Respondent's Exhibit QQ

IN THE UNITED STATES COURT OF FEDERAL CLAIMS
OFFICE OF SPECIAL MASTERS

THERESA CEDILLO and MICHAEL CEDILLO, as Parents and Natural Guardians of MICHELLE CEDILLO,	)	
Petitioners,	)	No. 98-916V
v.	)	Special Master George L. Hastings, Jr.
SECRETARY OF HEALTH AND HUMAN SERVICES,	)	
Respondent.	)	

DECLARATION OF NICHOLAS CHADWICK, PhD
UNIVERSITY OF MANCHESTER

1. From October 1994 until January 1998, I worked for a PhD under the supervision of Dr Andrew Wakefield at the Royal Free Hospital School of Medicine, Faculty of Medicine, University of London, and under Professor Ian Bruce of the University of Greenwich. I was awarded my PhD in 1998. I am currently a post-doctoral research associate at the University of Manchester.

2. I completed my undergraduate degree in biochemistry at Durham University in 1993. After reading a newspaper article about Dr Wakefield's work on Crohn's disease, I went to talk to him at the Royal Free Hospital about his work. He offered me a job, and I worked for him as a technician for a year from October 1993 before starting a PhD under his supervision in October 1994.

3. I worked on Crohn's disease for seven years, starting in October 1993 at the Royal Free Hospital and continuing at the University of Oxford, where I worked on the immunology of Crohn's disease in the laboratory of Professor Derek Jewell at the John Radcliffe Hospital until 2000. Crohn's disease is one of the two most common inflammatory bowel diseases.

4. At the time when I arrived at the Royal Free Hospital, Dr Wakefield had found that granulomas in the gut in Crohn's disease patients were blocking the blood supply and causing damage to tissue. Dr Wakefield was looking for viruses which gave rise to these granulomas; however, it was not clear to me at this time why Dr Wakefield was focussing his search specifically on measles virus. During my first year (1994), whilst working as a technician for Dr Wakefield, I used two laboratory techniques, in situ hybridisation and in

situ PCR, to look for measles virus RNA in gut tissue samples from patients with Crohn's disease. I was trying to repeat the work carried out in 1993, which was published in Wakefield et al. "Evidence of persistent measles virus infection in Crohn's disease" Journal of Medical Virology 39(4), 345-353. I also used a technique called NASBA (Nucleic Acid Sequence Based Amplification), which is an isothermal RNA based amplification technique. Most of these experiments were carried out with Dr Wakefield and a colleague named Ronnie Ray.

5. I was very enthusiastic about the work to begin with, but began to have doubts as time went on. These doubts were mainly about the use of immunohistochemistry tests, in particular the specificity of the antibodies we were using to detect measles virus. Also, the in situ PCR methods used by Ronnie Ray never produced convincing results, and nobody in the laboratory believed that his methodology worked. Towards the end of my PhD, I went to University College London (UCL) to learn a new in situ PCR method, which I demonstrated was able to identify measles virus in SSPE tissue. Sub-acute sclerosing panencephalitis (SSPE) is a rare, invariably fatal degenerative disease of the brain caused by wild measles infection. Brain tissue from SSPE patients is often used as a positive control in measles virus detection in tests. I also learned new methods by visiting Professor Ian Bruce (my other supervisor) at the University of Greenwich. However, since I was based at the Royal Free, Dr Wakefield was my main supervisor.

6. Towards the end of 1996, as I was entering the last year of my PhD, we began to look at examples from autistic patients. I am not really clear why Dr Wakefield decided to look at the association of autism and MMR vaccine; however, there was nowhere to go with the Crohn's work that I had been carrying out, and nobody else at the Royal Free seemed interested in getting involved in it. Dr Wakefield thought that the extra data from the autistic patients would be useful for my PhD thesis.

7. When gut biopsies were collected from autistic patients during colonoscopy, I was present in the operating theatre and would collect the biopsy material immediately and put it into liquid nitrogen and store it at -70°C. I was also present when lumbar punctures were carried out on autistic children to investigate cerebrospinal fluid. Once I had extracted the RNA from a sample, I would make a cDNA copy of the RNA and check for the presence of a housekeeping gene (U1A) to indicate that the RNA in the sample had not degraded.

8. In addition to looking for measles virus in gut biopsy material and cerebrospinal fluid (CSF), I looked for the evidence of measles virus in peripheral blood mononuclear cells (PBMCs). The earlier work of Dr Kawashima, which involved looking for measles virus in patients with autoimmune hepatitis, had suggested that there was a possibility that measles virus might be present in PBMCs. I did not think this would be very surprising because it was known that measles virus infected immune cells. Dr Wakefield established a link with Dr Kawashima after he invited him to give a talk at our Christmas meeting in 1995 or 1996. By this time, I had been working for a while on testing PBMCs for measles virus, but had

obtained no positive results. Dr Wakefield was not convinced that my results were actually negative and wanted someone else to repeat the work. I believe that despite my negative results, Dr Wakefield was convinced that measles virus was present in these cells. I think that Dr Wakefield may not have realised that Dr Kawashima employed the same testing methods as we were using, except that Dr Kawashima tested for the H gene rather than the N gene. I was never able to find a satisfactory answer to the question of why Dr Kawashima chose to test the H gene given that the N gene was much more abundant and, therefore, one should be able to find more RNA if one tests for N gene rather than H gene.

9. Dr Wakefield had two theories as to why we had not been able to obtain positive results for measles virus in PBMCs. The first was that the sequence of the measles virus in samples from these autistic children was so mutated that the primers would not bind to it. Dr Wakefield made reference to cases of SSPE when mutations had been found in wild measles virus. The second hypothesis was that proteins bound to the N gene and prevented the primers and enzymes from binding and amplifying the sequence when PCR was carried out. I carried out PCR for both H and N gene for all samples; there was no logical reason why H gene should be detected but not N gene. I was always able to find positive N gene results when testing SSPE tissue samples.

10. I used to send samples of RNA to Dr Kawashima for PCR testing. I experienced some difficulty in communicating with Dr Kawashima, who seemed reluctant to discuss his work. The first time I sent samples to Dr Kawashima, I included SSPE RNA and negative control uninfected, and infected Vero cells sent as ethanol precipitates. These were sent on dry ice as a stable way of sending the samples. One week later, I got a fax from Dr Kawashima saying that he had got positive results for measles virus from the ethanol precipitate, yet he also communicated that the precipitate sample still contained ethanol. I did not believe it was possible to detect RNA in this form. Dr Kawashima had apparently not removed the ethanol from the sample before testing it, as would be normal laboratory practice. On subsequent occasions, I sent freeze-dried samples of RNA that contained no ethanol to Dr Kawashima.

11. On one occasion, Dr Kawashima reported that he had obtained positive results for measles virus in PBMC samples from autistic children where I had obtained negative results from the same samples. In order to confirm the identity of the PCR product as measles virus, I routinely carried out sequencing. This also enabled the genetic identity of each product to be determined, i.e., whether the virus was wild-type, vaccine or lab strain. When I analysed the results sent by Dr Kawashima, I found that the positive results from these autistic patient samples contained the same sequence of measles virus as that found in tissue samples from patients with SSPE used as positive controls. It therefore appeared that Dr Kawashima had contaminated the PBMC samples from the autistic patients with measles virus from the SSPE positive controls. Each of the SSPE positive controls I had been using had quite specific changes in its sequence and so it was easy to determine when a sample

had been contaminated from this source. I mentioned this to Dr Wakefield, but he did not seem to take any particular notice of these results.

12. Where I did generate positive results for measles virus in PCR experiments, I am confident that every single one was as a result of contamination. I have gone back through my lab notes and found that during my PhD research I obtained only nine positive PCR results for measles, all of which were sent for sequencing. I used a sequencing service at Hinxton Hall, which was as cheap as carrying out the experiments myself. Hinxton Hall would email the results of the sequencing back to me. Sequencing revealed that all of the positive results were attributable to contamination, usually with a lab strain Hu2. Hu2 is a strain of measles virus isolated from a child with measles and used as a positive control in experimental work. I believe that the source of this contamination was measles virus from samples on which I had previously carried out PCR. Before sequencing, I cloned the PCR product into a plasmid, and this plasmid was then used to infect bacteria that were grown on plates in the laboratory. These bacteria reproduce, making more copies of the PCR product, and enabling the product to be sequenced more readily. I carried out this cloning in the same laboratory as the PCR reactions and there were therefore lots of bacteria containing products of measles virus in the same laboratory. Later on in my PhD work, in an attempt to avoid contaminating samples at an early stage of the experiment, I processed a number of Crohn's tissue samples at Greenwich University. Out of these 60 samples, only one was positive for measles virus, and this was as a result of contamination.

13. Towards the end of my PhD work, I also carried out detection tests for mumps and rubella viruses, using pre-published methods. All the results I obtained were negative.

14. I believe that Dr Wakefield was aware of my negative PCR test results at the time that he submitted his paper entitled "Ileal Lymphoid Nodular Hyperplasia, Non-Specific Colitis and Pervasive Developmental Disorder in Children," which was published in the Lancet in February 1998. Dr Wakefield relied on the positive results received from Dr Kawashima despite the fact that I had told him about Dr Kawashima's positive measles results, which turned out to be contamination from SSPE positive controls. I thought I had made it quite clear to Dr Wakefield that Kawashima's results were a result of contamination and were not true positives. I specifically asked Dr Wakefield not to include me on the list of authors of the Lancet paper because I was not comfortable with the fact that we had found lots of negative results for measles virus in tissues from the autistic children.

15. I also had reservations about the immunohistochemistry done to detect measles virus. One of my main reservations was the fact that Dr Wakefield had used an antibody from Porton Down (RAd 68), which had recently been reported to have cross reactivity with other proteins. There were similar problems with some of the monoclonal antibodies that had been used. I believe this may be why Dr Wakefield did not publish the measles immunohistochemistry data.

16. I was aware that Dr Andrew Anthony was carrying out some other work with Dr Wakefield trying to detect measles virus using a technique called immunogold. This work was done elsewhere in the hospital but the results were not communicated to me. Dr Wakefield tended to work separately with people from different departments and had separate laboratory meetings with each individual rather than bring them together as was the normal practice amongst scientists.

17. I made it clear to Dr Wakefield that I wanted to leave the Royal Free Hospital when my funding ran out in September 1997, but Dr Wakefield kindly provided me with further funding until February 1998. During this time I worked on a new in situ PCR method at UCL.

I solemnly declare that the contents of the foregoing are true to the best of my knowledge, information, and belief.

Executed on this 23rd day of May, 2007.

Nicholas Chadwick, PhD
Research Associate Faculty of Life Sciences
Leukaemia Research Fund
University of Manchester