UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF PENNSYLVANIA

UNITED STATES OF AMERICA ex rel., STEPHEN A. KRAHLING and JOAN A. WLOCHOWSKI, Plaintiffs, v. MERCK & CO., INC., Defendant.	Civil Action No. 10-4374 (CDJ)
IN RE: MERCK MUMPS VACCINE ANTITRUST LITIGATION THIS DOCUMENT RELATES TO: ALL ACTIONS	Master File No. 12-3555 (CDJ)

REPORT OF ROBERT MALONE, MD, MS

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I. MY BACKGROUND AND QUALIFICATIONS

I, Robert Malone, am a medical doctor and clinical research scientist with more than 30 years of experience in developing, designing, implementing, reviewing, interpreting and managing vaccine, bio-threat and biologics clinical trials and clinical development strategies. This includes my work on roughly 65 Phase 1, 2 and 3 clinical trials, the vast majority of which I served as the Medicare Director, Medical Monitor and/or Principal Investigator. This work also includes my direct bench research and research laboratory oversight in the development and performance of a variety of laboratory tests including ELISA and Plaque Assay-based tests.

I have worked on clinical trials relating to, among other things, HIV, Influenza (seasonal and pandemic), Plague, Anthrax, VEE/EEE/WEE, Tularemia, Tuberculosis, Ebola, Zika, Ricin toxin, Botulinum toxin, and engineered pathogens. This work has included vaccine product development, manufacturing, regulatory compliance, and testing (manufacturing release and clinical) aspects. My oversight responsibilities have included clinical trial design, regulatory and ethical compliance, and laboratory assay strategy, design, testing and performance. For many years, I consulted for one of the largest domestic U.S. immunogenicity clinical laboratory testing companies. I also worked on clinical trials conducted at vaccine-focused Clinical Research Organizations.

I have extensive research and development experience (bench to bedside) in the areas of pre-clinical discovery research, clinical trials, vaccines, gene therapy, bio-defense, and immunology. I have over twenty years of management and leadership experience in academia, pharmaceutical and biotechnology industries, as well as in governmental and non-governmental organizations. I have also provided confidential commercial intelligence gathering, evaluation and analysis services under subcontract to three major international vaccine development and manufacturing companies.

At the request of my colleagues at the Department of Defense/Defense Threat Reduction Agency, I managed their Ebola project and developed the contracts necessary to move the PHAC/rVSV ZEBOV vaccine forward quickly. I recruited organizations to team with USAMRIID/WRAIR to develop the immunoassays, put WHO leadership in touch with Pentagon leadership to expedite the initial WRAIR clinical trials, recruited the government of Norway to help fund the clinical research, recruited a management team, helped write and edit the clinical

trials developed by the World Health Organization and led the development of the BARDA and DTRA contracts - yielding over $200M in resources.

I was the Medical Director in Vaccines at Beardsworth Consulting Group, Inc., Director of Clinical Development/Medical Affairs in Influenza at Solvay Pharmaceuticals, Inc. (now AbbVie), Senior Medical Director of Summit Drug Development Services, and an Associate Director in Clinical Research at Dynport Vaccine Company, LLC. I have been a chair, committee member or reviewer on numerous government study sections and research programs (including for the NIH and DOD) relating to vaccines and biodefense. I have also served on the editorial board or as a reviewer on numerous academic journals relating to vaccines and infectious diseases, including serving as Editor in Chief of the *Journal of Immune Based Therapies and Vaccines*. I hold 15 domestic patents and have authored dozens of articles, many of which concern viruses and vaccination. I have been invited to chair or present at over 40 conferences on topics relating to viruses and vaccines.

I have been a professor of topics in biology and laboratory research at University of Maryland (Baltimore School of Medicine in the Department of Pathology), University of California, Davis (Department of Medical Pathology), University of California, San Diego, and Kennesaw State University. At University of Maryland, I set-up and ran a successful immunology research laboratory. At Kennesaw State University, I taught a survey course focused on advanced product development and regulatory aspects of biotechnology and vaccine products. At UC Davis, I taught, among other course, a class in the principles of pharmacology and toxicology. I was also the director and founder of the Gene Therapy program at UC Davis.

I received my MD at Northwestern University and a MS (Master of Science) at University of California, San Diego. I completed a year-long Global Clinical Scholars Research Training Program at Harvard Medical School, and a Clinical Pathology Internship and a Postgraduate Research Fellowship (funded by Bank of America and the Giannini Foundation Medical Research) at UC Davis. I was also trained at the Salk Institute Molecular Biology and Virology laboratories. I maintain an active license to practice medicine in the state of Maryland. A copy of my current curriculum vitae, which includes my publications and a listing of government performance grants and contracts I have received over the last five years, is attached as Appendix A. I have not previously provided expert testimony in any litigation.

II. SCOPE OF ASSIGNMENT

I was retained by Relators and the Private Plaintiffs in the two above-referenced actions to provide my opinions on the reliability and clinical relevance of the clinical trials Merck conducted in support of its (1) Supplemental Biologics License Application to the FDA to lower the mumps end-expiry potency specification for M-M-R II, (2) Biologics License Application to the FDA for ProQuad, and (3) Supplemental Biologics License Application to the FDA for M-M-R II formulated with rHA. I also was retained to provide my opinions on the submissions Merck made to the FDA in support of these license applications.

In preparing this report and reaching my conclusions herein, I have reviewed pleadings in this case, documents produced in discovery, deposition transcripts, academic literature and other publicly available material. Appendix B contains a list of the materials that I have considered or relied upon in reaching my conclusions. I also have relied on my training, knowledge and experience in vaccine research and clinical trials over the past 30 years. Relators and Private Plaintiffs are compensating me for my work on this matter at an hourly rate of $350.00/hour. My compensation does not depend on the outcome of this litigation or my conclusions.

III. SUMMARY OF OPINIONS

A summary of my opinions is as follows:

1. Merck's AIGENT assay failed to provide a reliable or clinically relevant measure of protection against mumps.

2. Merck's wild-type mumps ELISA assay failed to provide a reliable or clinically relevant measure of protection against mumps.

3. Merck misrepresented the AIGENT results in its submission to the FDA of the preliminary subset analysis.

4. Merck misrepresented the Protocol 007 results in its Supplemental Biologics License Application to the FDA to lower the mumps end-expiry potency specification for M-M-R II.

5. Merck misrepresented the wild-type ELISA results in its Biologics License Application to the FDA for ProQuad.

6. Merck misrepresented the wild-type ELISA results in its Supplemental Biologics License Application to the FDA for M-M-R II formulated with rHA.

7. Merck has conducted no clinical studies that demonstrate or support how well M-M-R II (as of 2005) or ProQuad protect against mumps.

8. The M-M-R II and ProQuad package inserts are inaccurate, false and misleading in claiming and/or suggesting Merck has conducted clinical studies demonstrating these vaccines afford protection against mumps.

IV. BACKGROUND AND CONTEXT

A. Introduction to Mumps

1. Mumps Disease

Mumps disease is caused by infection with mumps virus, which is a member of the Paramyxoviridae family of viruses.[1] Like other Paramyxoviridae viruses such as measles virus and respiratory syncytial virus, mumps virus has a single-strand structure of RNA that allows it to mutate easily.[2] More than one strain of mumps virus can circulate at any given time, and the type of strain circulating in a given region can change over time.[3] Currently, there are twelve identified distinct strains of mumps virus in the world, categorized by variations in their genetic structure and labeled as different Genotypes.[4] Genotype A was the predominant strain of mumps virus roughly fifty years ago, but currently Genotypes C, D, E, G, and H are most common in the Western Hemisphere while Genotypes B, F and I predominate in Asia.[5]

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[1] *See* Mumps for healthcare providers, at https://www.cdc.gov/mumps/hcp.html

[2] Rubin, S., M. Eckhaus, L. J. Rennick, C. G. Bamford and W. P. Duprex (2015). "Molecular biology, pathogenesis and pathology of mumps virus." J Pathol 235(2): 242-252.

[3] Jin, L., C. Orvell, R. Myers, P. A. Rota, T. Nakayama, D. Forcic, J. Hiebert and K. E. Brown (2015). "Genomic diversity of mumps virus and global distribution of the 12 genotypes." Rev Med Virol 25(2): 85-101.

[4] Johansson, B., T. Tecle and C. Orvell (2002). "Proposed criteria for classification of new genotypes of mumps virus." Scand J Infect Dis 34(5): 355-357; Jin, L., B. Rima, D. Brown, C. Orvell, T. Tecle, M. Afzal, K. Uchida, T. Nakayama, J. W. Song, C. Kang, P. A. Rota, W. Xu and D. Featherstone (2005). "Proposal for genetic characterisation of wild-type mumps strains: preliminary standardisation of the nomenclature." Arch Virol 150(9): 1903-1909.

[5] Cui, A., Z. Zhu, Y. Hu, X. Deng, Z. Sun, Y. Zhang, N. Mao, S. Xu, X. Fang, H. Gao, Y. Si, Y. Lei, H. Zheng, J. He, H. Wu and W. Xu (2017). "Mumps Epidemiology and Mumps Virus Genotypes Circulating in Mainland China during 2013-2015." PLoS One 12(1): e0169561; May, M., C. A. Rieder and R. J. Rowe (2018). "Emergent lineages of mumps virus suggest the need for a polyvalent vaccine." Int J Infect Dis 66: 1-4; Nojd, J., T. Tecle, A.

Mumps commonly presents with pain, tenderness, and swelling in the cheek and jaw area due to infection of one or both parotid salivary glands.[6] This swelling, called parotitis, lasts for at least two days, peaks in one to three days, and then subsides during the next week to ten days. The swelling is often preceded by non-specific symptoms such as muscle pain, headache, malaise and fever. Long term clinical complications associated with childhood mumps include deafness and encephalitis. Mumps has historically been one of the leading causes of childhood deafness. In adults, mumps complications include infection and inflammation of the testes (orchitis), ovaries (oophritis) or female breast (mastitis). Mumps infection during pregnancy carries a spontaneous abortion rate of up to 25%. Complications of mumps in adults can include infection of the pancreas (pancreatitis), deafness, inflammation of the membranes surrounding the brain and spinal cord (meningitis), and inflammation of the brain (encephalitis).[7] There is no specific antiviral drug treatment or prevention for mumps infection.[8]

2. Mumps Vaccines

The first mumps vaccine licensed in the United States used inactivated mumps virus and was available between 1950 and 1978. This vaccine induced only short-term immunity with low effectiveness in protecting vaccinees from mumps. Since then, vaccine developers in Japan, the former Soviet Union, Switzerland and the United States have created more than ten different live, attenuated mumps virus vaccines.[9] A live, attenuated virus vaccine uses an active (live) virus that has been weakened through serial passaging or otherwise to make it less harmful yet still potent enough to stimulate a protective adaptive immune response. Live, attenuated virus

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Samuelsson and C. Orvell (2001). "Mumps virus neutralizing antibodies do not protect against reinfection with a heterologous mumps virus genotype." Vaccine 19(13-14): 1727-1731; Kidokoro, M., R. Tuul, K. Komase and P. Nymadawa (2011). "Characterization of mumps viruses circulating in Mongolia: identification of a novel cluster of genotype H." J Clin Microbiol 49(5): 1917-1925.

[6] *See* Mumps for healthcare providers (Avaliable at https://www.cdc.gov/mumps/hcp.html).

[7] *See* WHO position paper on Mumps vaccines, No. 7, 2007, 82, 49–60 (Available at http://www.who.int/immunization/wer8207mumps_Feb07_position_paper.pdf).

[8] Cox, R. and R. K. Plemper (2015). "The paramyxovirus polymerase complex as a target for next-generation anti-paramyxovirus therapeutics." Front Microbiol 6: 459.

[9] *See* WHO position paper on Mumps vaccines, No. 7, 2007, 82, 49–60 at http://www.who.int/immunization/wer8207mumps_Feb07_position_paper.pdf; Demicheli, V., A. Rivetti, M. G. Debalini and C. Di Pietrantonj (2012). "Vaccines for measles, mumps and rubella in children." Cochrane Database Syst Rev(2): CD004407.

vaccines are considered a biological product and are produced by growing and then purifying large amounts of live virus under carefully controlled conditions.

In the United States, the Center for Biologics Evaluation ("CBER") of the Food and Drug Administration ("FDA") licenses and regulates the sale and manufacture of biological products. There are two currently-licensed mumps-containing vaccines available for sale in the United States, M-M-R II and ProQuad. Both are live, attenuated virus vaccines developed and manufactured by Merck. They are both derived from the same "Jeryl Lynn" strain of mumps virus, which Dr. Maurice Hilleman (the Merck scientist who led development of the vaccine) obtained from his daughter in 1963 when she contracted mumps.[10] The Jeryl Lynn strain is a Genotype A strain of mumps. The initial iteration of this vaccine (Mumpsvax, a standalone monovalent mumps vaccine) was originally licensed in 1967 and in one form or another has been the sole mumps vaccine sold in the United States for roughly 50 years. Merck discontinued production of Mumpsvax in 2009.[11]

M-M-R II (Measles, Mumps, and Rubella Virus Vaccine Live), originally licensed in 1978, is a live, attenuated virus vaccine for measles (rubeola), mumps, and rubella (German measles), and was developed by combining the previously licensed monovalent vaccines for these three diseases. This trivalent vaccine is a sterile lyophilized preparation of (1) Attenuvax (Measles Virus Vaccine Live); (2) Mumpsvax (Mumps Virus Vaccine Live); and (3) Meruvax II (Rubella Virus Vaccine Live). From at least 1991 to 2007, the M-M-R II package insert specified that each 0.5 mL dose should contain at least 20,000 $TCID_{50}$ (tissue culture infectious

[10] Hilleman, M. R. (1966). "Advances in control of viral infections by nonspecific measures and by vaccines, with special reference to live mumps and rubella virus vaccines." Clin Pharmacol Ther 7(6): 752-762; Hilleman, M. R. (1992). "Past, present, and future of measles, mumps, and rubella virus vaccines." Pediatrics 90(1 Pt 2): 149-153; Hilleman, M. R., E. B. Buynak, R. E. Weibel and J. Stokes, Jr. (1968). "Live, attenuated mumps-virus vaccine." N Engl J Med 278(5): 227-232; Buynak, E. B. and M. R. Hilleman (1966). "Live attenuated mumps virus vaccine. 1. Vaccine development." Proc Soc Exp Biol Med 123(3): 768-775; Hilleman, M. R., R. E. Weibel, E. B. Buynak, J. Stokes, Jr. and J. E. Whitman, Jr. (1967). "Live attenuated mumps-virus vaccine. IV. Protective efficacy as measured in a field evaluation." N Engl J Med 276(5): 252-258.

[11] CDC, Q&As about Monovalent M-M-R Vaccines, https://www.cdc.gov/vaccines/hcp/clinical-resources/mmr-faq-12-17-08 html.

doses) of mumps virus.[12] Since at least 2007, the M-M-R II package insert has specified that each 0.5 mL dose should contain at least 12,500 $TCID_{50}$ of mumps virus.[13]

ProQuad (Measles, Mumps, Rubella and Varicella Virus Vaccine Live), originally licensed in 2005, is also a combined live, attenuated virus vaccine containing measles, mumps, and rubella vaccines, with the addition of a varicella vaccine. ProQuad is a combination of (1) the components of M-M-R II and (2) Varicella Virus Vaccine Live (the Oka/Merck strain). The package inserts for ProQuad specify that each 0.5 mL dose of the vaccine should contain at least 4.3 $\log_{10}$ (20,000) $TCID_{50}$ of mumps virus.[14]

B. Immunogenicity Assays: PRN and ELISA Tests

Immunogenicity is the ability of a vaccine to elicit a measurable adaptive immune response.[15] Most immunogenicity assays (tests) measure the existence and relative levels of specific antibodies, which are protective proteins produced by the immune system in response to the presence of a foreign substance.[16] In the context of vaccine research (as opposed to clinical diagnostic use), an immunogenicity test is typically used to establish whether the serum of a test subject is seropositive or seronegative. A seropositive test result should indicate that a subject has developed a specific and clinically relevant immunologic response to the foreign substance

[12] MRK-CHA00542860; MRK-CHA01519087, at '088 (CBER approved Merck's sBLA to "include a change in the labeled potency of the mumps component from no less than 20,000 $TCID_{50}$ to no less than 12,500 $TCID_{50}$ per dose at end of expiry" on December 6, 2007).

[13] MRK-CHA00018611 (FDA approved Merck's request to amend its Product License to revise the minimum potency specification for mumps virus to 20,000 $TCID_{50}$ on December 10, 1990).

[14] https://www.merck.com/product/usa/pi_circulars/p/proquad/proquad_pi.pdf; https://www.merck.com/product/usa/pi_circulars/p/proquad/proquad_pi_4171.pdf.

[15] *See* Siegrist C-A. Vaccine immunology. In: Plotkin SA, Orenstein WA, Offit PA, editors. Vaccines, sixth edition. Philadelphia (PA): Elsevier Saunders; 2012, Chapter 2. For descriptions and definitions of key terms relating to vaccine immunogenicity and efficacy testing, *see* Guidelines on clinical evaluation of vaccines: regulatory expectations; WHO Technical Report Series, No. 1004, 2017, Annex 9 (Available at http://www.who.int/biologicals/expert_committee/WHO_TRS_1004_web_Annex_9.pdf); Guidelines on procedures and data requirements for changes to approved vaccines; WHO Technical Report Series No. 993, 2015, Annex 4 (Available at http://apps.who.int/medicinedocs/en/m/abstract/Js22021en/); and Guidance for Industry; Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products. Office of Communications, Division of Drug Information Center for Drug Evaluation and Research Food and Drug Administration. April 2016 (Available at https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM192750.pdf).

[16] Titers are a measure of the amount of antibodies in a person's blood.

being tested. A clinically relevant immunologic response is one that provides protection from an adverse effect of exposure to the foreign substance (virus, bacteria, allergen or toxin). A seronegative result should indicate that a subject has not developed such a clinically relevant immunologic response. In general, seroconversion occurs when a test subject's pre-vaccination serum is seronegative and post-vaccination serum is seropositive (or has demonstrated a clinically relevant increase in antibodies). An immunogenicity test can be used as a surrogate for predicting clinical protection, but only if it measures a functional immune response or has been shown to correlate to protection from disease. A measurable functional immune response is one that directly relates to a plausible mechanism of action for protection occurring in a patient. The two predominant tests used in mumps immunogenicity testing are the Plaque Reduction Neutralization (PRN) assay and the Enzyme-Linked Immunosorbent Assay (ELISA). [17] PRN assays measure the functional capacity of serum (or other fluids) to neutralize virus infectivity in cells as compared to ELISA assays that only measure the binding capacity against an antigen or mixture of antigens. [18]

[17] Örvell, C. (1988). Paramyxoviridae: Mumps Virus. Laboratory Diagnosis of Infectious Diseases Principles and Practice. New York, NY, Springer: 507-524; Bashe, W. J., Jr., T. Gotlieb, G. Henle and W. Henle (1953). "Studies on the prevention of mumps. VI. The relationship of neutralizing antibodies to the determination of susceptibility and to the evaluation of immunization procedures." J Immunol 71(2): 76-85; Linde, G. A., M. Granstrom and C. Orvell (1987). "Immunoglobulin class and immunoglobulin G subclass enzyme-linked immunosorbent assays compared with microneutralization assay for serodiagnosis of mumps infection and determination of immunity." J Clin Microbiol 25(9): 1653-1658; Mauldin, J., K. Carbone, H. Hsu, R. Yolken and S. Rubin (2005). "Mumps virus-specific antibody titers from pre-vaccine era sera: comparison of the plaque reduction neutralization assay and enzyme immunoassays." J Clin Microbiol 43(9): 4847-4851; Berger, R. J., M. (1980). "Comparison of five different tests for mumps antibodies." Infection 8(5): 180–183; Buynak, E. B., J. E. Whitman, Jr., R. R. Roehm, D. H. Morton, G. P. Lampson and M. R. Hilleman (1967). "Comparison of neutralization and hemagglutination-inhibition techniques for measuring mumps antibody." Proc Soc Exp Biol Med 125(4): 1068-1071; Kumakura, S., H. Shibata, T. Isobe, M. Hirose, M. Ohe, N. Nishimura, K. Onoda, A. Nagai and S. Yamaguchi (2013). "Comparison of hemagglutination inhibition assay and enzyme immunoassay for determination of mumps and rubella immune status in health care personnel." J Clin Lab Anal 27(5): 418-421; Pipkin, P. A., M. A. Afzal, A. B. Heath and P. D. Minor (1999). "Assay of humoral immunity to mumps virus." J Virol Methods 79(2): 219-225; Cusi, M. G., S. Fischer, R. Sedlmeier, M. Valassina, P. E. Valensin, M. Donati and W. J. Neubert (2001). "Localization of a new neutralizing epitope on the mumps virus hemagglutinin-neuraminidase protein." Virus Res 74(1-2): 133-137; Orvell, C., T. Tecle, B. Johansson, H. Saito and A. Samuelson (2002). "Antigenic relationships between six genotypes of the small hydrophobic protein gene of mumps virus." J Gen Virol 83(Pt 10): 2489-2496.

[18] Latner, D. R., M. McGrew, N. J. Williams, S. B. Sowers, W. J. Bellini and C. J. Hickman (2014). "Estimates of mumps seroprevalence may be influenced by antibody specificity and serologic method." Clin Vaccine Immunol 21(3): 286-297.

1. PRN Immunogenicity Assays

The PRN test is considered a functional immunogenicity assay.[19] It is designed to measure the amount of antibody in the test subject's blood (or serum) that will bind to a virus and block its ability to infect cells. These antibodies are referred to as neutralizing antibodies. Non-neutralizing antibodies may also bind to a virus but they fail to block the virus from infecting cells. For a PRN test to serve as a valid functional immunogenicity assay, it must distinguish between neutralizing and non-neutralizing antibodies. [20]

The PRN immunogenicity assay uses pre-vaccination and post-vaccination samples of the test subjects' (patients') blood. Pre-vaccination samples are taken before the test subjects have been inoculated with the vaccine; post-vaccination samples are taken after inoculation. The blood is processed to remove blood cells and blood clotting proteins to create serum, which is generally comprised of proteins (including antibodies), electrolytes, carbohydrates, antigens, hormones, and plasma. Various dilutions of the serum are then mixed with a constant amount of virus. Then, after sufficient time for the two to interact, the mixture is placed onto the surface of a single layer of cultured cells (cells which are grown in a controlled, artificial environment) inside a clear plastic well.[21]

After the sample is placed in the plastic well, the virus is allowed to replicate and spread infection within the well. The test is stopped by the addition of chemicals that stop the virus, essentially freezing the process in its tracks, and these chemicals also bind the virus mixture and cell layer to the clear plastic well on which they have grown. The plastic well is then treated

[19] *See* Guidelines on clinical evaluation of vaccines: regulatory expectations; WHO Technical Report Series, No. 1004, 2017, Annex 9 (Available at http://www.who.int/biologicals/expert_committee/WHO_TRS_1004_web_Annex_9.pdf), p. 518, Section 5.2 Characterization of the immune response ("In general the clinical development programme should include a description of the magnitude of the immune response, including an assessment of functional antibody (for example, antibody that neutralizes viruses or toxins, or antibody that mediates bactericidal activity or opsonophagocytosis) if this can be measured.").

[20] *See* Guidance for Industry (Draft), Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), Center for Devices and Radiological Health (CDRH). April 2016 (Available at https://www.fda.gov/downloads/Drugs/Guidances/UCM192750.pdf) (FDA regulatory guidance concerning general principles, methods, and confounding factors in developing and validating assays).

[21] There are often several wells in a single plate, so one plate will often contain several test samples.

with a stain or dye that helps distinguish the infected and uninfected cells so they can be examined with the naked eye or under a low powered microscope to identify holes or other differences in the cell layer indicating viral infection. These holes, known as plaques, are created when the virus infects and alters cells. The PRN test results typically are read by turning the plastic plate upside down and using a felt-tipped marker to identify each plaque in the wells, although more advanced automated methods are also used.

The number of plaques in a given test sample at various dilutions is used to determine whether the sample is seropositive or seronegative based on comparison to plaque counts in control samples using a pre-defined cutoff value for seropositivity. The dividing line between what is considered a seronegative or seropositive plaque count is referred to as the serostatus cutoff. A sample is considered to have seroconverted when the pre-vaccination sample is seronegative and the post-vaccinations sample is seropositive (or meets a predefined fold-rise increase in titer). For a PRN test to be fit for the purpose of measuring clinically relevant neutralizing antibodies, the serostatus cutoff (or fold-rise increase in titer) must reflect a level of neutralizing antibodies that provides protection from infection or disease. Selecting an appropriate serostatus cutoff is critical to the accuracy, predictive value and clinical relevance of a PRN or any other immunogenicity test.

The manual plaque counting process is subjective and vulnerable to bias, either conscious or unconscious. For this reason, it is critical to establish, document and abide by proper training processes, procedures, internal controls and quality assurance throughout the plaque counting exercise. The plaque counters must be well-trained, blinded to the nature of the sample being tested (including the treatment group and whether it is a pre- or post-vaccination sample), and closely monitored for consistency, precision and accuracy. Without proper procedures, controls and blinding, the PRN assay cannot provide a reliable measure of the number of plaques in the samples being tested. It likewise cannot provide a reliable measure of the serostatus of the sample or of whether a particular test subject has seroconverted.

2. ELISA Immunogenicity Assays

Unlike PRN immunogenicity assays, which are designed to measure only those antibodies that functionally neutralize the subject virus, ELISA immunogenicity assays measure all antibodies that bind to the subject virus or a selected antigen subset of the virus whether or

not they actually neutralize the virus. For example, there are many different antibodies in the serum of someone who has been infected with mumps or vaccinated with a live attenuated mumps vaccine which will bind to the many different mumps proteins, and each of these anti-mumps antibodies bind to different proteins (or antigens) and have different binding characteristics, functions and activities.[22] Furthermore, some parts of mumps virus proteins are like those present in related viruses (like measles or respiratory syncytial virus), and antibodies produced in response to those viruses may cross-react with mumps proteins in an ELISA assay.

The typical ELISA assay does not distinguish between these different characteristics, functions and activities, but rather just measures the presence of any and all antibodies present in a sample that bind to whatever antigen is being tested. ELISA assays are not functional assays because they simply measure binding activity, not whether this activity has any impact on viral infection and replication. Like the PRN assay, the ELISA is an assay that uses transparent plastic plates with many separate wells or chambers. There are many different variations of the basic ELISA method.

In a direct ELISA, the subject virus (or a test sample such as serum containing virus) is coated onto a plastic well. Then, antibody is added to the same well. The antibody chosen for this step is attached to a particular enzyme used to measure the results of the test. After allowing the subject virus or serum to interact with the added antibody, the sample is washed off, releasing any unbound antibody and leaving behind only the antibodies bound to the sample. Then, a chemical that changes color in the presence of the linked enzyme is added to the sample. If any of the antibody/enzyme mixture remains bound to the sample after washing, the sample in the plastic well will change color. This color change, which may be quantified in Ab units or as indicating a specific titer, is detected using a machine and interpreted by computer software. Like the PRN, the serostatus cutoff established for a clinical ELISA test can be a single measure of Ab units or a fold-rise increase between pre- and post-vaccination samples and serve as a type of dividing line between what is considered seronegative and seropositive.

[22] Latner, D. R., M. McGrew, N. J. Williams, S. B. Sowers, W. J. Bellini and C. J. Hickman (2014). "Estimates of mumps seroprevalence may be influenced by antibody specificity and serologic method." Clin Vaccine Immunol 21(3): 286-297; Orvell, C., T. Tecle, B. Johansson, H. Saito and A. Samuelson (2002). "Antigenic relationships between six genotypes of the small hydrophobic protein gene of mumps virus." J Gen Virol 83(Pt 10): 2489-2496.

The indirect ELISA is the type of ELISA typically used to measure the overall antibody response in a given serum sample to a vaccine. The first step of an indirect ELISA is the same as that of the direct ELISA where the subject virus or antigens derived from the virus are coated onto a plastic well. Then the serum sample to be tested is added to the well. The binding antibodies within the serum sample attach to the virus in the well and everything else is washed off. Then a second set of antibodies linked to a particular enzyme (as in the direct ELISA method) is added to the well. These antibodies attach to the already bound antibodies in the sample and the excess is washed off. Finally, a chemical known to change color in the presence of the linked enzyme is added and the resulting color change is detected, measured, and interpreted.

3. The Need to Correlate ELISA to a Functional Test for Mumps Immunogenicity Testing

Since the ELISA does not distinguish between neutralizing and non-neutralizing antibodies, it is generally understood that the ELISA method cannot provide a relevant measure of clinical protection for mumps unless it is correlated to a clinical outcome (e.g., protection from viral disease),[23] PRN or other type of functional assay. Scientists at the CDC have examined the specific nature of the relationship between the mumps PRN and ELISA and noted, "there is poor correlation between mumps neutralization titers and ELISAs that measure the presence of mumps-specific IgG levels."[24] These scientists determined that human anti-mumps antibodies predominantly bind to the NP protein in the mumps virus, and that this interaction does not neutralize the mumps virus. Thus, ELISA assays designed to measure clinically relevant mumps seroconversion need to differentiate among the various mumps virus proteins that are bound by the antibodies in the sample. If not, the results risk overestimating protection due to an antibody response that has no relationship to neutralization and no known functional significance. The CDC scientists emphasized this point in their analysis:

[23] Sanz, J. C., B. Ramos, A. Fernandez, L. Garcia-Comas, J. E. Echevarria and F. de Ory (2018). "Serological diagnosis of mumps: Value of the titration of specific IgG." Enferm Infecc Microbiol Clin 36(3): 172-174.

[24] Latner, D. R., M. McGrew, N. J. Williams, S. B. Sowers, W. J. Bellini and C. J. Hickman (2014). "Estimates of mumps seroprevalence may be influenced by antibody specificity and serologic method." Clin Vaccine Immunol 21(3): 286-297.

> One of the most important conclusions from these data is that care must be taken when interpreting ELISA results, especially those based on whole-virus antigen. While it may be reasonable to assume that an individual who is seronegative by ELISA likely does not have neutralizing antibody, no assumptions can be made about the levels of neutralizing antibodies in individuals who are seropositive by ELISA. Measurements of seroconversion that mostly detect NP antibody without taking into account the level of neutralizing antibody may overestimate the level of protection.[25]

Scientists at Merck have reached the same conclusion on the limited utility of the ELISA test in measuring mumps seroprotection unless it is correlated to a functional assay like a PRN. In criticizing an ELISA test SmithKline Beecham performed comparing its MMR product Priorix to Merck's M-M-R II, Drs. Scott Thaler and Joseph Heyse of Merck pointed to the failure of SmithKline to correlate the ELISA to a PRN or other functional assay. They emphasized that a "correlation between ELISAs and functional assays such as neutralization . . . must be individually demonstrated for each vaccine." They pointed to the case of the Swiss Berna vaccine Triviraten for support:

> There is precedent to support our concern about relying solely on ELISA values. For instance in the case of the Swiss Berna vaccine Triviraten, which contains the Rubini mumps strain, and which apparently had excellent ELISA seroconversion rates. However, the efficacy of the vaccine has been estimated to be as low as 20% against wild-type mumps. In one recent publication when several volunteers were vaccinated with Triviraten, all seroconverted according to either ELISA or indirect immunofluorescence results, but none developed neutralizing antibody to wild-type mumps.[26]

Drs. Thaler and Heyse concluded that "without demonstrating a correlation between the immunologic response and protection from circulating wild-type mumps infection, we question whether an ELISA assay can be assumed to correlate with protection from wild-type disease . . .

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[25] Latner, D. R., M. McGrew, N. J. Williams, S. B. Sowers, W. J. Bellini and C. J. Hickman (2014). "Estimates of mumps seroprevalence may be influenced by antibody specificity and serologic method." Clin Vaccine Immunol 21(3): 286-297. Dr. Mark Pallansch, the current Director of the Division of Viral Diseases at the CDC similarly testified to the need to correlate ELISA tests to a functional assay like a PRN: "[U]sually you will compare an ELISA to some other reference method to try to achieve some correlation – high correlation with what is considered a gold standard. In the specific case of mumps, there isn't really a true gold standard as it relates to protection. Therefore, neutralization has been the de facto gold standard, so you would want to see ELISA results in categorizing response versus response as measured by neutralization." Pallansch Dep. 127:14-24.

[26] MRK-CHA00088592, at '593.

and that the reliance on ELISA assays alone is insufficient to support the contention that a vaccine such as Priorix will protect against wild-type infection."[27]

Merck apparently took a similar position before the New Zealand regulatory authorities in criticizing Priorix ELISA-based immunogenicity data as not providing any meaningful indicator of protection against disease:

> Priorix immunogenicity . . . has been reported only by ELISA, which measures total antibody levels including non-neutrali[z]ing as well as neutrali[z]ing antibodies. It is important for a vaccine to produce the right type of antibodies for maximum efficacy. The Priorix [package insert] does not mention neutrali[z]ing antibodies . . . . In the case of another mumps vaccine, the Rubini strain, published studies have shown that Rubini vaccine, while it induces high seroconversion rates by fluorescent antibody tests, induces very low rates by neutrali[z]ing antibody tests. It has been shown to confer only about 15% protection against mumps.[28]

C. Merck's Mumps Immunogenicity Test Protocol 007

Protocol 007, "A Study of M-M-R^{TM} II at Mumps Expiry Potency in Healthy Children 12 to 18 Months of Age" (NCT00092391), was a Phase III pivotal clinical trial using both a PRN and ELISA to measure the immunogenicity of the mumps component of M-M-R II at various potencies. Merck developed the Protocol in response to CBER's discovery in the late 1990's that the mumps component of M-M-R II was not always meeting the minimum potency of 20,000 $TCID_{50}$ (4.3 $\log_{10}$) specified on the product label for the full 24-month shelf life (end-expiry period). CBER learned of this compliance issue when in the course of its review of M-M-R II, Merck disclosed it was treating this potency specification as a minimum release potency, not a minimum expiry potency. This compliance issue was exacerbated by Merck's finding that

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[27] MRK-CHA00088592, at '593. *See also* MRK-CHA00667054, at '061 ("These recent developments have increased the governmental agencies awareness of the importance of a functional assay to measure the true efficacy of vaccines. . . . For mumps, ELISA appears to be more sensitive than [PRN]. However, sera positive by ELISA may lack antibody to HN protein, which may be why ELISA is not the preferred assay to assess protection from mumps."); at '063 ("For mumps virus vaccine strains, studies have shown that seroconversion rates based on ELISA GMTs may not correlate with results from neutralizing antibody assays, a more sensitive and clinically relevant assay. ***For this reason, it is important to test mumps virus antibody levels by neutralization assay methods.***") (emphasis in original); MRK-CHA00017826, at '826 ("The immunogenicity assessment is a measure of whether or not the vaccinee responded to the vaccination in some detectable way. This response then needs to be correlated with protection from diseases. Historically, the functional assays have been judged to be a good surrogate marker of protection.").

[28] MRK-CHA00260815, at '816.

since the mid-1990's the mumps component of M-M-R II was decaying at a faster rate than Merck had historically observed.[29]

Protocol 007 was designed to address CBER's stated concern that lots of M-M-R II that failed to meet the mumps end-expiry potency specification did not provide sufficient protection against mumps.[30] This clinical trial was necessary because CBER rejected Merck's proposal to rely on the lack of outbreaks (at the time) together with the original licensure study data to demonstrate the vaccine's effectiveness at potencies below the end-expiry specification.[31]

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[29] *See* MRK-CHA00207690, at '706-07 ("During communications with CBER in 1996-98, it became evident that the agency did not agree with our proposal that the specifications . . . were the minimum release potencies for M-M-R®$_{II}$. Instead, they defined these specifications as end-expiry potencies. . . . It was determined that the mumps component could not meet its specifications if current manufacturing practices were followed."); MRK-CHA00094161, at '165-66 (same); MRK-CHA00587859, at '859 ("Routine stability study testing has observed an apparent decrease in the stability of the mumps component in Merck's live virus vaccines."); MRK-CHA00284623, at '623 ("[I]t does appear that there was a statistical shift in losses over time for mumps and that it occurred around 1994."); MRK-CHA00549487, at '488 ("These stability analyses show a trend to less stabile [mumps] product in recent years."); MRK-CHA00020396, at p. 4 ("CBER expressed concern over the apparent acceleration in the rate of decay of mumps potency over the shelf-life of M-M-R® II.").

[30] *See* MRK-CHA00590949, at '949 ("[I]t was noted that there is an increasing trend in stability losses for mumps in mumps containing vaccines. This trend was addressed initially in several ways: . . . Merck launched a Mumps Expiry Trial, aimed at demonstrating an expiry limit of 3.7 $\log_{10}$ $TCID_{50}$/dose for mumps M-M-R family of vaccines."); MRK-CHA00019225, at '226 ("CBER asked Merck to demonstrate that the mumps 'expiry' specification could be met as per their interpretation. To address this issue . . . Merck committed to a clinical study to evaluate lower end expiry potencies and a new functional antibody assay was developed at the request of CBER to be used for that study."); MRK-CHA00094161, at '163 (same); MRK-CHA00020635, at '036 ("In response to concerns raised regarding the immunogenicity of the Jeryl Lynn™ mumps strain at vaccine expiry, a clinical trial was designed and is presently underway . . . [and] a plaque reduction neutralization (PRN) assay was developed for use in this trial."); MRK-CHA00040705, at p. 4 ("Current manufacturing release specifications for M-M-R®II do not support the minimum effective dose for mumps (4.3 TCID50) . . . . An end expiry trial to establish a lower effective dose was conducted."); MRK-CHA00095320, at '320 ("I wanted to re-emphasize that there is no question that this trial [Protocol 007] is necessary for regulatory purposes. . . . We have only limited clinical data at this dose [4.3] and no data at all with the trivalent below 4.1."); MRK-CHA00334863, at '865 ("Drivers for Mumps Expiry Trial . . . Reduction in the labeled potency for mumps is necessary to ensure compliance with shelf-life claim").

[31] *See* MRK-CHA00020425, at '425 ("A requirement was set forth by CBER to use a functional neutralization assay for [Protocol 007] . . . due to: 1-The efficacy statements in MMR®II label are based on old, limited data and an assay that is no longer used by Merck."); MRK-CHA00207690, at '706 ("Arguments for the demonstrated immunogenicity at lower potencies of the monovalents and the apparent effectiveness of Merck's release strategy, due to the virtual eradication of disease in the US and Finland where the product was used exclusively were further rejected, because of the small number of children used in the studies, and the circumstantial nature of the justification."); MRK-CHA00615152, at '157 (same); MRK-CHA00019225, at '226 (same); MRK-CHA00094161, at '163 (same); MRK-CHA00615152, at '157 ("However, the minimum immunizing dose studies were performed in a relatively small number of individuals . . . and have not been repeated in recent years or with the trivalent M-M-R®$_{II}$ vaccine []. Therefore, in order to determine the minimum expiry potency for M-M-R®$_{II}$, it was essential to demonstrate the immunogenicity of M-M-R®$_{II}$ at the reduced titers expected at expiry."); MRK-CHA00019225, at '228 (same). *See also* MRK-CHA00017605, at '607 ("No data exist for mumps at the expiry potency Merck has selected. A clinical immunogenicity trial is necessary to provide these data.").

Protocol 007 was also conducted to support Merck's application to the FDA to lower the mumps end-expiry potency specification. Merck ultimately used the data from this clinical trial to support its Supplemental Biologics License Application (sBLA) to lower the mumps end-expiry potency specification for M-M-R II to 12,500 $TCID_{50}$ (4.1 $\log_{10}$).

1. Merck's Development and Design of Protocol 007

The Protocol 007 testing was primarily designed to compare by PRN assay the mumps neutralizing immune response elicited by three different doses of M-M-R II: 4.8 $\log_{10}$ $TCID_{50}$, the control dose; and 4.1 and 3.8 $\log_{10}$ $TCID_{50}$, as the tested end-expiry doses.[32] As set forth in Clinical Study Report (CSR), there were two primary objectives (endpoints) of the study:

> 1. To demonstrate a similar immune response to mumps virus by neutralization among subjects receiving M-M-R™II containing an expiry dose of mumps virus concomitantly with VARIVAX™ in comparison to subjects receiving M-M-R™II containing a release dose of mumps concomitantly with VARIVAX™.
>
> 2. To demonstrate an adequate immune response by mumps virus neutralization among subjects receiving M-M-R™II containing an expiry dose of mumps concomitantly with VARIVAX™.[33]

To satisfy the first objective (non-inferiority), the control group and expiry potency group "were considered similar if the proportion of children who developed neutralizing antibodies to mumps in the expiry potency group was not more than 5.0 percentage points lower than the proportion of subjects who developed neutralizing antibodies to mumps in the control group."[34] To satisfy the second objective (adequacy), "the lower bound of the 95% two-sided confidence interval on the observed response be >90%, where the observed response is the proportion of children 12 to 18 months of age who develop neutralizing antibodies to mumps."[35]

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[32] MRK-CHA00224982. The original tested expiry doses were set at 4.0 and 3.7 $\log_{10}$ $TCID_{50}$ but were adjusted to 4.1 and 3.8 $\log_{10}$ $TCID_{50}$ after the "reassignment of the mumps house standard [] potency". *See* MRK-CHA00224982, at '5005.

[33] MRK-CHA00224982, at '017. Among the secondary objectives of the test was "[t]o demonstrate similar immune responses to measles, mumps, and rubella (seroconversion rates by ELISA) among children who receive M-M-R™II containing an expiry dose of mumps virus concomitantly with VARIVAX™ compared to children who receive M-M-R™II containing a release dose of mumps virus given concomitantly with VARIVAX™." MRK-CHA00224982, at '017.

[34] MRK-CHA00224982, at '014.

[35] MRK-CHA00224982, at '014.

Based on prospective endpoint statistical power analysis calculations, the study was designed to enroll roughly 1,770 test subjects (590/group). The data analysis plan for the study required exclusion of immune response data from subjects with detectable anti-mumps immunity prior to vaccination (pre-positives). Serum samples were obtained for serologic analysis prior to vaccination, at six weeks post-vaccination, and at one-year post-vaccination. All collected samples were tested at Merck by a PRN (pre-vaccination, and six weeks post-vaccination) and ELISA assay (pre-vaccination, six weeks post-vaccination, and one-year post-vaccination). The study enrollment was initiated on February 26, 1999 and completed on July 20, 2001. The cutoff date for in-house data was October 26, 2001.[36]

In the design and development phase of the study, CBER required Merck to use a functional assay (such as a PRN test) to measure immune responses to mumps vaccine if Merck was going to use the testing results to support a change in the mumps potency end-expiry specifications.[37] In order to support a change in these potency specifications to the 4.1 or 3.8 $\log_{10}$ $TCID_{50}$ Merck was testing, Merck sought to match the 96% seroconversion rate on the M-M-R II label by demonstrating a neutralizing antibody seroconversion rate of at least 95% (with a lower bound of 90% using a 95% confidence interval).[38] Internal Merck documents indicate a

[36] MRK-CHA00224982, at '019.

[37] MRK-CHA00001255, at '255 (CBER's Dr. Carbone "can not accept ELISA for the mumps end expiry and only will only accept neutralization data . . . since neutralization reflects protection."); MRK-CHA00020425, at '425 ("requirement was set forth by CBER to use a functional neutralization assay"); MRK-CHA01898773, at '775 ("CBER has indicated that a functional antibody assay (e.g., WT Neut) will be required to establish equivalence."); MRK-CHA00040705, at p. 8 ("New functional antibody assay developed at the request of CBER."); MRK-CHA01386177, at '177 ("CBER considers a neutralization assay essential for establishing efficacy w[h]ere you need to define effectiveness for a product - the mumps end expiry trial . . . ."); MRK-CHA02021754, at '756 (CBER stating, "[a]s the immunological correlate for efficacy of mumps vaccination, Merck has developed an assay to measure anti-mumps antibodies in the serum of vaccinated subjects.").

[38] MRK-CHA00040705, at p. 8 ("note our label specifies a 96% SCR based on mumps neutralizing antibodies"); at p. 13 ("End expiry dose must have an acceptable antibody response[.] Lower bound of the 95% two-sided confidence interval > 90%."); MRK-CHA00137671, at '696 ("We confirm that a lower bound of 90% and a 5 percentage point equivalence margin will be used for the primary acceptability and equivalence hypotheses regarding mumps neutralization."); MRK-CHA00209674, at '675 ("A mumps neutralization using a wild type virus strain which results in ≥95% seroconversions rate is required for the M-M-R®II end-expiry trial."); MRK-CHA00336905, at '906 ("CBER requirement to demonstrate ≥95% seroconversion by a neutralization assay (90% lower limit)"); Krah Dep. 727:2-3 (FDA "indicated the requirement of a 95 percent seroconversion.").

concern by Merck that failing to meet this seroconversion rate would require Merck to lower the 96% mumps seroconversion rate on the label.[39]

CBER also insisted that Merck test against a wild-type mumps virus indicator strain, which is one actually circulating in the real world, as opposed to the attenuated Jeryl Lynn mumps virus strain, which is the weakened mumps virus used in the vaccine.[40] Merck engaged in preliminary testing with its standard PRN assay using various indicator virus isolates, including the attenuated Jeryl Lynn mumps virus strain as well as several wild-type mumps virus strains. With this initial developmental testing, Merck measured PRN seroconversion rates of approximately 90% for the Jeryl Lynn vaccine strain, and seroconversion rates of roughly 66% to 75% for the various wild-type strains that were tested.[41]

This original testing did not yield ≥ 95% wild-type PRN seroconversion rates. Therefore, after consultation with CBER, Merck made two fundamental changes to its standard PRN assay.

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[39] MRK-CHA00198869, at '869 ("Summary to date → 70% conversion rate . . . If this is not resolved and doesn't change → label may have to be changed from 96% to 75%"); MRK-CHA00273309, at '309 ("With JL as the test isolate, the PRN SCR is ~90%, and with L01 as the test isolate, the PRN SCR is ~70-80%. . . . Should CBER raise the issue of the 96% SCR in the current label, the extensive field experience of the vaccine will be emphasized. Should this approach to defending the 96% SCR in the label not be successful, one option may be to propose including the range of observed SCRs in the label."); MRK-CHA00625629, at '630 ('We are basing the expected rate of 96% on the package circular . . . . I selected 94% as the alternative case because the clinical monitor and I feel that if the response to mumps in the control is lower than this, we should generally be concerned (given what is in our circular)."); MRK-CHA00086292, at '292 ("CBER insistence on a WT neutralization assay will likely result in SCR of 70-80% . . . . Risks: a) Label may be adversely affected with perceived change in efficacy."); MRK-CHA00040705, at p. 26-27 ("Short-term options under evaluation • Change label to reflect lower than 96% protection . . . • Change label to reflect antibodies* against mumps in 96% of subjects vaccinated (as measured by ELISA) *not neutralizing antibodies as is currently reflected on the label"); MRK-CHA01725276, at '342 ("Short term options under consideration: . . . Change package circular to reflect < 96% protection against mumps by neutralizing antibodies."); MRK-CHA01648951, at '952 ("The label currently indicates that a single dose of MMRII results in a mumps seroconversion rate of 96%; if we are held to this as the 'acceptable historical standard' there is a chance that we may have a problem. We may need to consider the possibility that the SC rate at expiry may be lower and plan for this contingency now.").

[40] MRK-CHA00026466, at '470 ("CBER has indicated that the vaccine passage Jeryl Lynn™ is not suitable for use in the PRN and has established a requirement to use a "wild-type" mumps strain to evaluate vaccine-induced immune responses."); MRK-CHA00020635, at '636 ("[A]t CBER's request, the study incorporated a wild-type mumps isolate as a test strain on the *premise that neutralization of a wild-type isolate would act as a surrogate for protection from disease*.") (emphasis in original);

[41] *See* MRK-CHA01898773, at '774 ("With JL as the test isolate, the SCR is ~90%, and with LO1 as the test isolate, the SCR is ~70-75%."); MRK-CHA00020428, at '429 ("Current status Neut. assays support >90% SCR for JL and a range up ~ 70-75% for wild type virus."); MRK-CHA01927351, at '353 ("Merck's current assay showed: 90% seroconversion rate for JL[;] 66% . . . for Lo1[;] 56% . . . for JL2"); MRK-CHA00026466, at '471 ("Results of a series of pilot PRN assays of pediatric sera against Jeryl Lynn™, Lo1 and JL2 mumps strains showed respective seroconversion rates of 91% [], 69% [] and 56% [].").

The first change was to use a low-passage Jeryl Lynn mumps vaccine strain (rather than a true wild-type strain) as the indicator virus. Second, Merck added rabbit anti-human Immunoglobulin G (anti-IgG) to the clinical serum samples for the purpose of, as Merck characterized it, enhancing the sensitivity of the assay.[42] This technique was previously used by Dr. Hiroshi Sato in the 1970s, who found that the addition of anti-IgG yielded up to a 100-fold enhancement in measured plaque neutralization titer responses.[43] CBER required "appropriate validation" of Merck's use of anti-IgG in the assay.[44] Merck referred to this modified PRN test, designed specifically for the Protocol 007 study, as the anti-IgG enhanced neutralization test or the AIGENT.

In further development of the AIGENT assay, Merck measured a higher level of seropositive results in the pre-vaccination samples than had been expected in an unvaccinated population. Merck observed a pre-positive rate as high as 24% at certain anti-IgG dilutions.[45] Merck tested various dilutions of anti-IgG to identify and select the dilution of anti-IgG that yielded test results indicating a pre-positive rate within Merck's pre-determined target range of 10% or less.[46]

2. The AIGENT Validation

After Merck completed its design of the AIGENT assay, it conducted a validation study, which is required for primary endpoint tests used in pivotal clinical trials to ensure the AIGENT testing method was suitable and reliable for its intended purpose.[47] Validation studies measure and characterize various attributes of the assay, the most important of which include assay

[42] MRK-CHA00224982, at '5038.

[43] Sato, H., P. Albrecht, J. T. Hicks, B. C. Meyer and F. A. Ennis (1978). "Sensitive neutralization test for virus antibody. 1. Mumps antibody." Arch Virol 58(4): 301-311; Sato, H., P. Albrecht, S. Krugman and F. A. Ennis (1979). "Sensitive neutralization test for rubella antibody." J Clin Microbiol 9(2): 259-265.

[44] MRK-CHA00001258, at '260.

[45] MRK-CHA00016632, at '633 (referencing "unexpectedly high pre-positive rate" of 22%); MRK-CHA00273243, at '243 ("we do not plan to apply this assay [AIGENT] to other development efforts as the pre-pos rate is relatively high"); MRK-CHA00065695, at '700 (showing pre-positive rates of 8-24% depending on dilution of anti-IgG); MRK-CHA00026912,at '916 (same).

[46] *See* Sec. V.A.3.

[47] *See* 2013 *FDA Guidance for Industry regarding Bioanalytical Method Validation* ("For pivotal studies that require regulatory action for approval or labeling . . . the bioanalytical methods should be fully validated.").

specificity and reproducibility. For the AIGENT assay, specificity meant the ability to assess mumps neutralizing antibodies in the presence of other antibodies and components (matrix) of the subject serum and added anti-IgG reagent. Specificity was a critical parameter in the AIGENT assay to ensure the seroconversion rates being measured reflected only mumps neutralizing antibodies and did not also include non-mumps specific neutralizing activity or non-neutralizing activity. Reproducibility (or reliability) refers to the consistency of assay results over time under standardized testing conditions.

The AIGENT validation report was completed on February 27, 2001.[48] The report described the validation studies Merck performed concerning specificity as follows:

> [S]pecificity was assessed by measuring residual mumps neutralization titers following absorption of sera with measles, mumps or rubella virus extracts. Sera was also absorbed with mock extract or incubated with culture medium. The mock extract was made from the uninfected vero cells in which each virus was grown, and the culture medium is the medium in which the cells are grown.[49]

With respect to specificity, the validation report provided the following result summary:

> There is a reduction in neutralization titers with the measles and rubella antigens that is similar to the reduction obtained with the mock extract. Relative to the other antigens tested, absorption with the mumps antigen resulted in a further reduction in neutralization in half of the samples tested, indicating the specificity of the assay for measuring mumps antibodies.[50]

According to the validation report, in the first specificity experiment Merck pre-absorbed four pediatric sera samples using mumps, measles, rubella or mock extracts, or incubated them with culture medium, and tested the pre-absorbed serum samples in the AIGENT assay using serial two-fold dilutions from 1:32 through 1:4,096.[51] Neutralization was then assessed by the reduction in indicator virus plaques formed after running the AIGENT assay. Consistent with

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[48] MRK-CHA00016988. The validation report was completed more than two months after Merck began performing the AIGENT assay on Protocol 007 clinical sera. MRK-CHA00682341 (assay run Dec. 6, 2000).

[49] MRK-CHA00016988, at '001.

[50] MRK-CHA00016988, at '989.

[51] These tests did not include absorbing any sera samples with varicella extracts despite the concomitant administration of the Varivax and M-M-R II vaccines to the subjects in Protocol 007.

AIGENT standard operating procedures, the titer was defined as the highest serum dilution providing ≥ 50% reduction in plaques.

For an assay with high specificity, the expected result would be significantly higher neutralizing titers (lower levels of plaque forming virus) in the samples pre-absorbed with measles, rubella, or mock cell extracts, or culture medium, compared to that of the samples pre-absorbed with mumps extract. The non-mumps extracts should have little or no effect on removing mumps neutralizing antibodies by pre-absorption, so these antibodies would remain in the serum and be detected as inhibiting mumps virus infection and replication during testing. The culture medium used in this test functions as a negative control, which (presumably) lacks antigens available to bind and remove any serum antibodies that might act to block mumps virus infection and replication in the test. Pre-absorption with measles, rubella or mock cell extracts should yield similar results to pre-absorption with culture medium.

Pre-absorption with mumps extract should reduce available mumps-binding serum antibodies in the tested serum samples. This mumps extract pre-absorption would result in a reduction in neutralizing antibody titers in the pre-absorbed serum sample due to depletion during the initial pre-absorption step of the antibodies capable of binding mumps (they would no longer be available to bind mumps during testing). Such a result would demonstrate that the observed neutralization is specific to mumps and not related to the presence of antibodies that bind to the measles, rubella or mock extracts or the culture medium during the pre-absorption step. Merck's results, set forth in the table immediately below, did not show this outcome.[52]

Table 11
Specificity Experiment 1 - Neutralization Titers of Pediatric Sera

		Absorbing Antigen			
Sample	**Medium**	**Mock**	**Mumps**	**Measles**	**Rubella**
5 - 13	512	<32	<32	<32	<32
5 - 14	2048	512	<32	512	32
5 - 16	≥4096	512	256	512	256
5 - 26	256	<32	<32	<32	<32
Mock Plaque Average: 24.5 MKY Control Titer: 512					

Merck applied the same approach in its second specificity experiment except it used adult sera instead of pediatric sera and tested two of them at more (and higher) dilutions. The results

[52] MRK-CHA00016988, at '002.

of this experiment, set forth in the table immediately below, likewise did not consistently show the expected outcome of higher titers (akin to those observed when pre-absorbing using culture medium) in the samples absorbed with measles, rubella or mock extracts compared to that of the samples absorbed with mumps extract.[53]

Table 12
Specificity Experiment 2 - Neutralization Titers of Adult Sera

		Absorbing Antigen			
Sample	Medium	Mock	Mumps	Measles	Rubella
AS	2048	64	<32	64	256
CM	8192	512	<32	512	128
MKY	1024	<32	<32	<32	<32
DK	<32	<32	<32	<32	<32
Mock Plaque Average: 33.8 MKY Control Titer: 1024					

Dr. Joseph Antonello, a Merck statistician who co-authored the validation report, testified at his deposition that the specificity results were "peculiar," "unexpected," and "not strong" in demonstrating the assay was specific to mumps.[54]

3. The AIGENT Testing

The AIGENT testing was conducted in Dr. David Krah's laboratory at Merck's Research Laboratories Division in Merck's West Point, Pennsylvania facility. The bulk of the testing involved counting the plaques at the various dilutions being tested in the pre- and post-vaccination samples and the assay controls. Dr. Krah and his most senior staff virologist Mary Yagodich both directly participated in the plaque counting process and oversaw the plaque counting that was done by the other staff virologists in Dr. Krah's lab. The plaque counting commenced in December 2000 and was largely completed by July 30, 2001. The testing was done in two phases: the preliminary subset phase, which involved roughly one-third of the

[53] MRK-CHA00016988, at '002. These results also showed that one of the adult test subjects (DK) showed a negative neutralization response at all dilutions, including when merely incubated with culture medium, suggesting a significant lack of mumps neutralizing antibodies even at the highest serum concentration tested.

[54] Antonello Dep. 115:6-9 ("In retrospect, looking at the specificity results in this study, they're peculiar. And I think today I might push back a little more on the lab given these results."); 118:14-15, 120:6-8 ("Not the result that I would expect. . . . So I don't understand why that would be the case. So it's an unexpected result."); 121:16-18, ("I'd say these results don't show you that it can distinguish measles and rubella."); 123:25-124:7 (purpose of validation report is to conclude with some level of confidence that assay is specific to antigen being tested and AIGENT validation results "are not strong in that regard"); at 128:21-129:2 ("In looking back now, I would question this result. . . . It's not what I expected, would expect now.").

samples being tested (approximately 600 samples); and the final phase, which involved the balance of the samples.

Merck's original plan was for Dr. Krah and his staff to conduct the preliminary subset phase and to outsource the balance of the testing to the lab of Dr. Richard Ward of the Cincinnati Children's Hospital. However, Merck ultimately decided to conduct all the testing in Dr. Krah's lab. According to Alan Shaw, Merck's then Senior Director of Virus and Cell Biology and Dr. Krah's immediate superior, Merck decided against the outsourcing because of the "heightened importance" of the testing and concerns that Dr. Ward's lab would be "unable to match th[e] level of precision" needed for the test:

> Due to an urgent need for high-precision data to support label changes for M-M-R®II, David Krah's laboratory will be carrying out neutralization tests on approximately 3000 infant serum samples over the next five months. This activity was originally scheduled for transfer to a contract laboratory. Two things have conspired to make this transfer unacceptable from a strategic perspective. First, issues arising following a CBER inspection in MMD have placed a heightened importance on this data set. Second, a preliminary run of about one-third of the serum set has revealed an unanticipated tightness of data from the Krah laboratory. We doubt that the contract lab would be able to match this level of precision.[55]

As explained by Dr. Krah, the plaque counting process worked as follows. Starting from the point where the assay plates were stained and the plaques became visible, Dr. Krah or one of his staff members would look at the clinical sera and control plates (typically with a light box to better visualize the plaques), mark the plaques with a pen, and write the plaque count somewhere on the plate. Then the counter would transcribe the plaque count on a handwritten counting sheet and an Excel spreadsheet where calculations were performed to determine the various plaque counts and titers measured at various dilutions. These calculations were used to determine whether the assays were valid or invalid and whether the samples were seropositive or

[55] MRK-CHA00014739, at '739; MRK-CHA00014746, at '746; MRK-CHA00014829, at '829; MRK-CHA00015702, at '702. *See also* MRK-CHA00014731, at '731 ("The plan for the remaining samples had been to send them to an outside contract laboratory. Recognizing the tightness of the first data set, we realize that an outside laboratory would not be able to reproduce this kind of precision."); MRK-CHA00014744, at '744 (same); MRK-CHA00014749, at '749 (same); MRK-CHA00015719, at '719 (same).

seronegative. They also used the calculated results to determine whether paired pre- and post-vaccination samples could be counted towards seroconversion.[56]

For the interim analysis, Drs. Krah along with Emilio Emini, who at the time was Merck's Vice President of Vaccine and Biologics Research, reviewed the plaque counts as they were completed. When Drs. Emini and/or Krah determined that a recount was warranted, Dr. Krah asked the original counter to recount the particular plate at issue. The counter would then go back to the assay plate, erase from the plate the original plaque count markings and then rewrite on the plate the new plaque count. Then the counter would cross out the previously recorded count on the original handwritten counting sheet and record the new counts. For the majority of the recounts, the counter documented no justification of the reason for the change in the plaque counts. For the remainder of the testing, the counting and recounting procedure was essentially the same, except the plaque counts were entered into an electronic workbook that automatically flagged samples for recounting.[57]

On August 6, 2001, after the vast majority of the plaque counting process had been completed, two representatives from CBER performed an unannounced on-site inspection of Dr. Krah's lab. Following the inspection, CBER issued Merck a Form 483 citing several observations relating to the AIGENT testing and plaque counting procedure, including the changing of raw data without justification.[58] Following the inspection (which included two follow-up visits on August 10 and September 14), CBER described these concerns in more detail, noting (1) "there is no guarantee that the [plaque count] numbers on the worksheet were the original data, even at the time of transfer of count from plate to work sheet," and (2) "a selective re-review of specific assays or wells was undertaken after data analysis of pre- and post-neutralizing antibody titers (e.g., with specific knowledge of matched samples and of pre- or

[56] *See generally* Krah Dep. 418-50.

[57] *See generally* Krah Dep. 418-50.

[58] MRK-CHA00052249, at '250.

post-vaccination status of samples), providing clear opportunity for selective bias to the change."[59]

On February 4, 2002, Merck formally responded to CBER's concerns arising out of the August 6, 2001 inspection. In particular, Merck defended the changes to the AIGENT data with which CBER took issue, stating (1) "the application of the extravariability criteria improves the quality of data obtained and does provide accurate, scientifically sound data for use in decision making," and (2) the changes Merck made to the AIGENT data with which CBER took issue "were made for appropriate reasons," and emphasizing (3) "that laboratory personnel were, and remain, blinded to sample identity other than bleed dates required to pair pre- and post-vaccination sera. This reduces the potential for changes being made which could bias the resulting analysis of the total data set." Nevertheless, Merck proposed and sought CBER's concurrence with "us[ing] the original PRN assay results in the evaluation of the 007 trial," asserting that "[a] reliable record of originally recorded assay results exists, i.e., results before recounting."[60] After consultation with CBER, Merck relied on the "original" AIGENT results going forward.[61]

4. The WT ELISA Validation

At the same time Merck was working through the AIGENT testing, it also was developing and conducting a mumps "wild-type" (WT) ELISA test as a supporting assay to be used in analysis of Protocol 007 serum samples. The mumps antigens used with this WT ELISA were derived from the same low passage version of the Jeryl Lynn mumps vaccine strain Merck used in the AIGENT. Merck completed development and validation of this WT ELISA assay on December 4, 2000. Merck selected 10 ELISA antibody (Ab) units as the threshold for determining seropositivity (serostatus cutoff) for the WT ELISA.[62] This cutoff value was based

[59] MRK-CHA02021754, at '756-57. *See also* MRK-CHA00052249, at '252 ("Dr. Carbone [CBER] stated that if changes in the data were made after results were calculated and selective wells re-reviewed, then the practices were not consistent with GLP.").

[60] MRK-CHA00000410, at '418, 421-422 (Serial No. 80).

[61] MRK-CHA00064011, at '012 ("In these responses, Merck . . . proposed that the original results prior to retesting be used. . . . A decision to accept Merck's proposal was reached the following week . . . .").

[62] MRK-CHA00761129.

on pilot studies that used a panel of 72 selected samples that previously scored as "negative" using Merck's then-existing non-WT ELISA. Twelve of these samples were post-vaccination negatives in the non-WT ELISA test and the rest were pre-vaccination negatives.

As detailed in the validation report, Merck conducted the validation test using only two test criteria, a 5Ab or 10Ab serostatus cutoff. Merck did not assess the statistical impact of using higher serostatus cutoff levels on serostatus outcomes and discordance between the two tests. Using a 5Ab cutoff resulted in a pre-vaccination discordance from the non-WT ELISA of 3 out of 56 samples (5.3%), and a post-vaccination discordance of 3 out of 10 samples (30%). Using a 10Ab cutoff resulted in a pre-vaccination discordance from the non-WT ELISA of 0 out of 56 samples, and a post-vaccination discordance of 2 out of 10 samples (20%). These results are set forth in the table below.[63]

Table 5
Comparison of Serostatus Assignments Using Cutoff of 10 and 5 Ab units

Serostatus	10 Ab units		5 Ab units	
Assignment	Pre	Post	Pre	Post
Negative	56	8	53	7
Positive	0	2	3	3

Based on these results, Merck proposed a 10Ab serostatus cutoff for the WT ELISA.[64]

5. The AIGENT/WT ELISA Correlation

Before allowing Merck to use its WT ELISA to support regulatory decision making, CBER required that Merck correlate the assay to results obtained using its AIGENT assay.[65] CBER insisted on this correlation to ensure the WT ELISA seroconversion results and the

[63] For this analysis, 6 of the 72 selected samples were excluded as extravariable.

[64] MRK-CHA00761129, at '134.

[65] *See* MRK-CHA00761482, at '483 ("It is essential that ELISA testing methods be validated against a working neutralization assay to demonstrate the absence of such problems [false positive and false negative results]. In addition, the assignment of cut-off values for the ELISA needs to be justified."); MRK-CHA00791315, at '319 ("[W]e contended that there was reasonably good agreement between the two assays in terms of serostatus classification when using a cutoff of 10Ab . . . . Concluding that the two assays agree reasonably well was important for the purpose of arguing that the ELISA was an acceptable substitute for the neutralization assay."); MRK-CHA01898773, at '775 ("[E]vidence of a correlation between the current assays and a WT Neut assay, or evidence of correlation with protective efficacy, would allow the current EIA-based assays to be used.").

serostatus cutoff Merck used to get those results were clinically relevant and reflected actual protection from the disease.[66]

In addressing this CBER requirement, Merck conducted a correlation study between the two assays (WT ELISA/AIGENT) using two versions of the AIGENT data, which Merck characterized as the "original" and "corrected" versions of the AIGENT preliminary subset data. On June 10, 2002, Merck submitted to CBER (in Serial No. 86) the results of the correlation study using the "original" AIGENT data.[67] Merck used the correlation to justify to, and seek concurrence from, CBER in its selection of 10Ab as the serostatus cutoff for the WT ELISA assay.[68] Merck represented to CBER that "agreement between the ELISA and AIGENT assays was found to be quite good."[69] Specifically, Merck represented an overall agreement rate of 90.4%, with agreement rates of 87.3% for pre-vaccination samples and 93.6% for post-vaccination samples. The overall agreement based on seroconversion, but excluding pre-positive samples in either assay, was 93.4%.[70]

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[66] MRK-CHA00331831, '831 ("CBER requests additional justification for the cutoff chosen for the mumps ELISA. The observation that the ELISA cutoff is sufficiently high to accurately classify pre-vaccination sera as negative is useful, but insufficient by itself as it does not relate to seroprotection."); MRK-CHA00561467, at '467 (same); MRK-CHA00846454, at '454 ("PRN is a functional assay–correlate of protection. ELISA is not a functional assay but an antibody assay. We need to convince CBER that the ELISA will provide equivalent results to PRN and thus equate (bridge) to protection."); MRK-CHA00331831, at '833 ("Biological relevance of the 10Ab ELISA units cutoff: . . . CBER was not satisfied with the rationale as this does not relate the cutoff in any fashion to seroprotection but rather is circular in that Merck is verifying that Merck's historical experience with the legacy ELISA assay is consistent with the outcome of this new assay."); MRK-CHA00818776, at '778 ("Justification is required for the new mumps cutoff of 10 ELISA antibody units . . . . CBER requested that the ELISA results be compared to the mumps Plaque Reduction Neutralization (PRN) assay. CBER would like the rationale for the new cutoff to be linked to a biologically relevant reference standard."); MRK-CHA01583397, at p. 10 ("CBER: Provide biological justification . . . Does the AIGENT assay support the WT ELISA cutoff of 10Ab units?"); MRK-CHA00198876, at '878 ("CBER requires that SCR reflect protective efficacy."); MRK-CHA00126963, at '970 ("[T]he appropriateness of the cutoff employed in the ELISA for seropositivity should be supported by data demonstrating some relevance with protective levels of antibody (e.g., neutralizing antibody)."); MRK-CHA01927351, at '351 ("in the mumps end expiry [] we are trying to establish clinical protective efficacy").

[67] MRK-CHA00761628.

[68] MRK-CHA00761628, at '629-630 ("This submission is in response to CBER's request for additional information regarding the cutoff chosen for the Mumps WT ELISA comparing the ELISA cutoff to the AIGENT assay cutoff . . . . CBER concurrence is requested for . . . Mumps WT ELISA cutoff of 10Ab units.").

[69] MRK-CHA00761628, at '672. *See also* MRK-CHA00224982, at '032 (CSR) (referencing "excellent correlation between mumps PRN and ELISA").

[70] MRK-CHA00761628, at '673-674.

These correlation results were derived from cross-classification analyses Merck performed comparing the pre- and post-vaccination serostatus of the subject samples as measured in the AIGENT and WT ELISA tests. For three of these calculations, the correlation percentages were based on a ratio of the number of samples with the same serostatus classification measure from both assays to the total number of samples. This was done using a combined pre- and post-vaccination sample set, the pre-vaccination samples alone, and the post-vaccination samples alone. The fourth calculation was a ratio of the number of samples that had the same seroconversion result (either positive or negative) in both assays divided by the total number of samples, excluding any sample that was pre-positive in one or both assays. A summary of these cross-classification analyses is shown in the tables below.

Table 4
Sero-Status Cross-Classification for M-M-R®II 007 Pre- and Post-Vaccination Samples

		AIGENT		
		>=32	<32	Total
ELISA	>=10	469	29	498
	<10	69	456	525
	Total	538	485	1023

Table 5
Sero-Status Cross-Classification for M-M-R®II 007 Pre-Vaccination Samples

		AIGENT		
		>=32	<32	Total
ELISA	>=10	3	7	10
	<10	58	442	500
	Total	61	449	510

Table 6
Sero-Status Cross-Classification for M-M-R®II 007 Post-Vaccination Samples

		AIGENT		
		>=32	<32	Total
ELISA	>=10	466	22	488
	<10	11	14	25
	Total	477	36	513

Table 7
Sero-Conversion Cross-Classification for M-M-R®II 007

		AIGENT		
		>=32	<32	Total
ELISA	>=10	400	19	419
	<10	10	13	23
	Total	410	32	442

Following Merck's submission of these correlation results, CBER concurred with Merck's proposal to use a WT ELISA cutoff of 10Ab.[71]

D. Merck's Submissions of Protocol 007 Data to the FDA

1. The Interim AIGENT Results

On March 12, 2001, Merck submitted to CBER an interim analysis of the preliminary subset of the AIGENT assay.[72] The subset covered 600 of the test subjects and was based on what Merck characterized as the "corrected" AIGENT data. This subset incorporated the plaque count changes Dr. Krah and his staff made as part of the recounting procedure Drs. Krah and Emini had employed during the testing. Merck reported to CBER (1) "Mumps neutralizing antibody seroconversion rates at mumps vaccine doses of 4.9 and 4.0 $\log_{10}$ $TCID_{50}$ are comparable (94.1% and 93.3% respectively)"; and (2) "The seroconversion rate at 3.7 $\log_{10}$ $TCID_{50}$ . . . while somewhat lower . . . is also well within the historical seroconversion range . . . that have been associated with high field effectiveness in clinical trials."[73] Merck further reported that the "seroconversion rate range seen in this preliminary data is consistent with other neutralization data which were associated with high levels (97%) of protection."[74]

Merck also submitted the "corrected" interim AIGENT data to CBER in response to a February 9, 2001 Warning Letter concerning CBER's finding that certain mumps vaccine

[71] MRK-CHA00000561, at '561.

[72] MRK-CHA00017036 (Serial 63).

[73] MRK-CHA00017036, at '045.

[74] MRK-CHA00017036, at '048.

products had failed to meet the minimum potency specifications.[75] In light of this finding, CBER requested that Merck provide "an analysis of Mumps stability data describing the range of potencies [Merck] would expect the various Mumps Vaccine products to reach at the two-year expiration date."[76] Among the data and information Merck submitted in this response was a summary of the preliminary results from its AIGENT testing.[77] Merck concluded that these results, along with the other information it submitted "taken together provide evidence that M-M-R®II is effective through the predicted range of potencies post-release within a 2 -year expiration date."[78]

Merck also relied on its interim analysis of the "corrected" AIGENT data in its April 20, 2001 Biologics Product Deviation Report (BPDR) to CBER on certain M-M-R II lots that failed to comply with the mumps end-expiry potency specifications.[79] Merck referred back to the summary of the "corrected" AIGENT preliminary results it submitted in response to the February 9 Warning Letter. Merck stated that M-M-R II with a mumps potency as low as 3.9 is "efficacious and the possibility of seroconverting is essentially equivalent to that of a child who receives a dose at 4.9 or 4.0 log$TCID_{50}$/dose."[80] Merck told CBER that based on this preliminary data, "potency values in the range of 3.9 log$TCID_{50}$/dose or above are not likely to

[75] MRK-CHA00209399, at '402 ("Our investigators reported that the data in your firm's files showed that a number of Mumps Vaccine stability samples representing lots manufactured before the formulation was changed during February 2000 failed to meet the minimum potency specification.").

[76] MRK-CHA00209399, at '402.

[77] MRK-CHA00585231, at '237 ("Preliminary data from end-expiry clinical trials for M-M-R®II . . . have recently become available. . . . This seroconversion rate range is consistent with several older field efficacy studies that demonstrated seroconversion rates ranging between 83 and 93 %, which still afforded high levels of protection against mumps infection.").

[78] MRK-CHA00585231, at '237. *See also* MRK-CHA00207690, at '708 ("In addition, and more seriously, [CBER] challenged the efficacy of marketed product at the lowest predicted potencies (below label claim). . . . With regard to product efficacy, we provided an interim analysis of an ongoing mumps end-expiry trial to justify efficacy of lower potency product. CBER accepted the Merck response."); MRK-CHA00615152, at '159 (same); MRK-CHA00019225, at '230 (same); MRK-CHA00094161, at '167 (same); MRK-CHA00322038, at '041 ("Provided interim analysis of mumps end-expiry trial data to justify efficacy of lower potency product.").

[79] MRK-CHA00754233, at '234 ("Four of the five lots tested yielded results below the label claim of 4.3 log $TCID_{50}$/dose, but higher than the projected worst-case values of less than 3.7 log$TCID_{50}$/dose.").

[80] MRK-CHA00754233, at '236.

lead to a lack of immunity against mumps" and that "no further action is warranted" for its out-of-specification lots.[81]

2. The Final AIGENT Results

On December 19, 2003, Merck submitted to CBER the Protocol 007 Clinical Study Report (CSR), which contained the complete set of AIGENT serum analysis data.[82] These data were submitted in support of Merck's Supplemental Biologics License Application (sBLA) to lower the end-expiry potency specification for the mumps component of M-M-R II. The submitted results covered the full universe of test subjects and comprised what Merck characterized as the "original" AIGENT data. The final AIGENT results Merck reported were as follows: (1) for the candidate mumps expiry dose of 3.8 $\log_{10}$ $TCID_{50}$, a seroconversion rate of 89.3% (with a 95% confidence interval range of 86.1-92.0%); (2) for the candidate mumps expiry dose of 4.1 $\log_{10}$ $TCID_{50}$, a seroconversion rate of 93.3% (with a 95% confidence interval range of 90.5-95.5%); and (3) for the 4.8 $\log_{10}$ TCID50 control arm of the study, a seroconversion rate of 92.2% (with a 95% confidence interval range of 89.3-94.6%).[83]

Based on these results, Merck concluded "M-M-R™II with a mumps expiry dose of 4.1 $\log_{10}$ $TCID_{50}$ is highly immunogenic and induces an acceptable mumps-specific neutralizing antibody SCR."[84] Merck further concluded that these results "support the effectiveness of M-M-R™II containing a mumps virus potency of no more than 4.1 $\log_{10}$ $TCID_{50}$ and the lowering of the mumps virus end expiry potency from the currently assigned potency of 4.3 $\log_{10}$ $TCID_{50}$ to no less than 4.1 $\log_{10}$ $TCID_{50}$."[85]

3. The Final WT ELISA Results

The CSR Merck submitted on December 19, 2003 also contained the final WT ELISA results Merck submitted to CBER in support of its end-expiry sBLA. The final WT ELISA

[81] MRK-CHA00754233, at '236.

[82] MRK-CHA00224982.

[83] MRK-CHA00224982, at '005.

[84] MRK-CHA00224982, at '178.

[85] MRK- CHA00224982, at '175.

results Merck reported were as follows: (1) for the candidate mumps expiry dose of 3.8 $\log_{10}$ $TCID_{50}$, a seroconversion rate of 94.1% (with a 95% confidence interval range of 91.9-95.9%); (2) for the candidate mumps expiry dose of 4.1 $\log_{10}$ $TCID_{50}$, a seroconversion rate of 97.4% (with a 95% confidence interval range of 95.8-98.6%); and (3) for the 4.8 $\log_{10}$ TCID50 control arm of the study, a seroconversion rate of 98.0% (with a 95% confidence interval range of 96.5-98.9%).[86]

As with its final AIGENT results, Merck reported to CBER that the ELISA results "support the effectiveness of M-M-R™II containing a mumps virus potency of no more than 4.1 $\log_{10}$ $TCID_{50}$ and the lowering of the mumps virus end expiry potency from the currently assigned potency of 4.3 $\log_{10}$ $TCID_{50}$ to no less than 4.1 $\log_{10}$ $TCID_{50}$."[87] Merck further represented that the "mumps wild-type ELISA used in this study was shown to correlate with the PRN assay . . . and previous studies have established a strong correlation between the development of mumps-specific neutralizing antibodies and vaccine efficacy."[88]

4. Merck's Supplemental Biologics License Application

On January 29, 2004, Merck submitted to CBER the sBLA, which proposed lowering the mumps end-expiry potency specification for M-M-R II.[89] This sBLA included the results of Protocol 007 including the final AIGENT and WT ELISA results contained in the CSR. Merck summarized these results as follows:

> The clinical data described herein demonstrate that M-M-R™II with a mumps virus potency within the release range (based on a vaccine lot containing a mumps virus potency of 4.8 $\log_{10}$ $TCID_{50}$ per dose). Lowering the mumps virus potency to 4.1 $\log_{10}$ $TCID_{50}$ per dose maintains > 90% seroconversion using a mumps neutralization assay, thus preserving the excellent safety and efficacy profile of the vaccine.[90]

[86] MRK-CHA00224982, at '005.

[87] MRK-CHA00224982, at '175.

[88] MRK-CHA00224982, at '175.

[89] MRK-CHA00000032.

[90] MRK-CHA00000032, at '110.

This sBLA contained several other representations concerning the clinical relevance of the Protocol 007 results, including (1) "The PRN assay was used as the primary endpoint because it is a functional assay that measures the ability of the vaccine-induced immune response to inhibit viral replication in vitro, and can, therefore, be considered a surrogate for vaccine effectiveness";[91] (2) "In agreement with CBER/FDA, the mumps-specific PRN assay was developed and used as a surrogate for vaccine effectiveness";[92] and (3) "Study results showed that M-M-R™II with a candidate mumps end-expiry potency of 4.1 $\log_{10}$ $TCID_{50}$/dose induced acceptable levels of mumps-specific antibodies by PRN assay."[93]

Following Merck's submission of the sBLA, Merck made several additional submissions to CBER that relied on the Protocol 007 data. These were submitted in response to specific CBER questions concerning the clinical relevance of the Protocol 007 data. In particular, on July 27, 2004, CBER requested further assurances that "the cutoff employed in the ELISA for seropositivity should be supported by data demonstrating some relevance with protective levels of antibody (e.g., neutralizing antibody)."[94] Merck responded on November 17, 2004 by referring back to the AIGENT/WT ELISA correlation it provided in Serial 86, which it characterized as "provid[ing] information on the clinical relevance of the chosen ELISA cutoff for seropositivity."[95]

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[91] MRK-CHA00000032, at '111-112. *See also* MRK-CHA00000315, at '321 (Merck responding to CBER questions seeking additional support for sBLA: "The purpose and underlying of this [sBLA] file is to provide clinical data supporting a reduction in expiry potency of the mumps component of M-M-R®II. This clinical data provides evidence that a mumps end expiry potency of 4.1 log10 TCID50 dose in M-M-R®II at the end of its shelf life is not statistically different to that of the product at release [based on Mumps Plaque Reduction Neutralization (PRN) assay used as a surrogate marker for vaccine efficacy].") (brackets in original); MRK-CHA00030994, at '994 (CBER's Steve Rubin characterizing AIGENT as an assay "for measuring vaccine immunogenicity as a surrogate for efficacy in a clinical trial"). Merck likewise represented to the public that Protocol 007 was a surrogate for clinical efficacy through the poster presentation on Protocol 007 it gave at a October 2, 2004 meeting of the Infectious Diseases Society of America. MRK-CHA00337141, at '144 ("Antibody response measured by mumps-virus specific plaque reduction neutralization (PRN) assay was used as a surrogate of vaccine efficacy.").

[92] MRK-CHA00000032, at '116.

[93] MRK-CHA00000032, at '118. *See also* at '122 ("M-M-R™II with a mumps end-expiry potency of 4.1 $\log_{10}$ $TCID_{50}$/dose . . . [i]s highly immunogenic and induces acceptable mumps specific antibody responses by PRN.").

[94] MRK-CHA00126963, at '970.

[95] MRK-CHA00126963, at '963, 967. Merck made an identical submission on November 12, 2004 in response to CBER's request for similar assurances in connection with Merck's license application for ProQuad. MRK-CHA00846405, at '409, 414 (stating Serial 86 provides "information on the clinical relevance of the chosen ELISA cutoff for seropositivity").

Merck again relied on Serial 86 in responding to CBER's questioning on the clinical relevance of the WT ELISA assay it performed. On December 3, 2004, CBER notified Merck of its finding that the sBLA was insufficient, finding "that the information and data submitted are inadequate for final approval at this time."[96] As part of its response to CBER's request for additional justification for the WT ELISA data Merck submitted in Protocol 007, Merck again pointed to Serial 86 to support the clinical relevance of the 10Ab serostatus cutoff:

> CBER requested the mumps ELISA seropositive cutoff be justified via use of known mumps neutralizing and non-neutralizing sera. Merck submitted these data (June 2002, serial # 86) and believes that they provide helpful supportive information on the clinical relevance of the chosen ELISA cutoff for seropositivity.[97]

Merck again relied on Serial 86 in response to CBER's initial rejection of the AIGENT results. On October 17, 2005, CBER found the AIGENT data Merck submitted in support of the sBLA were "inadequate to support the label change."[98] Merck responded by pointing to Serial 86 and what it characterized as the "strong correlation (93.6%) between ELISA and PRN serology results."[99] From this Merck argued that the ELISA results were sufficient to support the sBLA despite the deficiencies CBER found with the AIGENT results.[100]

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[96] MRK-CHA00000315, at '356.

[97] MRK-CHA00000315, at '331.

[98] MRK-CHA00560257, at '257. Included among the deficiencies CBER identified were: (1) "A substantial amount of sample data was excluded from the analyses [only 437 out of 672 subjects were included] . . . preclud[ing] a conclusion of success;" and (2) "The control lot failed the acceptability criteria." CBER recommended that if Merck wanted to pursue the sBLA it "support the proposed label change by correlating these study data and/or other relevant mumps vaccine immunogenicity data with the immunogenicity data from the original efficacy studies." CBER further stated that Merck "could also consider shortening the [mumps] end-expiry dating period based upon these data." MRK-CHA00560257, at '257-258.

[99] MRK-CHA00000393, at '400. Notably, in citing to Serial 86, Merck appears to misstate to CBER what the actual agreement between the two assays was. Merck cites a 93.6% correlation rate when Serial 86 provides a 90.4% overall agreement rate and a 93.4% overall agreement rate for seroconversion. *See* MRK-CHA00761628, at '672.

[100] MRK-CHA00000393, at '404 ("The observed mumps ELISA results (SCR 98.0%) and the strong correlation between ELISA and PRN assay provide indirect evidence about the likely outcome for the missing data in the 4.8 $\log_{10}$ $TCID_{50}$ mumps potency group.").

E. FDA's Approval of Merck's Supplemental Biologics License Application

On May 18, 2007, CBER issued its final rejection of the AIGENT data Merck submitted in support of the sBLA:

> Our review finds that the information and data submitted are inadequate for final approval at this time. We cannot accept use of multiple imputation analyses of the PRN data to support the lowering of mumps vaccine end-expiry potency. However, the science related to immunogenicity testing of M-M-R®II has substantially evolved since our initial testing requirements. Use of ELISA data to evaluate the effect of differences in product potency on immunogenicity is now acceptable.[101]

CBER stated that Merck's sBLA to the lower the mumps end-expiry potency for M-M-R II could be supported by two analyses:

> 1. Product consistency
>
> We request a demonstration of consistency between Sublot 3 (4.8 $\log_{10}$ $TCID_{50}$ mumps potency) in the present study [Protocol 007] and two other lots used in previous MMR studies, e.g., Protocols 010-012, with mumps potency of at least 4.8 $\log_{10}$.
>
> . . .
>
> 2. Non-inferiority
>
> If consistency among the three lots is demonstrated as described above, the ELISA results of the three lots are pooled to form a control group (C). Non inferiority of Sublot 2 (4.1 $\log_{10}$ $TCID_{50}$ mumps potency) from this supplement will be demonstrated by comparing ELISA results of this sublot (T) with ELISA results of the pooled control group which has at least 4.8 $\log_{10}$ mumps potency.[102]

On June 5, 2007, Merck amended this end-expiry sBLA by providing additional information in support of the consistency and non-inferiority tests CBER outlined above: "These responses include the statistical analysis requested by CBER for product consistency and non-inferiority based on the ELISA assay and support the change in mumps end expiry potency to 12,500 $TCID_{50}$."[103] The data Merck used for the product consistency statistical analysis were the Protocol 007 WT ELISA results from the 4.8 $\log_{10}$ control lot, the Protocol 009 WT ELISA

[101] MRK-CHA00000368, at '372.

[102] MRK-CHA00000368, at '372-373.

[103] MRK-CHA00000368, at '368.

results from the subjects vaccinated with M-M-R II without rHA (discussed below), and the Protocol 012 WT ELISA results from the subjects vaccinated with M-M-R II (discussed below). The data Merck used for the non-inferiority statistical analysis were a pool of these three consistency lots which were compared to the Protocol 007 WT ELISA results from the 4.1 $\log_{10}$ testing lot.

On July 20, 2007 CBER requested additional follow up on Merck's sBLA amendment, including the underlying WT ELISA data (from Protocols 007, 009 and 012) supporting the submission.[104] On August 8, 2007, Merck responded to CBER's request, providing the "XPT file for each study [and] a document describing the contents of the data set."[105] These were "the same files that were submitted with the original filings of these [Protocol 007, 009, and 012] studies."[106] For Protocol 007, this included "a description of the immunogenicity analysis data set," and "prevaccination and postvaccination serology results."[107]

On December 6, 2007, CBER approved Merck's sBLA to "include a change in the labeled potency of the mumps component from no less than 20,000 $TCID_{50}$ to no less than 12,500 $TCID_{50}$ per dose at end of expiry."[108]

F. FDA's Approval of ProQuad

CBER allowed Merck to conduct a non-inferiority study (compared to M-M-R II) to support its ProQuad Biological License Application (BLA) because in CBER's view "MMRV is essentially composed of licensed products and the efficacy of those products has already been demonstrated."[109] CBER also accepted Merck's proposal that for its ProQuad BLA, "assays other than virus neutralization, such as the proposed [WT] ELISA, can be used to measure

[104] MRK-CHA00000140, at '145.

[105] MRK-CHA00000140, at '186.

[106] MRK-CHA00000140, at '186.

[107] MRK-CHA00000140, at '161.

[108] MRK-CHA01519087, at '088.

[109] MRK-CHA00846451, at '451.

mumps virus immunogenicity for this study."[110] However, as it did with Protocol 007, CBER requested that the serostatus "cutoff employed in the ELISA for seropositivity should be supported by data demonstrating some relevance with protective levels of antibody (e.g., neutralizing antibody)."[111]

On August 27, 2004, Merck submitted its BLA for ProQuad.[112] In support of its application, Merck submitted the results of five clinical trials (Protocols 009, 011, 012, 013 and 014) "designed to show that ProQuad™ could provide the same level of protection to the same intended population as M-M-R™II and VARIVAX™."[113] Merck represented that the results of these trials (1) "confirm that ProQuad™ is generally well tolerated and highly immunogenic," and (2) "confirm that ProQuad™ is similar (non-inferior) to M-M-R™II and VARIVAX™ given concomitantly . . . with respect to immunogenicity and safety [and] suggest that ProQuad™ can be used in place of M-M-R™II and VARIVAX™ to prevent measles, mumps, rubella, and varicella."[114]

In relying on these immunogenicity tests, Merck had previously sought and obtained CBER's agreement that for Merck's ProQuad BLA, "[e]valuation of immunogenicity using validated assays could be used as a surrogate measure for efficacy."[115] For three of the studies (012, 013 and 014), Merck relied on the WT ELISA Merck developed for Protocol 007 using the low-passage Jeryl Lynn mumps vaccine strain and 10Ab serostatus cutoff.[116] Merck represented these test results could be used as a correlate of protection against mumps:

> Merck [] has assessed the correlation between neutralizing antibody (as measured in a plaque reduction neutralization [PRN] assay) and a wild-type enzyme-linked immunosorbent assay (ELISA) []. The overall agreement rate was 93.6% [].

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[110] MRK-CHA00846451, at '451.

[111] MRK-CHA00846451, at '451.

[112] MRK-CHA00157572.

[113] MRK-CHA00158126, at '137.

[114] MRK-CHA00158126, at '156, '158.

[115] MRK-CHA00158126, at '135. *See also* MRK-CHA00821967, at '969 ("we're banking on immunogenicity data to serve as surrogate markers for efficacy").

[116] MRK-CHA00158320, at '371; MRK-CHA00158126, at '138.