification -- well, first, you would give it a positive, I suppose. And then you would say: How many copies if there is a positive result?

A That's right. It is always safer to say that something is there or not there, than to not say: Well, you know, there is 5,025 copies. It's always safer to say yes or no.

Q Now, in addition to the problems with the standard curve, in a lab that you know or strongly suspect has significant problems with contamination, are any of the positives reliable?

A I have seen gene positives in cases -- reliable, is that the question?

Q Yes.

A Or any positives when some occurred, for example?

Q A positive result when you know that there is a problem of contamination?

A If you have a lot of copy numbers, for example, the high-copy numbers in your standard curve, they are reliable because contamination typically

occurs at the higher end of the spectrum, so Ct's of 35 onwards.

If you have a Ct in your standard curve, for example, of 15, you'll believe that. If you have a Ct of 25, you probably still believe it. But if the Ct in your unknown is 35, and the Ct in your negative control is 35, then clearly you don't know what is what.

We have a rule-of-thumb: If there's a difference of 5 Ct's between a positive and a negative control, and the lowest Ct that your unknown has, you might want to believe it, but we would still recommend that you repeat the assay.

But anything closer than 5 Ct's, you would have to report it as a positive in the NTC. So, in principle, if your NTC is positive, you always doubt your assay. If your unknowns are at very high Ct's, you also doubt your assays; and you would certainly repeat it.

There is a very neat trick you can use to confirm the validity of the result, the quantitative result. Because we know from the PCR, if you double the amount of input, you reduce your Ct by one because ECT is a doubling.

So if I find that I'm getting unreliable

results at Ct's of 35 say, I would simply double or triple the amount of RNA that I put into my assay. What I would expect to see is that the Ct will go down by one or two, it could have doubled or tripled the amount of input of DNA.

If I see that, if I see the response that I expect, I would have more confidence in my data. And if I add ten times the amount of RNA and I still don't get a difference; or if I use denatured RNA, or formerly fixed RNA, and always get the same result, that is such classical evidence of contamination, that is just so typical of contamination.

So any high Ct is suspect, but there are ways of working on whether something is right or not. And you can use multiple curves, which you referred to earlier today; you can use sequencings which they call standard, which you should have done.

But, in the absence of all of that, you look at your controls. If your negative control is positive, at a Ct that is close to your unknown, you get worried.

Q Dr. Bustin, I'm just trying to keep up with the substrate, NCT?

A NCT.

SPECIAL MASTER HASTINGS: I think you said

NTC.

MS. CHIN-CAPLAN: I'm sorry, thank you.

THE WITNESS: No template control.

MS. CHIN-CAPLAN: Okay, thank you.

MS. BABCOCK: I don't have any further questions.

SPECIAL MASTER VOWELL: Your comments there raised this issue. What we're seeing in Michelle Cedillo's lab results from the Unigenetics Lab is a high-copy number?

THE WITNESS: Yes.

SPECIAL MASTER VOWELL: Does that mean that because there's a high-copy number that there were a large number of runs?

THE WITNESS: Of Ct's you mean?

SPECIAL MASTER VOWELL: Yes, of Ct's.

THE WITNESS: We can't tell because we haven't got the information. It is entirely subjective.

SPECIAL MASTER VOWELL: That's what I thought I understood, but I wanted to make sure.

THE WITNESS: It would have been better to record a Ct and a copy number with a standard curve. That would have been the ideal way to do it.

SPECIAL MASTER VOWELL: Thank you.

SPECIAL MASTER HASTINGS: Anything further for this witness?

MS. CHIN-CAPLAN: Just one last question.

SPECIAL MASTER HASTINGS: Go ahead.

FURTHER RECROSS-EXAMINATION

By MS. CHIN-CAPLAN:

Q Doctor, do you have any personal knowledge of Michelle Cedillo's sample?

A You mean have I analyzed it?

Q Yes.

A I have no idea. I don't know because I -- I can't tell you that because I just don't know. There is a list of names I suppose, but a: I can't refer to them; and b, I have no idea -- I never was interested in the names obviously of the people. I was just looking at basically numbers, looking at the samples. I have no idea.

Q Okay, thank you.

SPECIAL MASTER HASTINGS: All right. I take it that that concludes the testimony for today.

Tomorrow, I understand we have Dr. McCusker and Dr. Hanauer, is that correct?

MR. MATANOSKI: Yes, sir.

SPECIAL MASTER HASTINGS: Can you tell us which will go first?

MR. MATANOSKI: I believe it's going to be Dr. Hanauer first, sir.

SPECIAL MASTER HASTINGS: All right. Then, just for those who are following along from home, my understanding then is that on Friday we're planning to hear very briefly from Dr. Chadwick in the morning; and then Dr. Brent after that.

MR. MATANOSKI: That's correct, sir.

SPECIAL MASTER HASTINGS: And Monday, it's still Dr. Griffin and Dr. Fombonne.

MR. MATANOSKI: Correct, sir.

SPECIAL MASTER HASTINGS: All right. With that, we are adjourned for today. We're going to start again at 9:00 a.m. tomorrow morning.

Thank you all.

(Whereupon, at 4:45 p.m., the hearing in the above-entitled matter was adjourned, to reconvene Thursday, June 21, 2007, at 9:00 a.m.)

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REPORTER'S CERTIFICATE

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HEARING DATE: June 20, 2007

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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 Office of Special Masters.

Date: June 20, 2007

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