> These data support the use of the results of a wild-type ELISA as a correlate for protection.[117]

On October 5, 2004, CBER requested "additional data to support the appropriateness of the cutoff employed in the mumps WT ELISA for seropositivity, relative to the plaque reduction neutralization assay."[118] Specifically, "CBER requested the mumps ELISA seropositive cutoff be justified via use of known mumps neutralizing and non-neutralizing sera."[119] This followed from CBER's ultimate request for assurances that the ELISA cutoff "be supported by data demonstrating some relevance with protective levels of antibody."[120] Merck responded on November 12, 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."[121] On September 6, 2005, CBER approved Merck's ProQuad BLA, authorizing Merck to sell the vaccine in the U.S.[122]

G. FDA's Approval of M-M-R II with rHA

Until 2005, M-M-R II was manufactured using human serum albumin (HSA) as a stabilizer. Albumin is a protein used in the production of the viral bulks (pooled lots of measles, mumps, or rubella virus) which are blended to manufacture the final lots of M-M-R II. HSA comes from pooled human serum which is then pasteurized to inactivate viral contaminants. Because HSA is derived from humans, it is possible that adventitious agents (such as bacteria, viruses, prions, or fungi) in the albumin could impair the safety of the vaccine. According to Merck, "eliminating HSA from M-M-R™II would reduce greatly this theoretical risk." To this

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[117] MRK-CHA00158320, at '350 (citing Ref. 5.4: 107). While the 93.6% agreement rate derives from the correlation Merck performed using the "original" AIGENT results, Merck cited to and attached to its BLA the correlation study it conducted using the "corrected" AIGENT results. MRK-CHA00158299, at '304.

[118] MRK-CHA00846405, at '405.

[119] MRK-CHA00846405, at '409.

[120] MRK-CHA00846405, at '406.

[121] MRK-CHA00846405, at '409, '414. Merck relied on Serial 86 again in responding to CBER questions on the specificity and sensitivity of the WT Mumps ELISA Merck used to support its ProQuad BLA: "The serostatus cutoff has been evaluated against that of a mumps neutralization assay, and the data show good agreement between assays when using a cutoff of 10Ab . . . ." MRK-CHA00846087, at '090 (citing Serial 86).

[122] MRK-CHA00761865. The FDA's September 2005 approval was for the frozen version of ProQuad. Merck obtained FDA approval for a refrigerated version of ProQuad in 2006.

end, Merck sought CBER approval to replace the HSA in M-M-R II with albumin extracted from yeast (rHA) through an sBLA.[123]

As with ProQuad, CBER allowed Merck to demonstrate equivalence between the HSA and rHA formulations of M-M-R II using an ELISA test.[124] Merck did so in Protocol 009, which had as two of its primary objectives: demonstrating (1) "similar [] antibody response rates" between the two formulations of M-M-R II, and (2) that M-M-R™II manufactured with rHA will induce acceptable antibody response rates" to mumps.[125] For this testing, Merck employed the WT ELISA assay it developed for and used in Protocol 007.[126]

Just as it did with the mumps end-expiry sBLA (Protocol 007) and with the ProQuad BLA (Protocols 012, 013 and 014) CBER requested that Merck "provide AIGENT data in support of the ELISA cutoff" used in Protocol 009.[127] In response, Merck submitted Serial 53 (BBIND-10076), which referred back to Serial 86, stating "[t]his question was addressed previously, in the course of addressing comments to IND 1016 [mumps end-expiry sBLA]." Serial 53 also attached Serial 86, including the AIGENT/WT ELISA correlation report.[128]

On June 30, 2004, Merck submitted its sBLA for approval of M-M-R II formulated with rHA "[t]o address ongoing safety and sourcing concerns relating to human blood-derived products."[129] The submission relied on the results of Protocol 009 for immunogenicity data to support Merck's claim of equivalence between the HSA and rHA formulations of M-M-R II. Specifically, Merck represented Protocol 009 "support[ed] the replacement of HSA with rHA . . .

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[123] MRK-CHA00140056, at '079.

[124] MRK-CHA01386177, at '177 ("[I]n terms of why PRN and ELISA in the mumps end expiry and only ELISA in the MMRII/rHA – and this was CBER's explanation because we asked the same question regarding why the need for a PRN – CBER considers a neutralization assay essential for establishing efficacy where you need to define effectiveness for a product –the mumps end expiry trial is comparing release to expiry within the same product – however when you are comparing equivalence between two products – CBER considers ELISA sufficient.").

[125] MRK-CHA00140056, at '071.

[126] MRK-CHA00137854, at '854-55; MRK-CHA00138137, at '147; MRK-CHA00140056, at '109.

[127] MRK-CHA00124554, at '609.

[128] MRK-CHA00124554, at '588. '640.

[129] MRK-CHA00137854, at '854. Merck attached to this sBLA a copy of the Protocol 007 CSR Synopsis as a reference. MRK-CHA00141341. *See also* MRK-CHA00140053.

based on the following study results: M-M-R™II with rHA induced acceptable antibody response rates for . . . mumps . . . that are similar to those induced by M-M-R™II with HSA."[130]

In describing the WT ELISA used in Protocol 009, Merck stated the test had been correlated with functional assays that Merck had previously correlated to protection:

> Specific levels of serum antibodies to measles, mumps, and rubella as measured by hemagglutination inhibition [HI] and serum neutralizing antibody assays in field efficacy studies have been shown to correlate with protection against these diseases, and thus immunogenicity data can be used as a surrogate marker for vaccine efficacy. Correlation between the current assays (enzyme linked immunosorbent assay [ELISA]) and the assays used in the field efficacy studies (i.e., HI assay and serum neutralizing antibody assay) has been established.[131]

Merck likewise represented that the Protocol 009 results "suggest the M-M-R™II with rHA is highly immunogenic, well tolerated, and will be as effective as M-M-R™II with HSA in preventing measles, mumps, and rubella,"[132] and the switch to rHA "was not expected to affect the efficacy of the vaccine."[133] In August 2005, FDA approved the rHA change for M-M-R II.[134]

V. MY FINDINGS

A. Merck's AIGENT Assay Failed to Provide a Reliable or Clinically Relevant Measure of Protection Against Mumps

1. The AIGENT Assay Was a Results-Oriented Test

Pivotal clinical trials such as Protocol 007, which are designed to support new or supplemental drug or biologics licensing applications to the FDA, must be designed and

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[130] MRK-CHA00140056, at '197.

[131] MRK-CHA00138137, at '144-45 (internal cites omitted). Merck does not cite the prior mumps efficacy studies or any mumps studies to support the claimed correlations.

[132] MRK-CHA00140056, at '196.

[133] MRK-CHA00138137, at '147 ("No studies of the efficacy of M-M-R™II with rHA or M-M-R™II were performed in support of this application. . . . Given the structural and genetic similarities of HSA and rHA, as well as the excellent and high level of vaccine-induced immune responses observed for both treatment groups in Protocol 009, the replacement of HSA with rHA in the bulk manufacturing of M-M-R™II was not expected to affect the efficacy of the vaccine.").

[134] MRK-CHA00141909, at '909 ("We have approved your request to supplement your biologics license application . . . to replace the currently used Human Derived Serum Albumin (HSA) with Recombinant Human Albumin"). *See also* MRK-CHA00761865, at 865 (CBER's September 6, 2005 letter stating Merck is "hereby authorized to introduce or deliver for introduction into interstate commerce").

performed to demonstrate substantial evidence through adequate and well-controlled studies that the product satisfies the criteria required for licensing. Adequate and well-controlled studies must demonstrate a statistically significant outcome relating to a clinically meaningful endpoint. And they must be designed and carefully controlled to minimize bias. The data reported must be accurate and complete, and adequate quality control systems must be implemented, verified and used to ensure that the integrity and accuracy of the reported data are above reproach.[135]

Design and performance of clinical trials must not incorporate characteristics, features or tests which have been selected to favor one clinical outcome over another or to influence study or test performance outcomes to improve the chances of achieving a predetermined outcome or objective. Clinical trials designed to achieve one desired outcome over another, rather than to objectively assess study results and compare differences between study groups, will result in unacceptable study outcome bias.[136]

The need for objective and well-controlled studies is particularly important when performing human clinical research.[137] Accepted human research ethics requires a balance between risks incurred by study subjects and the benefits to society likely to be derived from the study.[138] A study designed with a bias towards achieving a predetermined outcome compromises the risk/benefit balance required to justify exposing patients to the risks and

[135] Schulz, K. F. (1995). "Subverting randomization in controlled trials." JAMA 274(18): 1456-1458; Begg, C., M. Cho, S. Eastwood, R. Horton, D. Moher, I. Olkin, R. Pitkin, D. Rennie, K. F. Schulz, D. Simel and D. F. Stroup (1996). "Improving the quality of reporting of randomized controlled trials. The CONSORT statement." JAMA 276(8): 637-639; Moher, D., S. Hopewell, K. F. Schulz, V. Montori, P. C. Gotzsche, P. J. Devereaux, D. Elbourne, M. Egger, D. G. Altman and G. Consolidated Standards of Reporting Trials (2010). "CONSORT 2010 Explanation and Elaboration: Updated guidelines for reporting parallel group randomised trials." J Clin Epidemiol 63(8): e1-37.

[136] Wood, L., M. Egger, L. L. Gluud, K. F. Schulz, P. Juni, D. G. Altman, C. Gluud, R. M. Martin, A. J. Wood and J. A. Sterne (2008). "Empirical evidence of bias in treatment effect estimates in controlled trials with different interventions and outcomes: meta-epidemiological study." BMJ 336(7644): 601-605; Gotzsche, P. C. (1989). "Methodology and overt and hidden bias in reports of 196 double-blind trials of nonsteroidal antiinflammatory drugs in rheumatoid arthritis." Control Clin Trials 10(1): 31-56; Jarvinen, T. L., R. Sihvonen, M. Bhandari, S. Sprague, A. Malmivaara, M. Paavola, H. J. Schunemann and G. H. Guyatt (2014). "Blinded interpretation of study results can feasibly and effectively diminish interpretation bias." J Clin Epidemiol 67(7): 769-772.

[137] FDA Guidance for Industry: E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) (Available at https://www.fda.gov/downloads/Drugs/Guidances/UCM464506.pdf); Guidelines for good clinical practice (GCP) for trials on pharmaceutical products; WHO Technical Report Series, No. 850, 1995, Annex 3 (Available at http://apps.who.int/medicinedocs/en/d/Jwhozip13e/; Handbook for Good Clinical Research Practice (GCP): Guidance for Implementation (Available at http://apps.who.int/medicinedocs/documents/s14084e/s14084e.pdf).

[138] FDA Guidance for Industry: Investigator Responsibilities — Protecting the Rights, Safety, and Welfare of Study Subjects (Available at https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM187772.pdf).

inconvenience of a clinical research study. A biased study also may provide false information as to the purported benefit (or lack of benefit) the study subject has received from participating in the study, such as whether the vaccine being injected into the test subject actually provides protection against the disease. These general ethical fundamentals are even more important in pediatric studies, where the study subject is unable to provide informed consent on their participation and must rely on the parent/guardian instead. [139]

Despite these well-accepted standards for avoiding bias in human clinical research, Merck designed the AIGENT as a results-oriented test to ensure it achieved its predetermined criteria of a seroconversion rate of at least 95%. This is evident from Merck's multiple efforts to "optimize" the assay to ensure it reached the desired result. As I discussed above and in more detail below, this included, among other things, using a vaccine strain rather than a true wild-type strain as the testing antigen, adding anti-IgG to artificially boost the neutralization response, ignoring the poor specificity of the assay, failing to adequately blind the plaque counters, and selectively identifying test samples for recounting.

The results-oriented nature of Protocol 007 is further demonstrated by the numerous internal documents Merck prepared setting forth the AIGENT assay development objectives. These documents describe an objective of designing a test that would achieve a minimum 95% seroconversion rate, not one that would accurately measure seroconversion by neutralizing antibodies (as a proxy for measuring protection against disease) as CBER required. [140] Some of these documents further define a results-oriented design objective of ensuring a level of pre-

[139] Committee On Bioethics. (2016). "Informed Consent in Decision-Making in Pediatric Practice." Pediatrics 138(2); 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):133-7.

[140] *See, e.g.,* MRK-CHA00019225, at '240 ("The goal throughout the development of the mumps PRN assay was to create an assay that achieved a 95% seroconversion rate."); MRK-CHA00561111, at '112 (same); MRK-CHA00026466, at '470 ("A plaque-reduction neutralization assay . . . is being optimized . . . with the goal of providing an assay that permits measurement of a ≥ 95% seroconversion rate."); MRK-CHA00065695, at '696 (AIGENT "Objective: Identify a mumps neutralization assay format using 'wild-type' mumps strain that permits measurement of ≥95% seroconversion rate in M-M-R®II vaccines."); MRK-CHA00026912, at '912 (AIGENT "Objective: Identify a mumps neutralization assay format using 'wild-type' mumps strain that permits measurement of ≥95% seroconversion rate in M-M-R®II vaccines."); MRK-CHA00016632, at '632 ("Modification of the standard mumps Nt assay to include . . . anti-human IgG . . . has been evaluated to determine whether the sensitized assay is capable of detecting ≥95% seroconversion in vaccines. This target has been established by CBER to demonstrate adequate immunogenicity of mumps vaccine in current use."). *See also* Krah Dep. 723:1-14 (Goal of AIGENT test was "to develop an assay that was capable of detecting a 95 percent seroconversion. . . . This assay was developed with the requirement to meet the seroconversion target and a target . . . pre-positivity rate.").

positives less than 10%.[141] Since Protocol 007 specified that immune response data from pre-positive subjects would be excluded from primary endpoint statistical analyses, a higher pre-positive rate risked compromising the statistical power of the study.[142]

Merck's results-oriented approach to designing the AIGENT assay for Protocol 007 is further demonstrated by comparing it to the objective Merck articulated for designing the standard PRN assay used in Protocol 006. For Protocol 006, Merck designed the test simply to "evaluate immune responses."[143] Merck acknowledged internally that it would have used this industry standard PRN design for Protocol 007 but it would not achieve Merck's predetermined seroconversion objectives:

> The M-M-RII Protocol 006 study used a straightforward, non-enhanced neutralization, using several different indicator viruses. The M-M-RII study used an anti-lgG enhanced neutralization and the low-passage Jeryl Lynn indicator virus. We would have used the same assay used in 006 for 007 except that we could not achieve the 90% seroconversion sensitivity with any of the wild-type mumps strains without enhancing the assay sensitivity. We could measure >90% seroconversion using the vaccine strain as the indicator, but CBER required us to use a 'wild-type' indicator virus for 007.[144]

CBER required that Merck demonstrate no clinically meaningful difference in protection from mumps after vaccination with M-M-R II at reduced, below end-expiry potencies compared to M-M-R II at the release potency. Critical to this assessment was to measure outcomes using a functional test designed to reliably predict protection from mumps. By designing and "optimizing" the AIGENT assay to achieve a predetermined seroconversion and pre-positive rate

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[141] *See, e.g.,* MRK-CHA00759836, at '838 (pointing to a pre-positive rate of "~10%" and a seroconversion rate of ">=95%" as "the metrics that drove [the] development" of the AIGENT); MRK-CHA00065695, at '699 ("Conclusion from previous testing with 1:4 anti-IgG[:] Measurement of ≥95% seroconversion in vaccines is achievable[;] Pre-positive rate is higher than desirable[;] Continue evaluation of results using optimized anti-IgG amount (target ≤10% pre-positive rate and ≥ 95% seroconversions)"); MRK-CHA00026912, at '915 (same).

[142] An excessively high pre-positive rate might indicate either inadequate test specificity or unexpectedly high prior exposure to subclinical mumps or related viruses (for example, respiratory syncytial virus). Surprisingly, the WT ELISA data from the Protocol 007 samples yielded a lower pre-positive rate than the AIGENT data. This observation is consistent with inadequate AIGENT test specificity playing a major role in the unexpectedly high pre-positive rate detected with the AIGENT assay.

[143] *See* MRK-CHA00336905, at '905 ("Mumps Neutralization Assay Development Objectives: Develop an assay using different wild-type mumps strains to *evaluate immune responses* to M-M-R®II and Priorix (**Protocol 006** Competitive Trial) . . . [;] Develop an assay which *permits detection of ≥95% seroconversion rates* in M-M-R®II vaccines (**Protocol 007** Expiry Trial)") (emphasis added).

[144] MRK-CHA00051640, at '640.

without regard to whether it provided a clinically meaningful measure of protection, the AIGENT assay failed to meet this critical criterion.

2. The AIGENT Assay Did Not Test Against a True Wild-Type Mumps Strain

In designing an immune response blood test to detect protective serum immunity, the test antigen plays a central role in the scientific and clinical relevance of the test results. So, if the purpose is to specifically measure whether a vaccine will produce a clinically protective immune response in the real world, then the target antigen used in the test must be representative of a wild-type strain, preferably a currently circulating strain. It is for this reason that CBER refused Merck's request to use the Jeryl Lynn mumps vaccine strain as the test antigen for the AIGENT test. *See* Sec. IV.C.1. While originally insisting on a true wild-type mumps strain, CBER eventually allowed Merck to use a low-passage version of the vaccine strain as a proxy for a wild-type strain.

In my opinion, Merck's use of a low-passage version of the Jeryl Lynn vaccine strain undermined the relevance of the AIGENT as a measure of clinical protection against currently circulating mumps viruses. It did so for two reasons. First, the low-passage vaccine strain Merck used was still an attenuated (or weakened) version of the original Jeryl Lynn wild-type strain from which it originated.[145] Second, the Jeryl Lynn strain which was circulating in the 1960's when Merck isolated it for development of its mumps vaccine is of a different genotype (Genotype A) than the mumps strains that have been circulating in the U.S. in more recent times (predominantly Genotype G).[146]

[145] Preparations of viruses that have been modified so that they do not cause disease are sometimes used for vaccines, and can be administered as "live" virus which is capable of a limited infection but no disease when injected. These changes are specifically engineered to produce a vaccine virus, and historically they were introduced in a random manner by forcing viruses to replicate again and again under artificial conditions – to evolve and adapt to unnatural growth conditions which then makes it difficult for the "serially passaged" virus to infect and grow in its natural host. Merck Jeryl's Lynn mumps vaccine is an example of this latter type of modified virus. In many cases, these "attenuation" modifications change parts of the virus which help viruses that are naturally circulating and infecting in the wild to escape protective immune responses produced in infected patients. As a consequence, "attenuated" viruses typically produce different immune responses relative to the natural "wild type" viruses from which they are derived.

[146] Viruses in nature evolve rapidly in response to a variety of selective pressures including the immune responses of those infected with the disease. If, for example, a virus displays an antigen that the host immune system can attack, then viral mutations that help a virus to escape immune attack will be selected and included in the next round of viral replication. Therefore, an immune response test using antigens from a mumps virus that was circulating in the 1960's may not be relevant for measuring immune responses to currently circulating mumps viruses.

The impact of the genetic differences between the historic Genotype A mumps strain and currently circulating strains on the protective activity of a live attenuated vaccine derived from Genotype A is not clear. However, what is apparent is that PRN titers and seroconversion rates will be impacted by whether the testing antigen is a true wild type strain (regardless of genotype) or some version of the vaccine strain. This was reflected in Merck's preliminary AIGENT testing when the seroconversion rates it measured when testing against various wild type strains were significantly lower than the rates Merck measured when testing against the Jeryl Lynn vaccine strain. *See* Sec. IV.C.1.

In my opinion, Merck's use of the low passage Jeryl Lynn Genotype A mumps virus as the AIGENT indicator virus biased the test towards yielding higher titer and seroconversion outcomes. Had Merck used a true wild type mumps indicator virus, as CBER originally requested, the titer and seroconversion rate results would have been lower as Merck's own preliminary testing demonstrated. Merck's use of the vaccine strain in its AIGENT testing thus further undermined the clinical relevance of the AIGENT assay for differentiating between those vaccine recipients who were protected from currently circulating mumps and those who were not. Notably, years after the AIGENT testing was complete, CBER's Steve Rubin apparently agreed with this view, stating his belief that Merck's use of the low passage Jeryl Lynn vaccine strain in the AIGENT testing was "stacking the deck" in favor of higher seroconversion results.[147]

3. The AIGENT Assay Did Not Properly Control for the Use of Anti-IgG

There are many ways that antibodies can act to block viral infection, replication, and spread in an infected patient. The industry-standard PRN test method is considered a functional assay because it measures antibodies that appear to act by directly interfering with virus infection and replication in the cells of a patient. By contrast, the ELISA assay is not a functional assay because it measures any antibody that binds to the test antigen(s), regardless of whether that antibody has any functional role in blocking virus infection and replication. Because it is

[147] MRK-CHA00030994, at '994-995 ("In this study, we used the JL vaccine virus in the assay because we wanted to look at the effectiveness of the vaccine-induced immune response against the vaccine strain itself. This would not be an acceptable practice for measuring vaccine immunogenicity as a surrogate for efficacy in a clinical trial (given that we are not interested in protection against vaccine virus exposure). Instead, a wild type virus will have to be used. Many years ago Merck argued for use of a low passage version of JL in such an assay, and we accepted (not my decision, I would not have been in favor of stacking the deck).").

designed to measure a functional immune response, the standard PRN assay is generally a more clinically relevant method for predicting vaccine-induced protection from disease compared to an ELISA.[148]

However, Merck's AIGENT assay was not a standard PRN test because of the addition of anti-human IgG in the form of rabbit antibodies. Merck had not used this anti-IgG modification prior to development of the AIGENT assay and has not reported using this method for analysis of any clinical study results since Protocol 007.[149] It also is a method of PRN modification rarely, if ever, used in the industry. It was developed in the 1970's by research scientist Dr. Hiroshi Sato during his training at the FDA as a method of increasing the sensitivity of a neutralization assay. I have been unable to find any academic literature or commentary on it since Sato's publications describing the method.[150] I have seen no indication that either the FDA or CDC has ever used this technique in their testing. According to one internal Merck document, "CBER does not use either complement or IgG to enhance sensitivity and feels that these maneuvers should not be necessary" for Protocol 007.[151]

Healthy humans do not normally make large amounts of antibodies that bind to their own antibodies. However, animals (including rabbits) will readily make antibodies that bind to human antibodies (anti-human antibodies) if those animals are injected with human antibody preparations. The AIGENT assay used rabbit anti-human antibodies (anti-IgG) in its serum preparation to increase what the assay scored as mumps neutralization activity. Anti-IgG binds to any human IgG whether or not it is naturally neutralizing. Through this process, the combination of anti-IgG and non-neutralizing human IgG antibodies form complexes that can acquire the ability to neutralize mumps. As a result, an anti-IgG enhanced PRN assay measures both serum antibody which directly neutralizes viral infection as well as antibody which binds

[148] *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).

[149] *See* Krah Dep. 588:6-589:14 (AIGENT was unique in its "combination of the anti-IgG, the JL 135 virus, and the immunostaining . . . . I am not aware or do not recall any other clinical trial in which it was used."). *See also* MRK-CHA00000393, at '405 ("The PRN assay used in the study was developed solely for the purpose of this clinical trial It was not used in any previous nor subsequent clinical trials.").

[150] 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.

[151] MRK-CHA00095050, at '050.

the surface of the mumps virus but does not normally cause neutralization. The addition of anti-IgG to a PRN assay has no relationship to anything that takes place during actual infection of a patient by a virus.

In this way, the use of anti-IgG converted the AIGENT assay from a functional neutralization test to something more akin to an ELISA assay. In my opinion, it did so by artificially boosting the amount and types of antibodies that scored as neutralizing by including a secondary anti-IgG antibody preparation that binds to any human IgG antibody present in a patient sample. According to internal Merck documents I reviewed, this artificial boosting in the neutralizing activity the AIGENT measured was significant, on the order of up to one-hundred fold,[152] which directly led to a significant increase in the seroconversion rates it measured.[153] Merck could have controlled for this artificial boost by using a negative serum control with anti-IgG (instead of the mock control it used which contained no serum) but failed to do so.[154] Merck also could have determined and accounted for the mechanism by which anti-IgG boosted apparent neutralization, but I have seen no evidence that Merck did so.

An additional issue I see with using anti-IgG is that it exacerbates artifacts associated with the so-called Prozone Effect, a type of testing artifact that can complicate interpretation of serology test results. Prozone Effects typically occur in low dilution serum samples where high concentrations of antibodies and other matrix components interfere with the binding of antibodies to the antigens being tested. This results in an apparent false negative or reduced signal response (relative to assay performance at higher test serum dilutions/lower serum

[152] MRK-CHA00336905, at '916 ("Enhancement of Neutralization with Anti-Human IgG . . . Typically enhances Nt titers ~100-fold . . . Increase affinity of binding of primary antibody to virus"); MRK-CHA00016335, at '341 ("Anti-IgG provides "~100-fold enhancement" in neutralization.").

[153] MRK-CHA00068546, at '546 (showing jump in seroconversion rates from 76% to 91% between standard PRN and AIGENT); MRK-CHA00016632, at '632 (same); MRK-CHA00026912, at '913-914 (showing seroconversion rates of 79.5% with standard PRN using JL strain and 96% with AIGENT using JL 135 strain).

[154] Diluent without serum cannot control for matrix or other non-specific effects that may be present in the tested serum samples. It is not clear whether Merck used anti-IgG in the mock serum control. Dr. Krah testified that they did. Krah Dep. 463:11-12. However, Mary Yagodich, who Dr. Krah described as the person "who developed the assay and was the most experienced in running the assay" (Krah Dep. 429:25-430:3), testified repeatedly that they did not use anti-IgG in the mock control. *See* Yagodich Dep. 81:7-9. Merck's failure to include anti-IgG in the mock control tests would have further amplified the artificial boost in measured neutralization caused by Merck's use of anti-IgG.

concentrations) because the measured neutralizing activity is suppressed.[155] Dr. Sato and his team identified this issue in their neutralization test studies involving use of anti-IgG. At low serum concentrations, they observed a measured enhancement of up to 100 fold in neutralization activity. However, in the Prozone region (high serum concentrations), the same amount of added anti-IgG had very little effect. The Prozone Effect artifact was thus more apparent and pronounced with the addition of anti-IgG. For this reason, Dr. Sato recommended against testing with anti-IgG in high serum (low dilution) concentration samples. If neutralization testing of high concentration serum samples was unavoidable, Dr. Sato recommended adding more anti-IgG to minimize the interference and allow the neutralization enhancement to occur.

Merck did not employ either of these methods in its AIGENT testing in a waythat eliminated or properly controlled for these previously reported Prozone Effect artifacts. It did not exclude measurements obtained with high serum concentration samples and it did not increase the level of anti-IgG it used in these samples. Instead, Merck defined a single concentration of added anti-IgG (1:6) and used this same amount throughout the dilution curve, including at higher concentrations of human serum then were recommended by Dr. Sato and his team. This resulted in an unusual and unpredictable non-monotonic response curve characterized by a decrease in the measured neutralization response with the high serum concentration samples and a significantly increased measured neutralization response with low concentration samples.[156] This translated into a bias against neutralization seropositivity for samples with low levels of antibody/virus binding (typically observed in pre-vaccination serum samples) and a bias in favor of neutralization seropositivity for samples with high levels of such binding. The practical consequence of this dynamic was suppressing the number of pre-positive

[155] The binding interference caused by the Prozone Effect is part of the larger Matrix Effects phenomenon caused generally by interference from uncharacterized molecules present in the serum sample. These matrix effects are usually most pronounced at very high or very low concentrations of patient serum. This would occur at the curved portions on either end of the S-shaped curve that typically forms when the assay results at different dilutions are plotted on a graph. The curved portion observed at very high serum concentrations is the Prozone region. Typically, only the linear portion of the response curve is used for calculating seropositivity results. The designated "cut-off point" is where the Prozone region ends and the linear portion begins, which to be most precise should be defined with the ultimate purpose of the test in mind (*i.e.*, distinguishing between subject likely to be protected from mumps infection and those at significantly greater risk of contracting mumps).

[156] *See* MRK-CHA00016988, at '7003 (AIGENT Validation Report) ("Given the non-monotonicity present in many of the individual profiles, a straightforward application of the Spearman-Karber method is not recommended for estimating the endpoint dilution in this assay.").

samples, an outcome Merck further facilitated, as discussed below, through a recounting procedure targeting pre-positives.[157]

In my opinion, Merck's use of anti-IgG in its AIGENT testing further undermined the relevance of the test as a measure of protection against mumps. The artificial boost in reported neutralization was unrelated to whatever true mumps neutralization was actually caused by the vaccine. And the Prozone Effect masked seropositivity results in the pre-vaccination samples, allowing Merck through further manipulation to minimize the number of pre-positives. Merck's documents strongly suggest Merck used anti-IgG to "optimize" the test to ensure the highest level of seroconversions with an acceptable level of pre-positives, regardless of actual mumps-neutralizing activity.[158]

The chart immediately below was prepared by Dr. Krah during his development of the AIGENT, and identifies the seroconversion and pre-positive rates associated with adding different dilutions of anti-IgG.[159] This summary chart in my view further demonstrates Merck's focus on seroconversion and pre-positive rates as the object of its anti-IgG "optimization" efforts.

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[157] Beyond manipulating the level of pre-positives and seroconversion rate outcomes in the AIGENT testing, Merck's use of anti-IgG had significant additional clinical trial implications. This is because Merck failed to identify those test subjects who genuinely had low levels of anti-mumps IgG antibodies prior to vaccination (whether it be from their mother, prior mumps exposure or prior infection by mumps-related viruses). It is important to accurately identify these test subjects because their immune response to vaccination with a live attenuated mumps vaccine are likely to differ from the general population and if not accounted for could undermine the resulting immunogenicity analysis.

[158] *See, e.g.,* MRK-CHA01634869, at '869-870 ("The goal of these studies is to determine if the anti-IgG can boost seroconversion rate measurements. . . . Follow-up studies are underway using additional pediatric sera to determine the amount of anti-IgG that provides optimum enhancement of post-vaccination titers without boosting pre-vaccination titers."); MRK-CHA00026466, at '472 ("Current studies are focusing on determining the optimum concentration of anti-IgG to boost post-vaccination titers but not shift pre-vaccination sera to a positive Nt response."); MRK-CHA00016632, at '632 ("Preliminary studies have focused on identifying an optimum concentration of anti-human IgG that provides adequate sensitivity to detect post-vaccination responses, but does not provide excess pre-vaccination positive titers."); MRK-CHA0064608, at '608 ("Titrate the amount of anti-human IgG in the mumps neutralization assay to determine the effect of the anti-IgG concentration on Nt activity of selected pre-vaccination sera. . . . This is being done to determine the maximum amount of anti-IgG that does not provide 'excess' neutralization in pre-vaccination sera."); MRK-CHA00026912, at '915 ("Conclusion from previous testing with 1:4 anti-IgG[:] Measurement of ≥95% seroconversion in vaccines is achievable[;] Pre-positive rate is higher than desirable[;] Continue evaluation of results using optimized anti-IgG amount (target ≤10% pre-positive rate and ≥ 95% seroconversions"): MRK-CHA00068546, at '546 ("We are also readdressing the anti-IgG concentration to see if we can reduce the assay sensitivity to reduce the pre-positive rate while maintaining sensitivity to detect seroconversion.").

[159] MRK-CHA00026912, at '916.

Results of Additional Anti-IgG Titrations Studies

• Compare Nt titers using 1:4, 1:6 and 1:8 anti-IgG and a panel of pediatric sera

• Results summary

	No. of sera/total for anti-IgG dilution		
Serum Classification	1:4	1:6	1:8
Pre-positives	7/29	7/58	2/26
	24%	12%	8%
Seroconversions	21/22	48/48	22/23
	95%	100%	96%

Internal Merck documents support my opinion that Merck did not use anti-IgG for a legitimate scientific purpose. In one document, Dr. Krah told Drs. Emini and Shaw that Merck's use of anti-IgG was simply a "back up" plan if Merck's standard PRN testing fell short of achieving the required seroconversion rate.[160] Another document attributes to Dr. Emini the view that the addition of anti-IgG made the neutralization test "very artificial."[161]

4. The AIGENT Assay Was Not Sufficiently Specific for Measuring Mumps Neutralizing Antibodies

Specificity in a clinical assay is often used to refer to how accurately the assay identifies as negative test subjects who are truly negative for the tested condition. For a mumps neutralization assay like the AIGENT, specificity is the metric that represents the fraction of the test samples which the assay correctly scores as seronegative (measured seronegative) over the number of test samples that truly lack sufficient mumps-neutralizing antibodies (true seronegative).[162] So, for example, an assay with 50% specificity would mean that for every 100

[160] MRK-CHA00336323, at '324 ("We plan to readdress the use of anti- human IgG to enhance Nt, as a back-up if we fall short of our 90+%").

[161] MRK-CHA00549464, at '471 ("In talking with Emilio [Emini], the neutralization is very artificial because of the IgG added; to avoid too many seropositives, very high initial dilutions were required. Thus, low level responders cannot be distinguished from nonresponders.").

[162] Conversely, sensitivity refers to how accurately the assay identifies as positive test subjects who are truly positive for the tested condition. For a mumps neutralization assay, sensitivity is the metric that represents the fraction of test samples that have sufficient mumps-neutralizing antibodies (seropositive) and which the assay scores as positive for seroconversion. In other words, specificity can be thought of as measuring for false positives and sensitivity for false negatives.

post-vaccination serum samples that were actually seronegative, the assay would score 50 of them incorrectly as seropositive. In this example (and assuming minimal pre-positives), such a test would result in a large overestimation of the actual seroconversion rate of the test samples. A mumps neutralization test with such a low level of specificity would have little, if any, ability to provide a measure of protection from disease or assess the relative immunogenicity of different vaccine preparations.

In my opinion, Merck's AIGENT test was exactly such a low specificity assay. Merck's AIGENT validation studies showed the AIGENT did not distinguish between mumps antibodies and antibodies that bound to the other cell and viral extracts that were tested. *See* Sec. IV.C.2. My opinion is supported by the testimony of the Merck statistician who co-authored the AIGENT validation report (Dr. Antonello). As I detailed above, he testified how surprised he was by the poor specificity results of the AIGENT validation studies.

As shown in the table I created below, in at least half of the validation subjects the titer response to cell extract, measles virus, and rubella virus antigen absorption was equivalent to the titer response to mumps virus antigen absorption. This means that in at least half the validation subjects, the AIGENT was no more specific for mumps antibodies than it was for antibodies binding to the measles, rubella or mock extracts. This represents a conservative conclusion because it does not consider that several of the absorption responses that were not equivalent to mumps were separated by only a single dilution.

Effect of Test Antigen Absorption Compared to Mumps

Subject	**Mumps** (Virus Antigen)	**Mock** (Cell Extract Antigen)	**Measles** (Virus Antigen)	**Rubella** (Virus Antigen)
5 - 13	<32	<32 (E)	<32 (E)	<32 (E)
5 - 14	<32	512	512	32
5 - 16	256	512	512	256 (E)
5 - 26	<32	<32 (E)	<32 (E)	<32 (E)
AS	<32	64	64	256
CM	<32	512	512	128
MKY	<32	<32 (E)	<32 (E)	<32 (E)
DK	<32	<32 (E)	<32 (E)	<32 (E)
% Equivalent Titer Response	-	50%	50%	62%

(E) = Equivalent Titer Response Compared to Mumps Absorption. Cells demonstrating equivalence are shaded.
Data obtained from Tables 11 and 12 of Merck AIGENT Validation Report (MRK-CHA00016988, at '7002)

Furthermore, as shown in a second table I created below, in all subjects with detectable neutralization titers, absorption with every antigen preparation tested reduced neutralization titers compared to the negative control (medium). A conservative interpretation of these comparisons

is that some fraction of the mumps neutralization signal detected by the AIGENT test can be attributed to antibodies that bind to cell extract antigens, measles virus antigens, or rubella virus antigens.

Effect of Test Antigen Absorption Compared to Medium (Negative Control)					
Subject	**Medium** (Negative Control)	**Mock** (Cell Extract Antigen)	**Mumps** (Virus Antigen)	**Measles** (Virus Antigen)	**Rubella** (Virus Antigen)
5 - 13	512	<32 (R)	<32 (R)	<32 (R)	<32 (R)
5 - 14	2048	512 (R)	<32 (R)	512 (R)	32 (R)
5 - 16	≥4096	512 (R)	256 (R)	512 (R)	256 (R)
5 - 26	256	<32 (R)	<32 (R)	<32 (R)	<32 (R)
AS	2048	64 (R)	<32 (R)	64 (R)	256 (R)
CM	8192	512 (R)	<32 (R)	612 (R)	128 (R)
MKY	1024	<32 (R)	<32 (R)	<32 (R)	<32 (R)
DK	<32 (N/A)	<32 (N/A)	<32 (N/A)	<32 (N/A)	<32 (N/A)
% with Titer Reduction Compared to Medium	-	100%	100%	100%	100%
(R): Reduced titer compared to medium (negative control) (N/A): Not applicable because no further reduction possible Data obtained from Tables 11 and 12 of Merck AIGENT Validation Report (MRK-CHA00016988, at '7002)					

In sum, these validation results demonstrate the poor specificity of the AIGENT assay. In addition, Merck's validation specificity studies did not account for potential cross-reactivity with varicella (despite the concomitant administration of Varivax and M-M-R II in Protocol 007) or other viruses related to mumps (such as respiratory syncytial virus, a common pediatric pathogen). Therefore, the validation testing did not even account for the full range of potential non-mumps specific neutralizing activity detected by the AIGENT.

The causes of this low specificity in the AIGENT attributable to cross-reactivity are not entirely clear to me because I have seen no evidence that Merck conducted any follow-up AIGENT specificity studies. According to Dr. Florian Schodel (former Director of Clinical Vaccine Research), there were none.[163] In deposition, Dr. Antonello was not able to provide any explanation for the observed lack of specificity of the AIGENT.[164] This low specificity did not appear to be a problem in the anti-IgG enhanced mumps neutralization assay Dr. Sato

[163] Schodel Dep. 352:24-353:3 ("there was not a formal specificity analysis performed, so I couldn't know what the exact specificity was.").

[164] Antonello Dep. 118:14-16 ("Not the [specificity] result that I would expect. Now, why that's happening, I don't – can't explain.").

designed,[165] suggesting that to the extent Merck's use of anti-IgG contributed to the problem it was because Merck used it without properly controlling for the artificial boost in neutralization. In any event, separate and apart from non-specificity attributable to cross-reactivity, Merck's improper use of anti-IgG was also a major contributing factor to the AIGENT assay's low specificity. As I discussed above, the addition of anti-IgG rendered the assay incapable of distinguishing between mumps-neutralizing antibodies and other mumps and non-mumps antibodies.[166]

5. The AIGENT Assay Data Was Changed Through Selective Recounting of Plaques

One of the most critical components of a plaque reduction neutralization test is the plaque counting process. The plaques that form in the cell plates in this testing process represent viral infection and therefore are the key metric the test uses to measure virus neutralization. An inaccurate plaque count directly undermines the validity of the assay results. A count that overstates the number of plaques can lead to an artificially low measure of the level of virus neutralization. Conversely, a count that understates the number of plaques can lead to an artificially high estimate of the level of virus neutralization. This kind of miscounting compromises the accuracy of what the assay scores as seropositive and seronegative, the ultimate seroconversion rate the assay measures, and the assessment of which test subjects should be excluded as pre-positives.

For these reasons, coupled with the inherently subjective nature of the plaque counting process and its vulnerability to bias, the plaque counting exercise must be carefully managed using strict procedures, internal controls and rigorous quality assurance oversight. The laboratory staff responsible for plaque counting likewise should be well-trained, appropriately blinded, and closely monitored. For the AIGENT testing there should have been strict procedures in place for counting plaques, identifying what circumstances would warrant a

[165] 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.

[166] Ironically, when questioned on whether the poor specificity results could be attributed to Merck's use of anti-IgG, Dr. Antonello testified "if everything was [anti-IgG] driven, the pre-vaccination samples would be testing positive in the assay as well. We'd have, you know, huge response in the pre-vaccination samples." Antonello Dep. 145:8-14.

recount, documenting those reasons when a recount is deemed necessary, and conducting the recount itself. In addition, the plaque counters should have been blinded as to whether they were counting a pre-vaccination sample, a post-vaccination sample, a positive control, a mock control, or an uninfected cell control to minimize any propensity for bias or the potential to (consciously or unconsciously) influence the results.

In my opinion, Merck's performance of the AIGENT assay failed with respect to each of these critical requirements. Merck did not follow any kind of strict plaque counting procedure, nor did it abide by a blinding protocol that kept hidden from the plaque counters the type of sample or control counted. Instead, Dr. Krah and his team, with Dr. Emini's direction, ran the assay according to an ad hoc set of rules designed to eliminate problematic plaque counts (pre-positives). And they were guided by an unblinded contemporaneous analysis of the plaque count results and directed to achieve the target pre-positive rates by selectively recounting specific results. This conclusion comes in part from my review of the CBER record following its August 2001 inspection of Dr. Krah's lab where CBER concluded, among other things, that Merck selectively re-reviewed plaque counts and made changes without documenting the reason.[167]

My conclusion is further supported by internal Merck documents and testimony which describe a deliberate selective re-counting strategy that Drs. Krah and Emini employed in an attempt to rectify the pre-positive problem observed in the AIGENT assay data. Dr. Krah, for example, testified that for the interim analysis of the preliminary subset of AIGENT data, he and Dr. Emini reviewed the plaque counts, fully aware of the serostatus and seroconversion results that they yielded, and sent back for recounting those samples they found problematic.[168] Dr. Krah further testified that for the balance of the AIGENT testing, Merck used an electronic workbook that automatically flagged for recounting those samples found to be problematic.[169]

Dr. Krah further testified that "[o]nce the plaque count sheets were available, calculations were done to determine seroconversion rates and also, at least in the first third of the testing by

[167] MRK-CHA02021754.

[168] Krah Dep. 432-442. Dr. Krah testified that a major focus of his recount strategy was targeting positive neutralizations observed at a single dilution. But given the nature of the interaction between the Prozone Effect and Merck's "optimization" of the anti-IgG dilutions to minimize pre-positives, these targeted results occurred predominantly in the pre-vaccination samples. By focusing on single dilution events, the recounting process was systematically biased towards recounting pre-positive samples.

[169] Krah Dep. 500:16-506:23.

assay, evaluating the number of pre-positives."[170] The pre-positive calculations according to Dr. Krah were done at the specific direction of Dr. Emini.[171] The ability of Drs. Krah and Emini to assess in real time the plaque counting results, analyze how they impacted pre-positive rates and seroconversion outcomes, identify those counts that led to undesirable results, and then selectively target them for recounting, arose from Merck's failure to conduct the AIGENT test in an appropriately blinded manner. It also demonstrates exactly why appropriate blinding in the plaque counting process is so critical in a PRN test. Dr. Krah admitted in a memo he wrote to his direct supervisor Dr. Shaw that the lack of proper blinding introduced potential bias into the plaque counting procedure: "I have not been blinded since I was using the workbook printout as a guide to check for extravariable/single dilution positive samples. . . . This may introduce a bias, but the changes have been both up and down (although largely up due to missed counts)."[172]

My view of the selective recounting of pre-positives and resulting bias that occurred is further supported by the testimony of Mary Yagodich, described by Dr. Krah as the person "who developed the assay and was the most experienced in running the assay."[173] She testified that Dr. Krah specifically directed her to recount pre-positive samples. She could not recall him directing her to recount pre-negative, post-positive or post-negative samples.[174] Relators Joan Wlochowski and Steve Krahling similarly detailed the selective pre-positive recounting strategy Merck employed in the AIGENT testing. As Ms. Wlochowski explained:

> [I]t was known across the lab staff that anything that we found pre-positive was unexpected. . . . if the results of the plaque counting would give you something that would generate a pre-positive result, they would continue to look for plaques to find additional plaques in order to get the result that was expected as far as not having a pre-positive. . . . [P]eople were recounting the plaques on the plates and

[170] Krah Dep. 628:7-11.

[171] Krah Dep. 628:25-629:2.

[172] MRK-CHA00052243, at '243. Merck's biased recounting approach is further evidenced by the significantly higher number of pre-positives contained in the "original" versus "corrected" AIGENT results. *See* MRK-CHA00544820, '828 (Table 2) and '841 (Table 2) (showing in the preliminary subset analysis 61 pre-positives in the "original data" and 38 pre-positives in the "corrected data").

[173] Krah Dep. 429:25-430:3. *See also* MRK-CHA00008835, at '835 ("Mary [Yagodich] is effectively the 'second in command' in the lab and knows a lot about how things work.").

[174] Yagodich Dep. 187:24-188:19.

> focused on counting the pre-positives because, again, it was not the expected and did not lead to the desired outcome.[175]

Mr. Krahling testified similarly concerning Merck's selective targeting of pre-positives:

> [Krah told me] that in order to meet the 95 percent efficacy FDA mandate, that we needed to cross out pre-positives when we found them and change them to pre-negatives. He said that we had to target a 10 percent pre-positive rate. And that the reason we needed to do that is because the FDA might not allow them to use that protocol or method including the rabbit antihuman IgG unless they change those results . . . . [Krah] told me specifically that we were targeting pre-positives. [He said] If you recount them, you need to count very liberally and find as many plaques as you can in order to switch the result from pre-positive to pre-negative . . . he was pretty clear that the directive was to change the results. He didn't order me to have to recount the plaques. He just said change the results.[176]

Even if Merck's selective targeting of pre-positives were based on Merck's view that pre-positives were an unexpected (rather than undesired) result, it still would not justify Merck's approach and would go against what Merck has described as its own accepted practice: "it is Merck's practice not to retest samples on the basis of clinical expectation since selective retesting would introduce bias and complete retesting would likely result in similar discrepancies based on assay variability."[177]

Merck's failure to abide by any strict plaque counting and recounting procedures is also demonstrated by the absence of any documented plaque counting protocol. It was not until August 1, 2001 that Dr. Krah formalized any written plaque counting procedure.[178] This was nine months into the plaque counting process, when the bulk of the AIGENT testing was complete, and just days before CBER's August 6, 2001 inspection of Dr. Krah's lab, which

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[175] Wlochowski Dep. 264:12-21, 279:16-20. *See also* Wlochowski Dep. 298:11-15 ("Dave Krah himself had told us on multiple occasions that we were also to – that the pre-positive results are unexpected and not a desired outcome."); 447:15-17 ("changes were being made on focusing on pre-positives which is falsifying data").

[176] Krahling Dep. 190:14-24, 192:9-19. *See also* Krahling Dep. 197:24-198:2 (Mary Yagodich told Krahling "we are not trying to change the results of anything other than the pre-positives."); 269:22-270:2-5 (Krah told Krahling that high pre-positives "would be a big red flag that the use of antibodies with an improper control, that this isn't a methodology that is providing reliable data. The point was to hide…that high pre-positive rate from the FDA so that the Protocol 007 could be a success.").

[177] MRK-CHA00760670, at '670.

[178] MRK-CHA00026864 (Krah memo titled "Review of mumps AIGENT neutralization data").

focused on the plaque counting process Merck employed.[179] At his deposition, Dr. Krah's only explanation for why it took him so long to draft a plaque counting protocol was that he was "busy" and "did not understand the need to have [the plaque counting process] documented as we were doing it."[180] Dr. Krah's explanation reveals a fundamental misunderstanding or rejection of one of the core requirements of conducting a reliable PRN test in compliance with accepted industry norms and Good Clinical Practice (GCP) guidelines.

The end result of Merck's uncontrolled and biased plaque counting process was a reduction in the pre-positive rate through targeted and unjustified recounting of undesirable plaque counts. The pretextual nature of the bulk of the plaque count changes is further evidenced from my review of the log Merck prepared *ex post facto* following CBER's August 2001 inspection, which purportedly set forth the justifications for the changes. The log is comprised of two portions. For the portion covering assays 120-01 to 275-01, 645 of the plaque count changes (roughly 20% of the changes listed) the "Scientific justification for correction" is "*Reason for change could not be stated with certainty*." For the other portion of the log, covering assays 741-00 to 786-01, 1-01, 2-01, and 8-01, for 252 of the plaque count changes (roughly 54% of the changes listed) the "Justification provided" is simply "*NA*."[181]

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For all the reasons set forth above, both independently and collectively – the assay's results-oriented design and approach, Merck's use of the low passage vaccine strain as the test antigen, Merck's improper use of anti-IgG, the assay's poor specificity, and ultimately, Merck's selective recounting of plaques – the AIGENT assay results were unreliable, inaccurate and invalid for any scientific or clinical purpose. They certainly provided no relevant measure of protection from disease and provided no support for Merck's various mumps-related applications to the FDA.

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[179] *See* MRK-CHA00026654, at '654 (as of July 30, 2001, AIGENT testing "nearing completion," and "[a]ll available sera have been assayed, and current testing consists of retesting individual sets of paired sera that provided invalid results in previous assays.").

[180] Krah Dep. 682:11-17.

[181] MRK-CHA00027469. Merck's biased recounting approach is further evidenced by the significantly higher number of pre-positives contained in the "original" versus "corrected" AIGENT results. *See* MRK-CHA00544820, '828 (Table 2) and '841 (Table 2) (showing in the preliminary subset analysis 61 pre-positives in the "original data" and 38 pre-positives in the "corrected data").

My opinion is reinforced by the following additional attributes of the AIGENT testing which further reflect the assay's lack of reliability and clinical relevance:

- *AIGENT Outsourcing.* Merck cancelled its plan to outsource the bulk of the AIGENT testing to Dr. Ward's lab at the Cincinnati Children's Hospital. Neither Dr. Krah nor Ms. Yagodich could explain the reason for the cancellation and both believed Dr. Ward's lab to be fully capable of conducting the AIGENT testing.[182] Dr. Shaw stated that the cancellation was based on a concern that Dr. Ward's lab would be unable to obtain the same results with the balance of the testing as Dr. Krah and his team obtained with the preliminary subset testing. Specifically, he pointed to the "heightened importance" of the AIGENT results, the "unanticipated tightness" of the data set run by Dr. Krah, and a concern that Dr. Ward would be unable to "match this level of precision."[183] Merck's decision indicates it had no confidence in its ability to transfer the AIGENT test and obtain similar results in a well-qualified, independent clinical laboratory testing environment. This suggests there were unique attributes of the AIGENT assay as performed in Dr. Krah's laboratory that could not be replicated in an independent clinical laboratory. This is inconsistent with the AIGENT being a well-developed, unbiased, reliable and rigorously validated clinical immunology test.

- *GCP Violations.* Merck engaged in extensive violations of the GCP requirements in its AIGENT testing.[184] These requirements must be followed in any clinical trial to ensure the testing is well controlled, scientifically sound and the results properly documented and accurately reflect the purpose for which the test is being conducted. Failure to abide by these requirements seriously undermines the reliability and accuracy of the test results. Merck's AIGENT testing was rife with GCP violations, some of which were identified by CBER in its August 2001 inspection.[185] Dr. Krah testified quite clearly to his lack of knowledge (still to this day) of GCP requirements and whether he conducted the AIGENT assay in compliance with them.[186] From my review of the documents and

[182] *See* Krah Dep. at 717:10-12 ("[T]he completion of the Protocol 007 testing was not done in Dick Ward's lab. The reasons for that I don't – I'm not aware of."); 718:1-4 (Krah could not think of anything about Ward or his lab that would have made them incapable of conducting the AIGENT testing.); Yagodich Dep. 249:13-18 (not aware of why outsourcing to Ward's lab was cancelled); 260:4-25 (confident Ward's lab was capable of running the AIGENT testing).

[183] MRK-CHA00014739, at '739; MRK-CHA00014746, at '746; MRK-CHA00014829, at '829; MRK-CHA00015702, at '702. Dr. Krah testified he had no idea what Dr. Shaw was referring to in telling Dr. Emini that with the "unanticipated tightness of the data" Ward's lab would be unable to match the level of precision Dr. Krah was able to achieve. Krah Dep. 728:23-25.

[184] *See* Handbook for Good Clinical Research Practice (GCP): Guidance for Implementation (Available at http://apps.who.int/medicinedocs/documents/s14084e/s14084e.pdf). Merck was also required to conduct Protocol 007 under CLIA certification (comprising the requirements under Clinical Laboratory Improvement Act of 1967, Clinical Laboratory Improvement Amendments of 1988, Food and Drug Administration Modernization Act of 1997, and Taking Essential Steps for Testing Act of 2012). I have seen no evidence that Merck did so.

[185] *See* MRK-CHA02021754.

testimony in this case, these violations included multiple failures to meet GCP requirements in the majority of key trial activities, including: (1) development of standard operating procedures,[187] (2) maintaining proper records, (3) quality assurance,[188] and (4) ethics committee review and approval of the protocol.[189]

- *Blinding/Re-Testing*. In addition to Merck's failure to blind Drs. Krah, Emini and the laboratory staff to the type of sample or control being counted, Merck violated its own blinding protocol. The protocol required that "personnel associated with the conduct of the study were blinded to group assignment for each subject."[190] However, mid-way through the AIGENT testing Merck identified for reanalysis 56 samples from the preliminary subset analysis that did not seroconvert (non-responders) or seroconverted but at a low dilution (low-responders). These results were analyzed by treatment group, shared within Merck and served as the basis for a Merck retesting plan to assess whether the AIGENT results were reliable.[191] This plan involved re-testing groups of these samples in the AIGENT at a higher initial concentration or in a standard PRN (no added anti-IgG) testing against both the Jeryl Lynn vaccine strain and the low-passage vaccine strain used in the AIGENT.[192] It also involved a comparison with the results of the WT ELISA assay. The results of the re-testing and comparison to the WT ELISA revealed a significant discordance among the various test results further undermining the reliability

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[186] *See* Krah Dep. 515:12-23 (doesn't know if any aspect of the AIGENT testing he led was GMP or GCP compliant); 496:4 ("I can't say with certainty" whether AIGENT plaque counting process was GMP compliant); 513:13-514:2 (doesn't know whether the AIGENT workbook was GMP or GCP compliant); 539:17-25 (doesn't know whether handling laboratory worksheets and raw data, notebook documentation, spreadsheet validation, calibration of pipettes, and QA audit procedures for the AIGENT testing was GMP or GCP compliant); 566:14-18 (doesn't know if AIGENT blinding protocol was GMP or GCP compliant); 496:24-25 (admitting he is not familiar with GCP).

[187] Merck's failure to develop and abide by SOPs in assessing the laboratory test outcomes resulted in specific citations by CBER. In addition, many other crucial SOPs were either missing, not cited, or not made available for review. The most notable of these was the SOP defining the "in house blinding rules" which (for example) should have provided clear guidance regarding the roles and constraints placed on those with access to unblinded interim data during the study (such as the unblinded biostatistician).

[188] Among the most consistent and egregious GCP violations was the repeated failure of Merck's quality assurance systems to mitigate quality risks throughout the study (from protocol implementation, to data management/blinding, and through clinical immunogenicity laboratory training and performance) and to provide effective oversight and review of critical processes as well as the integrity and accuracy of the data generated via critical processes.

[189] The Independent Ethics Committee (IEC)/Institutional Review Board (IRB) review and approval of the 007 trial protocol was based on a document which was confusing, incomplete and inaccurate. Furthermore, I have seen no evidence that Merck actively informed or otherwise kept the IEC/IRB apprised of the many substantial changes to the protocol which occurred during performance of the study.

[190] MRK-CHA00001270, at '1307.

[191] MRK-CHA00549518, at '519 (attaching AIGENT preliminary subset seroconversion results broken out by treatment group). *See* MRK-CHA00562246-247, at '246 ("Here are the results of the ELISA testing for the non-converters and low converters from the 007 subset analysis.").

[192] *See, e.g.,* MRK-CHA00065915, MRK-CHA00068448, MRK-CHA00068391, MRK-CHA00064825.

of one or both tests.[193] Moreover, this whole exercise demonstrated Merck's failure to comply with the blinding protocol it established (limited as it was) for the AIGENT testing, thus introducing further bias into the testing.

- *Excluded Results*. Merck ultimately had to exclude roughly one-third (668) of the AIGENT subjects because they were seropositive prior to vaccination (pre-positive), or invalid, missing or non-evaluable.[194] Such a high number of exclusions in a pivotal clinical trial is highly unusual and reflects serious failings in clinical trial management as well as design and performance of the test.

- *Control Lot Cohort Immunogenicity Failure*. The 4.8 $\log_{10}$ AIGENT test control arm failed to meet the 90% (lower bound) acceptability criteria stipulated as a Protocol 007 primary study objective.[195] Therefore the validity of the control cohort for use in statistical comparisons to the test end-expiry cohorts was compromised. Failure of the cohort to meet minimum statistical parameters raises concerns as to the reliability of both the measured potency of the samples used in the testing and the immunogenicity results measured by the testing. Merck recognized internally that this failure "of the control lot raises the question if the non-inferiority comparison is really valid."[196]

- *No Reliable Record of Original Data*. Based on my review of the laboratory record (Krah's primary laboratory journal and the AIGENT counting sheets), Merck had no reliable record of original data from the AIGENT test. Therefore, Merck's use of the "original" data (instead of the "corrected" data) to support the sBLA to lower the mumps end-expiry potency for M-M-R II did not cure the numerous defects in the AIGENT data. *First*, as discussed above, the entire plaque counting process was conducted in an ad hoc manner without any reasonable controls in place to ensure the counting (whether "original" or "corrected") was consistent, accurate or reliable. *Second*, despite the numerous unjustified changes Merck made to the "original" pre-positive counts, Merck did make some changes to the "original" counts to fix basic transcription errors. While these legitimate corrections account for a relatively small percentage of the "corrections" Merck made, there still were a sizeable number of them.[197] *Third*, Merck had no controls in place to ensure plaque-counting sheets were not discarded and in fact Relator Joan Wlochowski testified that she witnessed counting sheets being discarded by lab staff

[193] *See, e.g.,* MRK-CHA00562246, at '247 (showing AIGENT/WT ELISA discordance in roughly half of subjects); MRK-CHA00065915 (showing multiple discordant results between AIGENT and low dilution AIGENT tests), MRK-CHA00068448 (showing multiple discordant results between AIGENT and standard PRN tests).

[194] MRK-CHA00000393, at '399.

[195] MRK-CHA00000393, at '404.

[196] MRK-CHA00759061, at '061.

[197] *See* MRK-CHA00027469 (AIGENT data correction log) (identifying dozens of corrections based on transcription errors).

> during the AIGENT testing.[198] *Fourth*, Merck destroyed a large number of the assay plates from which the plaques were counted. As the primary source for the plaque counts, these plates should have been retained through the duration of the AIGENT testing. However, Dr. Krah testified that he discarded many of the assay plates during the AIGENT testing and did so without anyone from quality assurance confirming the counts on the plates matched the numbers recorded on the counting sheets.[199]

Finally, my opinions on the lack of reliability and clinical relevance of the AIGENT results is corroborated by the testimony of two of the Merck personnel most central to the AIGENT testing: Dr. Krah, who developed and ran the assay; and Dr. Antonello, who wrote the AIGENT validation report and ran the AIGENT/WT ELISA correlation study. Each of them was clear in their testimony that the AIGENT test was neither a reliable nor relevant measure of immunogenicity or protection against mumps:

> I would not be able to say that the AIGENT assay is the most accurate measure of mumps antibody. It's an assay that's intended as an imperfect model for looking at immune response in terms of antibody response to the vaccine. Whether it's accurate or not, that's beyond my expertise.[200]

> I have an opinion that the assay was reliable in measuring antibodies to mumps. As far as the impact on – or the conclusion about whether it was reliable assessment to immunogenicity, I can't say.[201]

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[198] Wlochowski Dep. 328:23-329:1 ("counting sheets that were used [in Protocol 007] were not controlled counting sheets"); Wlochowski Dep. 337:22-338:12 ("The data can be compromised. There is no way of ensuring that it hasn't been compromised. … The counting sheet is not in any way controlled with a numbering. It can be generated and destroyed without anybody knowing."); Wlochowski Dep. 338:14-345:24 (she witnessed others discarding counting sheets).

[199] Krah Dep. 487:23-488:4 ("Once that review was completed, my understanding was that those plates were no longer needed. The plaque counts on the counting sheet served as a primary data source, and in some cases assays were then discarded after the QA audit was completed."); Krah Dep. 488:5-16 (Dr. Krah stated that the QA auditor did not check the accuracy of counting against the raw data). *See also* Wlochowski Dep. 327:20-328:11 ("The well plate is the original data in this case. It would be a means to preserve the original raw data, maintain it through the end of the study. So, in my experience, while working in Dave Krah's lab, I did see him discard plates which had been sitting there since I had started in January through July after some escalations had happened internally. The very next day after being told that an internal audit would occur, Dave Krah came into the laboratory early in the morning, which he never does, I was in, taking plates and putting them in the autoclave and getting rid of them, which was not something I had ever witnessed him doing in my previous months working there."). Relator Wlochowski also witnessed lab staff wiping away original plaque count, further undermining that the "original" data was truly original. Wlochowski Dep. 381:6-10 ("[T]here were instances of wiping out the original plaque counts on the plate and repeating the plaque counts. So, therefore, again, I consider that the original data was not maintained.").

[200] Krah Dep. 754:17-23.

[201] Krah Dep. 412:3-9.

> I do not agree that the data were designed to indicate whether they were protected or not. They're looking at immunogenicity and antibody responses, not – to the best of my understanding, not correlating it with protection.[202]
>
> I would say that the AIGENT assay was developed to meet [] a specific requirement . . . have the capability of measuring antibody responses. [] I don't have an expectation of what the correlation of that assay would be with protection.[203]
>
> So I don't think [the AIGENT serostatus cutoff] was ever considered a protective level.[204]
>
> No. I don't think we knew – you know, it's known what a protective level, antibody level is. . . . So, no, [the AIGENT serostatus cutoff is] not indicative of protection against the virus. . . . It just means that if you're above that, your response is likely above the variability in the assay. And it's likely due to having been vaccinated, but it doesn't reflect protection.[205]

B. Merck's Wild-Type Mumps ELISA Assay Failed to Provide a Reliable or Clinically Relevant Measure of Protection Against Mumps

1. The WT ELISA Did Not Test Against a True Wild-Type Mumps Strain

Like the AIGENT test, Merck's WT ELISA test used a low-passage version of the Jeryl Lynn vaccine strain as the source of the antigen mixture against which it tested the vaccine. For the reasons stated above, Merck's use of the vaccine strain significantly undermined any potential clinical relevance of the ELISA results, especially as it related to any measure of protection against circulating mumps disease. *See* Sec. V.A.2.

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[202] Krah Dep. 754:2-7.

[203] Krah Dep. 772:13-20. *See also* Krah Dep. 726:9-17 ("Our – my goal and my understanding for developing the assay was to have an assay that would allow us to have the capability of measuring 95 percent seroconversion and have a pre-positivity rate of approximately 10 percent without – from my personal perspective, without considering the impact on accuracy."); Krah Dep. 599:15-21 ("My objective and our lab's objective was to develop an assay that would be capable of measuring 95 percent seroconversion. The clinical application is something that's beyond my responsibility of assigning.").

[204] Antonello Dep. 32:25-33:1.

[205] Antonello Dep. 151:5-22. *See also* MRK-CHA00791315, at '319 ("We don't really know what a clinically protective level is in either [AIGENT or ELISA] assay."); MRK-CHA00759061, at '061 ("[T]here is no clinical history/expectation/meaning that can be attached to the 90% response level in the PRN assay."); MRK-CHA00791315, at '315 (Merck's then Director of Clinical Vaccine Research Florian Schodel, stating "Agree with Joe [Antonello]- could not overemphasize the weakness of the PRN (50% specificity!!!!!!).").

2. Merck Did Not Correlate the WT ELISA Assay to a Reliable or Relevant Functional Assay

As discussed above, an ELISA assay must be correlated to a clinically relevant functional assay for it to provide any reliable measure of protection against disease.[206] *See* Sec. IV.B.3. This is reflected in numerous exchanges between CBER and Merck following the Protocol 007 testing and Merck's efforts to use the ELISA testing to support its desired end-expiry potency label change. CBER made it very clear that Merck needed to demonstrate a sufficient correlation between the two assays before CBER would be willing to accept the WT ELISA results for Protocol 007 and other CBER decision-making predicated on evidence of clinical relevance. *See* Sec. IV.C.5. Consequently, Merck conducted a correlation study of the ELISA results compared to the AIGENT results (both the "original" and "corrected" results), finding what it characterized as a high correlation between the two assays. For the all reasons I discuss above, the AIGENT assay was not reliable or relevant as a measure of protection against mumps. The AIGENT correlation therefore provided no support for the WT ELISA results having any clinical significance or providing any reliable measure of protection.[207]

3. Merck Overstated the AIGENT/WT ELISA Correlation

In my opinion, Merck also overstated the correlation calculated between the two assays because Merck excluded from its seroconversion comparison the samples that scored pre-positive in either assay. As reported in Serial 86, "with respect to sero-conversion [], the overall agreement rate between the assays was 93.4% (413/442), with the WT ELISA being only slightly more likely than the AIGENT to classify a sample as a seroconverter."[208] However, this calculation excluded the 68 samples that were pre-positive in either assay (61 in AIGENT; 10 in WT ELISA; 3 in both).

[206] An ELISA can also provide a reliable measure of protection against disease if it is demonstrated to meet statistical criteria as a validated correlate of protection through field efficacy or challenge studies. This has never existed for mumps.

[207] Dr. Antonello, who performed the correlation, recognized its limited utility in justifying the serostatus cutoff given the possibility the AIGENT results were not reliable: "[I]f the PRN doesn't necessarily represent truth for every sample, you don't know that those are the correct results. So I don't think you would want to tie your ELISA cutoff to be as close as possible to the PRN if the PRN is potentially inaccurate." Antonello Dep. 217:20-218:2.

[208] MRK-CHA00761628, at '638.

Merck's exclusion of pre-positives in this correlation calculation biases the result in favor of overstating the correlation between the assays and minimizing the apparent false positive rate of the WT ELISA with respect to seroconversion. Therefore, Merck's assertion to CBER of the "WT ELISA being only slightly more likely than the AIGENT to classify a sample as a seroconverter" is misleading due to the bias inherent on the correlation calculations that Merck based on a selected subset of the relevant data. It also ignored what should have been Merck's goal of minimizing false positives to ensure the WT ELISA did not overestimate seroconversions in test subjects.[209] This is an especially compelling concern in the clinical testing of children, particularly when the data is used in clinical decision making on re-vaccination of test subjects, like it was in Protocol 007.[210] Had Merck not excluded these pre-positives from the correlation calculation, the resulting seroconversion agreement rate would have dropped to 82%, or a decrease of more than 10% from what Merck reported to CBER.[211]

4. Merck Did Not Use a Clinically Relevant Serostatus Cutoff

The validation report for the WT ELISA, from which Merck's original selection of the 10Ab cutoff was derived, provides no support for the cutoff's relevance to clinical protection.[212] The validation report defined that "[t]he serostatus cutoff is the lowest antibody concentration that can be reliably distinguished from a panel of negative samples."[213] Therefore, the serostatus cutoff for the WT ELISA was by definition based only on assay performance characteristics (including statistically random assay variability). It had no relevance to clinical protection because the cutoff selection was not based on the ability of the assay to predict a protective antibody response to mumps.

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[209] This concern over false positives in the WT ELISA was reflected in CBER's communications with Merck regarding Merck's selection of the WT ELISA cutoff. *See, e.g.,* MRK-CHA00331831, at '833 ("CBER pointed out that a correlation rate of 92% was low . . . but noted that the ELISA seemed to be more conservative than the PRN in assignment of low sero-positives.").

[210] *See* CLIA.

[211] *See* MRK-CHA00544847 (showing 56 samples AIGENT pre-positive/WT ELISA seroconversion; 7 samples WT ELISA pre-positive/AIGENT seroconversion; 2 samples AIGENT pre-positive/WT ELISA non-responder; and 3 samples AIGENT pre-positive/WT ELISA pre-positive). The addition of these 63 discordant pairs and 5 concordant pairs changes the 413/442 ratio (93.4%) Merck reported to CBER to the true discordant ratio of 418/510, equaling 82%.

[212] MRK-CHA00761129.

[213] MRK-CHA00761129, at '134.

Further undermining Merck's selection of a 10Ab cutoff was the limited testing Merck performed to arrive at its selected cutoff value, and the bias Merck introduced in the testing sample set it used for the cutoff value determination. The validation report shows Merck only tested two of the many possible cutoff values, 10Ab and 5Ab. Exclusive investigation and analysis of these low cutoff thresholds introduced assay bias towards false positive seroconversion results.[214] This bias was further exacerbated by the absence of any consideration of the clinical relevance of the tested cutoffs to protection. The validation report also shows that the selection of the final cutoff value was based on a single run rather than multiple runs designed to increase the statistical validity of the analysis. The FDA recommends a minimum of five determinations of sample value to ensure the accuracy and precision of bioanalytical method validations for ligand binding assays (such as ELISA).[215]

Merck also introduced bias in the testing sample set results it reported to CBER by only testing post-vaccination negative samples or pre-vaccination samples which by definition were biased to low concentrations of mumps antibodies. This resulted in a form of sample selection bias which is at odds with regulatory guidance that the validation test sample set and resulting test calibration curve should be selected to represent the entire range of the tested analyte concentrations (titers), including the lower limit of quantitation.[216] The validation testing set did not include samples of known low-positive titers, which should have been included to evaluate and ensure consistent test accuracy and precision at the lower limit of quantitation. Moreover, Merck ignored the results of the post-vaccination sample set which showed a significantly higher discordance rate when analyzed at either a 10Ab or 5Ab serostatus cutoff value.

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[214] Notably, optimal ELISA assay cut points for serologic diagnostic mumps testing are significantly higher than 10 Ab. *See* 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.

[215] *See* Guidance for Industry, Bioanalytical Method Validation (Draft Guidance, Revision 1). U.S. Department of Health and Human Services Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), September 2013 (Available at https://www.fda.gov/downloads/drugs/guidances/ucm368107.pdf).

[216] *See* Guidance for Industry, Bioanalytical Method Validation (Draft Guidance, Revision 1). U.S. Department of Health and Human Services Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), September 2013 (Available at https://www.fda.gov/downloads/drugs/guidances/ucm368107.pdf).

The AIGENT/WT ELISA correlation likewise provided no support for 10Ab as a clinically relevant serostatus cutoff for the same reasons it provided no support for the WT ELISA assay as a measure of clinical protection. As with CBER's insistence that Merck correlate the WT ELISA and AIGENT assays, CBER also insisted that Merck demonstrate through the correlation that its selected 10Ab cutoff was clinically relevant. *See* IV.C.5. The alternative as set out by CBER was that Merck employ a cutoff based on a 4-fold rise in titers between pre- and post-vaccination samples.[217] Documents I have reviewed show that Merck acknowledged internally that with such a serostatus cutoff, Protocol 007 would be unable to reach the 95% seroconversion rate Merck was seeking in support of Merck's sBLA to lower the mumps end-expiry potency specification.[218]

Despite CBER's insistence on the clinical relevance of the 10Ab cutoff, and Merck's assertions of such clinical relevance, Dr. Antonello (the Merck statistician who calculated the cutoff) testified repeatedly that the cutoff had no relevance to clinical protection:

> Q: But in your work in calculating what you thought was the appropriate serostatus cutoff level for the mumps wild type ELISA assay, did seroprotection play any role in that exercise? A: Not that I'm aware of. . . . Q: So in your work in calculating it, did you take into account in any way the level of seroprotection that would be measured by the particular serostatus cutoff that you were calculating? A: I don't believe I did. Q: Why not? A: Because I don't believe a seroprotective level was defined for mumps. It was for, I remember, measles and rubella, and we used those for serostatus cutoffs for those assays, but there was no seroprotective level defined for mumps.[219]

[217] MRK-CHA00331831, at '835 ("It should be noted that if the questions about the justification and relevance of the mumps ELISA cutoff could be addressed (i.e. by correlating to PRN), then a 4 fold criterion would not be necessary. If, however there continues to be uncertainty about the biological / clinical relevance of the cutoff, it is expected that CBER would require a 4 fold rise criterion, as that would be necessary to demonstrate significant response to the vaccine."); MRK-CHA00544296, at '296 ("[I]f we are unable to provide sufficient reassurance about the clinical relevance of the ELISA cutoff (which in [CBER's Dr. Kathy Carbone's] mind means linking this to the PRN) then we may end up with some type of fold-rise criterion which I assume we would rather avoid if possible.")

[218] MRK-CHA00561416, at '418 ("There is some concern that CBER may require a fold rise in titers (from pre- to post-vaccination) in order to demonstrate that seroconversion has occurred. . . . If CBER required a 4-fold rise in titer (defined as <10 to ≥ 40), the seroconversion rates for these studies would range from 80.9% to 85.2%.").

[219] Antonello Dep. 66:25-67:21. *See also* Antonello Dep. 33:21-22 ("I'm not aware of [10Ab] being identified as protective level."); Antonello Dep. 233:7-12 (Antonello affirming "that the serostatus cutoff of 10Ab used for the wild type mumps ELISA did not in any way relate to seroprotection.").

Dr. Antonello further testified that the AIGENT/WT ELISA correlation he performed did not provide any support for the 10Ab cutoff having any relevance to protection:

> Q: Did the correlation that you performed between the wild type mumps ELISA assay and the AIGENT assay results provide, in your opinion, any support that the 10Ab serostatus cutoff was relevant to seroprotection? . . . A: I don't know what a protective level is. So . . . I can't address what a protective level is and whether that's protective or not protective. It just showed the relationship between the two assays. CBER inferred that to mean that that's good. That's not my role.[220]

As Dr. Antonello appreciated, the problem of using a cutoff that is lower than that associated with protection from disease is the risk the assay will classify as protected (seropositive) a test subject who is not (*e.g.*, clinically seronegative and at risk for mumps disease).[221]

* * *

For each of these reasons – Merck's use of the low passage vaccine strain as the source for the test antigens, Merck's failure to correlate the WT ELISA to a reliable or relevant functional assay, Merck's overstatement of the AIGENT/WT ELISA correlation results, and Merck's failure to use a clinically relevant serostatus cutoff – I conclude that the WT ELISA assay results were not a reliable or relevant measure of protection against mumps. They provided no support for Merck's various mumps-related applications to the FDA, or any other CBER decision making predicated on evidence of clinical relevance.

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[220] Antonello Dep. 235:11-236:1. *See also* Antonello Dep. 236:3-14 ("Q. As far as your performing the calculation, did it give you any comfort that the 10Ab serostatus cutoff that you calculated was relevant to seroprotection? . . . A. Yeah, I don't know what is protection. I did the comparison that was requested and showed how the two assays relate. What that means beyond that, that's not my area of expertise, how to interpret the results in that sense."); Antonello Dep. 240:9-15 ("I don't know what a protective level is. So for me I can't say that 10 is the correct protective level . . . So [correlation] doesn't give me greater confidence in that sense that 10 is a protective level.").

[221] Antonello Dep. 259:23-260:9 ("Q. But don't you run the risk if the serostatus cutoff is too low of classifying a true negative as a positive? A. Absolutely. . . . Increasing the cutoff decreases the probability that you'll get a false positive."). My opinion that the AIGENT/WT ELISA correlation did not support the clinical relevance of a 10Ab serostatus cutoff is further supported by the fact that, according to Dr. Antonello, the 10Ab cutoff did not even provide the best agreement. Dr. Antonello testified that a 16 Ab cutoff provided "better overall agreement between the two assays." Antonello Dep. 258:7-9. *See also* MRK-CHA00025762, at '762 ("A WT ELISA cutoff of 16 balances misclassifications between assays. As I recall, the recommendation at CAS [Clinical Assay Subcommittee] was to stick with 10Ab units."). He further testified that had Merck used this higher serostatus cutoff instead of 10Ab, the WT ELISA would have yielded lower seroconversion rates in Protocol 007. Antonello Dep. 251:16-23 ("Q. And is it your understanding that if Merck had selected for its wild type mumps ELISA assay a serostatus cutoff higher than 10Ab, then the seroconversion rates that it measured in the assay would have decreased? A. Yes. The higher you set the serostatus cutoff, the lower seroconversion rate.").

C. In Submitting or Relying on Protocol 007 for Its Various Mumps Related FDA Approvals, Merck Misrepresented the Clinical Relevance of the AIGENT and WT ELISA Assays and Results

1. Merck Misrepresented the AIGENT Results in Its Submission of the Preliminary Subset Analysis

As I previously discussed, Merck provided CBER the preliminary subset of the "corrected" results of the AIGENT testing (based on roughly one-third of the Protocol 007 test subjects) on several occasions to provide assurance that M-M-R II afforded sufficient protection against mumps at below end-expiry potencies. *See* Sec. IV.D.1. Merck provided these results in March and April 2001 in Serial 63, in response to a CBER Warning Letter relating to certain M-M-R II lots that had failed to meet the minimum mumps potency specifications, and in the BPDR that followed. In each of these submissions, Merck pointed to these "corrected" AIGENT results, which were the product of Merck's selective re-counting and alteration, to support its claims of mumps protection at the below end-expiry potencies: (1) "seroconversion rate range seen in this preliminary data is consistent with other neutralization data which were associated with high levels (97%) of protection;[222] (2) relying on preliminary AIGENT data for "evidence that M-M-R®II is effective through the predicted range of potencies";[223] and (3) preliminary AIGENT data shows M-M-R II with a mumps potency as low as 3.9 is "efficacious" and "not likely to lead to a lack of immunity against mumps."[224]

For all the reasons I set forth above, including the selective and unjustified plaque count changes that tainted the "corrected" results,[225] these statements do not accurately represent the clinical relevance of the AIGENT data or how it relates to protection against mumps. These data

[222] MRK-CHA00017036, at '048.

[223] 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.").

[224] MRK-CHA00754233, at '236.

[225] With respect to these "corrected" results, Merck made further misrepresentations to CBER in asserting the changes "were made for appropriate reasons," "improve[d] the quality of data obtained, " and "provide[d], accurate, scientifically sound data for use in decision making." Merck MRK-CHA00000410, at '418, 422-423.

provided no basis for any measure of efficacy, effectiveness or protection against mumps. They therefore provided no support for Merck's claims to CBER that M-M-R II afforded sufficient protection against mumps at below end-expiry potencies.

2. Merck Misrepresented the Protocol 007 Results in Its sBLA for the M-M-R II Mumps End-Expiry Potency Change

Merck likewise misrepresented the clinical relevance of the AIGENT and WT ELISA results in its sBLA for the M-M-R II mumps end-expiry potency change. Specifically, the sBLA and supporting Protocol 007 CSR contained numerous statements claiming Protocol 007, in both its design and results, demonstrated the protection against mumps afforded by M-M-R II at the tested potency of 4.1 $\log_{10}$ $TCID_{50}$:

> The clinical data described herein demonstrate that M-M-R^{TM}II with a mumps virus potency of 4.1 $\log_{10}$ $TCID_{50}$ per dose is as immunogenic and well tolerated as M-M-R^{TM}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 rate using a mumps neutralization assay, *thus preserving the excellent safety and efficacy profile of the vaccine*.[226]
>
> **Benefits and Risks Conclusions** In the 25 years following licensure in the United States, M-M-R^{TM}II has proven to be highly efficacious against measles, mumps, and rubella, highly immunogenic, and well tolerated . . . . The data presented here indicate with a high level of assurance that decreasing the mumps end expiry titer from 4.3 to 4.1 $\log_{10}$ $TCID_{50}$ per dose in children 12 to 18 months of age *will ensure that M-M-R^{TM}II remains a highly effective vaccine*.[227]
>
> 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 []. *Therefore, the mumps PRN assay and ELISA results from this study support the effectiveness of M-M-R^{TM}II* containing a mumps virus potency of no more than 4.1 $\log_{10}$ $TCID_{50}$ . . . .[228]
>
> **Efficacy** . . . No studies of the efficacy of M-M-R^{TM}II were performed in support of this application. In agreement with CBER/FDA, the mumps-specific PRN assay was developed and used as a *surrogate for vaccine effectiveness*.[229]

[226] MRK-CHA00000034, at '110 (sBLA) (emphasis added).

[227] MRK-CHA00000034, at '127 (sBLA) (emphasis added).

[228] MRK-CHA00224982, at '5036 (CSR) (emphasis added).

[229] MRK-CHA00000034, at '115 (sBLA).

> **Study Endpoints** . . . 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*.[230]
>
> The purpose of this study is to compare the ability of the three different formulations of M-M-R™II to make *protective antibodies against disease*. The results of this comparison will be used to determine how much of each of the virus components is necessary at the date of expiration to make a *protective antibody response*. . . . In order to make sure that the amount of each of the M-M-R™II components is *enough to protect children* even at the end of the vaccine storage period (24 months), or shelf-life, Merck & Co., Inc., could add additional amounts to the vaccine when it is produced. However, this would expose some children to high doses of the vaccine. Merck & Co., Inc., believes a better approach is *to make sure that lower doses of the vaccine will still be effective at stimulating antibodies*.[231]

Yet none of these representations of efficacy, effectiveness and protection in Merck's sBLA – which Merck identified as an "Efficacy Supplement" on the face of the application[232] –

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[230] MRK-CHA00000034, at '110 (sBLA). *See also* MRK-CHA00224982, at '5174 (CSR) ("Following agreement with CBER and in an attempt to demonstrate that mumps virus at end expiry potency was not only immunogenic but *effective in inhibiting viral replication,* a functional assay (Plaque Reduction Neutralization or PRN assay), aimed at measuring mumps-specific neutralizing antibodies, was developed and validated at MRL, and used to evaluate the primary immunogenicity hypotheses of the study.") (emphasis added); MRK-CHA00224982, at '5036 (CSR) ("**Appropriateness of Measurements . . . [A]**ntibody detection by ELISA does not reveal ability to block viral replication. For the purpose of this study, a functional [PRN] assay was developed and used to measure the ability of the vaccine-induced immune response to inhibit viral replication in vitro, and therefore possibly provide a *better indication of immune protection*.") (emphasis added; bold in original). *See also* MRK-CHA00224982, at '6152 (CSR) (Clinical Protocol 007-01) ("This [PRN] assay measures the ability of serum neutralizing antibody to *block infection of Vero cells* by the target mumps strain and thereby *inhibit plaque formation*. Because inhibition of infection by wild type mumps is *thought to be a good correlate of protection* from wild type mumps . . . .") (emphasis added). This language remained in the final 007 Clinical Protocol. MRK-CHA00224982, at '6177-79, '6189.

[231] MRK-CHA00224982, at '6197 (CSR) (Protocol 007-00 Generic Consent Form) (emphasis in original). The patient consent forms Merck used for Protocol 007 stated that the purpose of the study was "to make sure that M-M-R™II vaccine still has enough live viruses at the end of its 24 month storage period or 'shelf- life' to protect children from measles, mumps and rubella infections." MRK-CHA00126233, at '247. Merck also communicated through these forms that the results of the trial would determine "whether your child is protected from measles, mumps, rubella, or varicella," or whether "your child needs to be revaccinated." MRK-CHA00126233, at '241. *See also* MRK-CHA00126233, at '249-250 ("If your child responds to the vaccine with protective antibodies to measles, mumps, and rubella, your child will not need the routine 2nd shot of MMR given before entering school. You will be given written proof of protection against measles, mumps, and rubella with the date the blood was drawn."). Merck made similar representations of protection for test subjects at the Protocol 007 Investigators Meeting: "Advantages to Participation in this Trial for Subjects • Avoid unnecessary exposure in the future to higher levels of mumps vaccine virus. • A positive mumps neutralization titer almost certainly ensures protection from wild type infection."); MRK-CHA01888826, at '849.

[232] MRK-CHA00000034, at '038 (sBLA) ("TYPE OF SUBMISSION (check one) . . . [X] EFFICACY SUPPLEMENT").

are a correct statement of what Protocol 007 actually measured or what the results actually demonstrated. As I describe in detail above, both the AIGENT and WT ELISA tests were unreliable and had no relevance to efficacy, effectiveness or protection. Therefore, these key findings and assertions Merck submitted to the FDA in support of its sBLA for the M-M-R II mumps end-expiry potency change were false and unsupported.[233]

I have found numerous additional misrepresentations Merck made in this sBLA, including:

- *Correlation*. Merck made repeated representations on what it characterized as the "strong" or "excellent" correlation between the AIGENT and WT ELISA results and that "previous studies have established a strong correlation between the development of mumps-specific neutralizing antibodies and vaccine efficacy" and "[t]herefore, the mumps PRN assay and ELISA results from this study support the effectiveness of M-M-R^{TM}II . . . ."[234] In making these assertions, Merck mischaracterized the AIGENT/WT ELISA correlation and its clinical relevance to protection. Moreover, in supporting the

[233] Also unsupported were the dozens of representations Merck made in this sBLA and supporting materials claiming the Protocol 007 results demonstrated M-M-R II at the tested potencies induced mumps specific and mumps neutralizing antibodies. *See, e.g.,* MRK-CHA00000034, at '122 (sBLA) **("**M-M-R^{TM}II with a mumps end-expiry potency of 4.1 $\log_{10}$ $TCID_{50}$/dose . . . induces acceptable *mumps specific antibody responses* by PRN [and] [e]licits comparable levels of *mumps . . . specific antibodies* to those obtained in control subjects.") (emphasis added); MRK-CHA00224982, at '5007 (CSR) (**"**M-M-R^{TM}II with a mumps expiry dose of 4.1 $\log_{10}$ $TCID_{50}$ . . . induces an acceptable *mumps specific neutralizing antibody* SCR . . . . [and] induces comparable SCRs for . . . *mumps- . . . specific antibodies by ELISA* as M-M-R^{TM}II containing a release mumps virus potency of 4.8 $\log_{10}$ $TCID_{50}$.") (emphasis added); MRK-CHA00224982, at '5019 (CSR) ("All collected serum samples were tested by MRL for levels of *mumps-specific neutralizing antibodies* using the PRN assay.") (emphasis added); MRK-CHA00224982, at '5021 (CSR) ("The primary endpoint for comparing the mumps antibody response between the 2 groups was the proportion of initially seronegative subjects who developed *neutralizing antibodies to mumps* 6 weeks postvaccination in all treatment groups.") (emphasis added). *See also generally* throughout MRK-CHA00000034 and MRK-CHA00224982. As discussed in detail above, neither the AIGENT nor WT ELISA assays were capable of identifying mumps neutralizing antibodies.

[234] MRK-CHA00224982, at '5036, (CSR) ("The mumps wild-type ELISA used in this study was shown to correlate with the PRN assay [2.2.6] and previous studies have established a strong correlation between the development of mumps-specific neutralizing antibodies and vaccine efficacy [].") (citing AIGENT/WT ELISA correlation study "Using the 'Corrected' AIGENT Results"); '5177 (CSR) ("The mumps wild-type ELISA used in this study was shown to correlate with the PRN assay [2.2.6] and previous studies have established a strong correlation between the development of mumps-specific neutralizing antibodies and vaccine efficacy [1.1.10; 1.1.11; 1.1.12]. Therefore, the mumps PRN assay and ELISA results from this study support the effectiveness of M-M-R^{TM}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 $\log_{10}$ $TCID_{50}$.") (citing AIGENT/WT ELISA correlation study "Using the 'Corrected' AIGENT Results"); at '5032 (CSR) ("In agreement with CBER, the measurement of mumps neutralizing Ab by PRN at 1 year postvaccination was later eliminated in view of the excellent correlation between mumps PRN and ELISA."). *See also* MRK-CHA00224982, at '5062 (CSR) ("The mumps PRN assay correlates well with the mumps ELISA and therefore only the mumps ELISA testing was conducted for this endpoint [2.2.6]. Revaccinations for measles, mumps, and rubella were based solely on ELISA results.").

correlation study, Merck provided CBER with the study it conducted using the "corrected" AIGENT data, which CBER had previously precluded Merck from using to support its mumps end-expiry potency sBLA.

- *Use of oGOS Stabilizer*. Merck represented that its use in the Protocol 007 testing of M-M-R II doses formulated with an Optimized Gelatin-Medium O-Sorbitol (oGOS) stabilizer was supported by WT ELISA test using "a fixed seroprotective cutoff of 10 ELISA Ab units."[235] Since the marketed product used a GOS formulation, CBER required Merck to demonstrate "the equivalence in vaccine immunogenicity between M-M-R™$_{II}$ manufactured with GOS and M-M-R™II manufactured with oGOS."[236] Merck did so by comparing the WT ELISA results from Protocol 007 to the results from other immunogenicity studies of M-M-R II formulated with GOS. Several of these comparator studies also used the WT ELISA. Merck described the WT ELISA used in Protocol 007 and these comparator studies as an "assay [] modified to include a low-passage (passage 9) Jeryl Lynn strain virus and a fixed seroprotective cutoff of 10 ELISA Ab units."[237] However, as discussed above, the 10Ab cutoff was not a seroprotective level and in fact had no relevance to protection at all.

- *Mock Control*. Merck represented that the mock serum control used in the AIGENT assay contained anti-IgG: "The mock serum control consists of a sample containing virus, anti-IgG and the serum diluent, but no human serum. . . . Each assay also includes a mock serum control (containing virus, anti-IgG and serum diluent, but no human serum) that is used to calculate neutralization of test samples."[238] Yet Mary Yagodich testified repeatedly that the mock control did not contain anti-IgG.[239] Merck's failure to add anti-IgG to the mock control would further exacerbate the significant issues already discussed with Merck's uncontrolled use of anti-IgG in the AIGENT. The absence of an appropriate internal control prevents detection and correction for assay artifacts attributable to added anti-IgG or other matrix effects. The consequence of failing to include anti-IgG in the control therefore would further compromise the consistency and reliability of the AIGENT assay results.

- *Anti-IgG*. Merck made repeated representations that the AIGENT assay used anti-IgG "to increase neutralization efficiency," "enhance the sensitivity of the assay," and to "enhance 'primary neutralization.'"[240] These are incorrect statements of the impact of Merck's use of anti-IgG in its AIGENT testing. A more reasonable and accurate

[235] MRK-CHA00224982, at '5929 (CSR).

[236] MRK-CHA00224982, at '5025 (CSR).

[237] MRK-CHA00224982, at '5929 (CSR).

[238] MRK-CHA00224982, at '5038 (CSR) (emphasis added).

[239] *See* Yagodich Dep. 81:7-9.

[240] MRK-CHA00224982, at '5037, '6152, '6160 (CSR).

explanation of the impact of added anti-IgG is conversion of non-neutralizing antibodies to act in a manner that mimics the activity of antibodies that directly block virus replication and spread in a PRN assay. As discussed above, this process is artificial and does not mimic normal human physiology.

- *GCP*. Merck made repeated representations that in conducting the AIGENT test Merck complied with all GCP and related quality control standards: "The clinical study was carried out in accordance with current standard research approaches regarding the design, conduct, and analysis of clinical trials. The study was conducted following appropriate Good Clinical Practice (GCP) guidelines . . . ."[241] These statements are incorrect in light of Merck's multiple and extensive violations of GCP.

- *Live Virus Degradation.* Merck represented that the source lot for the M-M-R-II samples used in Protocol 007, which had an initial mumps potency following fill of 4.8 $\log_{10}$ (~63,000) $TCID_{50}$/dose, was "representative of a mumps potency within the release range for M-M-R™II."[242] However, since October 1999, the minimum mumps release potency of M-M-R II has been 5.0 $\log_{10}$ (100,000) $TCID_{50}$/dose, with a target manufacturing potency of 5.2 $\log_{10}$ (~160,000) $TCID_{50}$/dose, and (as of September 2006) a maximum mumps release potency of 5.5 $\log_{10}$ (~316,000) $TCID_{50}$/dose.[243] Therefore, Merck was incorrect in representing that the samples used in the clinical trial fell within the mumps potency release range of M-M-R II. This is significant because the potency at which a live virus vaccine is released can impact the level of protection it provides at the time of injection.[244] Merck's testing in Protocol 007 of samples that had anywhere from 37,000

[241] MRK-CHA00000034, at '115 (sBLA). *See also* MRK-CHA00224982, at '6094 (Protocol from 007 Study) "All corrections or changes made to any study data (e.g., source documents, workbooks, case report forms) must be appropriately initialed and dated, as per good research practice standards."; at '6030 ("Based on our review and to the best of our knowledge, this clinical investigation has been conducted in accordance with applicable Good Clinical Practice standards."); MRK-CHA00000034, at '114 (sBLA) (**"**The trial methodology, assessment of safety, serologic assays, and selection of endpoints were in accordance with established practices for conducting vaccine trials."); MRK-CHA00224982, at '6099 (CSR) (Clinical Protocol 007-00) ("[T]he SPONSOR agrees to . . . ensure that trials are conducted and data are generated, documented, and reported in compliance with . . . accepted standards of Good Clinical Practice, and all applicable federal, state, and local laws, rules and regulations relating to the conduct of the clinical study.").

[242] MRK-CHA00224982 (CSR), at '5000-01. *See also* MRK-CHA00224982, at '5018, '5024 (explaining that both control and testing samples originated from the same parent lot).

[243] Stannard Dep. Ex. 3 at pp. 2-3.

[244] During manufacturing, replication of live virus produces both live and defective virus particles. These defective virus particles are referred to as defective interfering particles because they often interfere with infection and replication of the live virus (by blocking receptors, inducing interferon activation, and by a variety of other mechanisms), and can also interfere with the immune responses generated by live virus. After manufacturing, both the live and defective virus particles degrade over time to yield inactive virus particles and viral degradation products (proteins, protein fragments, nucleic acids, and other antigens). These defective and inactive virus particles and virus degradation products can interfere with the immune response to the live virus and the protection afforded by live virus vaccines. *See, e.g.,* Killip, MJ, "Activation of the beta interferon promoter by paramyxoviruses in the absence of virus protein synthesis," Journal of General Virology, 2012 Feb; 93 (Pt 2): 299-307; Santak, M. "Accumulation of defective interfering viral particles in only a few passages in Vero cells attenuates mumps virus neurovirulence," Microbes and Infection, 2015 Mar; 17(3): 228-36; Andzhaparidze, OG, "Chronic non-cytopathic

to 253,000 fewer degraded live virus particles than the product actually on the market (as well as significantly fewer degraded defective virus particles) further undermined the reliability and relevance to protection of the clinical trial results.[245]

In addition to these misrepresentations in this sBLA and accompanying materials, I have found numerous additional misrepresentations Merck made to CBER following the sBLA submission. Among Merck's most significant misrepresentations were assertions that the WT ELISA results and the 10Ab serostatus cutoff used in the test were clinically relevant to protection. These were made in response to specific CBER questions concerning the clinical relevance of the Protocol 007 data.

- In responding to CBER's July 27, 2004 request for 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)," Merck referred 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."[246]

- In responding to CBER's December 3, 2004 request for additional justification for the WT ELISA, 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."[247]

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infection of human continuous cell lines with mumps virus," Acta Virologica, 1983 Jul; 27(4):318-28; Calain, P, "Generation of measles virus defective interfering particles and their presence in a preparation of attenuated live-virus vaccine," Journal of Virology, 1988 Aug; Naruse, H, "Studies on the adaptation of mumps virus to chick embryo," Medical Microbiology and Immunology, 1986; 174(6):295-304; Andzhaparidze, OG, "Mumps virus-persistently infected cell cultures release defective interfering virus particles," Journal General Virology, 1982 Dec; 63(2):499-503.

[245] The 37,000 and 253,000 amounts of degraded live virus particles are based on the difference between (1) the amount of live virus particles in the 4.8 $\log_{10}$ parent lot minus the amounts after artificial aging to the 4.1 $\log_{10}$ and 3.7 $\log_{10}$ testing potencies [for 4.1 $\log_{10}$, this is 63,000 - 12,500 = 50,500; for 3.7 $\log_{10}$, this is 63,000 - 5,000 = 58,000], and (2) the amount of live virus particles in the potency release range of 5.0-5.5 $\log_{10}$ minus the amounts at the 4.1 $\log_{10}$ and 3.7 $\log_{10}$ testing potencies [for 4.1 $\log_{10}$, this is 100,000/316,000 - 12,500 = 87,500/303,500; for 3.7 $\log_{10}$, this is 100,000/316,000 - 5,000 = 95,000/311,000]. At least one regulatory agency expressed concern to Merck over the impact of potency degradation in Merck's mumps vaccine. *See* MRK-CHA00626021, at '022 ("For several years Paul-Ehrlich-Institute has adopted the attitude that the maximum level of degradation of a live virus vaccine (in terms of potency loss) is 0.5 log, i.e. that the maximum acceptable shelf life should ensure that losses of potency go not beyond this figure.").

[246] MRK-CHA00126963, at '970; MRK-CHA00126963, at '963, 967.

[247] MRK-CHA00000315, at '331. *See also* MRK-CHA00000393, at '400 (Merck asserting WT ELISA results were sufficient to support the sBLA by citing to Serial 86 and what it characterized as the "strong correlation (93.6%)

Following Merck's repeated misrepresentations to CBER of the clinical relevance of the AIGENT/WT ELISA correlation, the WT ELISA data, and the WT ELISA serostatus cutoff, CBER reached the conclusion that "the science related to immunogenicity testing of M-M-R®II has substantially evolved."[248] Consequently, CBER allowed Merck to use the Protocol 007 WT ELISA data to support its mumps end-expiry potency sBLA. With its final submissions in support of this sBLA, Merck resubmitted this Protocol 007 WT ELISA data, which as I have explained was not reliable or in any way relevant to clinical protection. In addition, Merck further supported this sBLA by also including data from its ProQuad and rHA applications, which were generated by the same unreliable and not clinically relevant WT ELISA assay.[249]

In sum, these key representations and submissions Merck made to the FDA in support of its end-expiry sBLA were false and unsupported.

3. Merck Misrepresented the WT ELISA Results in Its Submission of the BLA for ProQuad

Merck similarly misrepresented the clinical relevance of the test results it submitted in its ProQuad BLA and follow-up submissions. These results were largely based on the same WT ELISA test Merck developed and used for Protocol 007.[250] In reporting these results to CBER, Merck made repeated misrepresentations on the reliability of these results, the AIGENT/WT ELISA correlation on which they were justified, and their relevance to the efficacy and clinical protection afforded by ProQuad:

> A formal efficacy trial was not conducted with ProQuad. The efficacy of the product was determined through the use of serologic correlates of protection

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between ELISA and PRN serology results."). 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.

[248] MRK-CHA00000368, at '372.

[249] MRK-CHA00000368, at '368; MRK-CHA00000140, at '186.

[250] *See* MRK-CHA-01634832, at '848 (ProQuad package insert, showing 3,735 of the subjects, roughly 80%, were tested using mumps WT ELISA assay).

> previously established in the evaluation of the efficacy of the monovalent measles, mumps, rubella, and varicella vaccines.[251]
>
> More recently, Merck & Co., Inc. 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) [Ref. 5.4: 107, 108]. The overall agreement rate was 93.6% (480/513). These data support the use of the results of a wild-type ELISA as a correlate for protection.[252]
>
> Because ProQuad™ is intended to replace the routine use of M-M-R™II and VARIVAX™ in children, the clinical development program was designed to show that ProQuad™ could provide the same level of protection to the same intended population as M-M-R™II and VARIVAX™.[253]
>
> Clinical data presented in this Application confirm that ProQuad™ is similar (non-inferior) to M-M-R™II and VARWAX™ given concomitantly (at separate injection sites) with respect to immunogenicity and safety. These data suggest that ProQuad™ can be used in place of M-M-R™II and VARIVAX™ to prevent measles, mumps, rubella, and varicella.[254]

Furthermore, when CBER sought assurances that the WT ELISA cutoff had "relevance [to] protective levels," Merck claimed the AIGENT/WT ELISA correlation "provid[ed] information on the clinical relevance of the chosen ELISA cutoff."[255] Again, as I discuss above, the WT ELISA tests (including the serostatus cutoff and AIGENT correlation on which they relied) were unreliable and had no relevance to protection. Therefore, these central

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[251] MRK-CHA00158126, at '130. *See also* MRK-CHA00158126, at '136 ("The efficacy of ProQuad was established through the use of immunological correlates for protection against measles, mumps, rubella, and varicella."); at '137 ("Because ProQuad is intended to replace the routine use of M-M-RII and VARIVAX in children, the clinical development program was designed to show that ProQuad could provide the same level of protection to the same intended population as M-M-RII and VARIVAX.").

[252] MRK-CHA00158320, at '350. While the 93.6% agreement rate derives from the correlation Merck performed using the "original" AIGENT results, Merck cited to and attached to its BLA the correlation study it conducted using the "corrected" AIGENT results. MRK-CHA00158299, at '304. *See also* MRK-CHA00158320, at '350 (AIGENT/WT ELISA correlation "support[s] the use of the results of a wild-type ELISA as a correlate for protection") (also citing to "corrected" AIGENT results); MRK-CHA00158320, at '365 ("The presence of detectable antibody by the . . . the neutralization assay or EIA [ELISA] for mumps . . . has generally been shown to have a strong correlation with protection from disease.").

[253] MRK-CHA00158126, at '137.

[254] MRK-CHA00158126, at '158.

[255] MRK-CHA00846405, at '409, '414. Merck relied on Serial 86 again in responding to CBER questions on the specificity and sensitivity of the WT Mumps ELISA Merck used to support its ProQuad BLA: "The serostatus cutoff has been evaluated against that of a mumps neutralization assay, and the data show good agreement between assays when using a cutoff of 10Ab . . . ." MRK-CHA00846087, at '090 (citing Serial 86).

representations and submissions Merck made to the FDA in support of its ProQuad BLA were false and unsupported.

4. Merck Misrepresented the WT ELISA Results in Its Submission of the sBLA for M-M-R II with rHA

Merck similarly misrepresented the clinical relevance of the Protocol 009 test results it submitted in its sBLA for switching to an rHA formulation of M-M-R II. These results were also based on the same WT ELISA test Merck developed and used for Protocol 007. In reporting these results to CBER, Merck falsely stated: (1) the results "suggest the M-M-R™II with rHA is highly immunogenic, well tolerated, and will be as effective as M-M-R™II with HSA in preventing measles, mumps, and rubella,"[256] (2) the switch to rHA "was not expected to affect the efficacy of the vaccine,"[257] and (3) "[t]he data presented in this application indicate with a high level of assurance that manufacturing . . . M-M-RII . . . with rHA in place of HSA . . . will ensure that M-M-RII remains a highly effective vaccine."[258]

Moreover, in justifying the use of the WT ELISA "as a surrogate marker for efficacy," Merck represented that the assay had been correlated with the field efficacy studies Merck conducted in support of the original licensing of its mumps vaccine:

> Specific levels of serum antibodies to measles, mumps, and rubella as measured by hemagglutination inhibition [HI] and serum neutralizing antibody assays in field efficacy studies have been shown to correlate with protection against these diseases, and thus immunogenicity data can be used as a surrogate marker for vaccine efficacy. Correlation between the current assays (enzyme linked

[256] MRK-CHA00140056, at '196.

[257] MRK-CHA00138137, at '147 ("No studies of the efficacy of M-M-R™II with rHA or M-M-R™II were performed in support of this application. . . . Given the structural and genetic similarities of HSA and rHA, as well as the excellent and high level of vaccine-induced immune responses observed for both treatment groups in Protocol 009, the replacement of HSA with rHA in the bulk manufacturing of M-M-R™II was not expected to affect the efficacy of the vaccine.").

[258] MRK-CHA00138137, at '157. *See also* MRK-CHA00140056, at '853 ("Serum levels of antibodies to . . . mumps . . . will be determined by ELISA. . . . Protective levels of antibody will be defined as . . . >10.0 ELISA antibody units for mumps (wild-type) IgG."), at '866 ("For a given antigen, at least 477 of the 515 (92.6%) evaluable subjects in the M-M-RII manufactured with rHA group must have 6-week postvaccination titers greater than the protective level in order to meet the acceptability criteria."), at '917 ("Subjects who do not have protective antibody levels to one or more of the components of the study vaccine at the Day 42 bleed will be offered revaccination with currently licensed vaccine.").

> immunosorbent assay [ELISA]) and the assays used in the field efficacy studies (i.e., HI assay and serum neutralizing antibody assay) has been established.[259]

This representation was incorrect because Merck never correlated its WT ELISA assay to the field efficacy tests Merck used in its original licensing studies.[260]

In addition, when CBER requested that Merck "provide AIGENT data in support of the ELISA cutoff,"[261] as it did with the ProQuad BLA, Merck again responded by referring CBER back to Serial 86.[262] As I have repeatedly explained, the WT ELISA assay (including the serostatus cutoff and AIGENT correlation on which they relied) was unreliable and had no relevance to protection. It also had never been correlated (bridged) to the original licensing studies, or any studies connected to protection.[263] Therefore, these representations and submissions Merck made to the FDA in support of its rHA sBLA were false and unsupported.

* * *

In sum, the AIGENT and WT ELISA data Merck provided CBER to support these various mumps-related applications and submissions were not reliable or relevant to protection. Nevertheless, Merck repeatedly represented to CBER that they were. Moreover, Merck failed to provide CBER a full and transparent disclosure of the data and Merck's internal assessment of its

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[259] MRK-CHA00138137, at '144-45 (internal cites omitted). Merck does not cite the prior mumps efficacy studies or any mumps studies to support the claimed correlations.

[260] Merck internal correspondence clearly documents that there were no bridging studies performed that would support the correlation between Merck's mumps ELISA assays and its historical PRN assays. It was for this reason that CBER originally required Merck to use a functional assay for Protocol 007. MRK-CHA00215930, at '942 ("The current serologic [ELISA] assays lack an established correlation with protective efficacy. CBER believes that these assays are therefore not appropriate to establish equivalence, and has indicated that neutralizing antibody to wild type virus would be acceptable."); MRK-CHA00273309, at '302 ("The current serologic [ELISA] assays lack an established correlation with protective efficacy and CBER has indicated that a functional antibody assay (e.g. WT neut) will be required to establish equivalence.")

[261] MRK-CHA00124554, at '609.

[262] MRK-CHA00124554, at '588, '640 (Merck submitted Serial 53, which referred back to Serial 86, stating "[t]his question was addressed previously, in the course of addressing comments to IND 1016 [mumps end-expiry sBLA]") (attaching Serial 86, including the AIGENT/WT ELISA correlation report).

[263] MRK-CHA00625837, at '838 ("CBER has repeatedly requested that there be some 'clinically valid justification' for serologic criteria. Unfortunately, there has been no bridge between the different assays used over the years which would permit a correlation between current serologic endpoints and clinical endpoints from an era in which there was a measurable mumps attack rate."); MRK-CHA00020425, at '425 ("A requirement was set forth by CBER to use a functional neutralization assay for [Protocol 007] . . . due to: 2-Lack of data that correlates currently used ELISA assays and efficacy for MMR®II.").

reliability and clinical relevance. In my opinion, these data and Merck's false, unsupported and incomplete submissions of the data did not (and were not suitable for submission to) support or substantiate the various mumps licensing approvals and regulatory decisions Merck sought and obtained from CBER relating to M-M-R II and ProQuad.

D. Merck Has Conducted No Clinical Studies That Provide Reliable or Clinically Relevant Data On How Well Its Mumps Vaccines Protect Against Mumps

1. The Original Mumps Studies Supporting Licensure Provide No Clinical Data Demonstrating M-M-R II or ProQuad Protection From Mumps

As I discussed above, one of the catalysts for Protocol 007 was CBER's concern over the level of mumps protection afforded by M-M-R II at below end-expiry potencies. *See* Sec. IV.C Merck's initial proposal to address CBER's concern was to rely on the mumps efficacy studies Merck performed in the 1960's in connection with the original licensing of Merck's monovalent mumps vaccine Mumpsvax. Merck claimed these original studies demonstrated sufficient mumps protection at below end-expiry potencies. CBER rejected Merck's proposal, finding the original licensing studies too old, based on too small a sampling, and not necessarily relevant to the mumps protection afforded by M-M-R II. CBER required Merck to conduct a new clinical trial (Protocol 007) to demonstrate the mumps protection M-M-R II provided at lower potencies and to support Merck's label change application:

> [C]orrelation between the Neut. assay data generated to support protective efficacy in the label were old - based on small number of studies . . . and furthermore were questioned as to w[he]ther they are still valid in predicting the current protective efficacy of the MMRII vaccine against present [mumps] wild type strains.[264]

> 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.[265]

[264] MRK-CHA00198876, at '877.

[265] MRK-CHA00020425, at '425. *See also* 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).

> 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.[266]

I agree with CBER's assessment that Merck's original mumps efficacy studies, conducted on the monovalent vaccine (not the current trivalent or quadravalent formulations), with a very small subject sampling, and with what I would consider antiquated testing methods, are not clinically relevant to ProQuad or M-M-R II (as it has existed since 2005 or earlier).

My opinion (and that expressed by CBER) is further supported by Merck's internal documents. They show Merck's own recognition that it had no clinical trial data supporting M-M-R II at the 4.1 $\log_{10}$ $TCID_{50}$/dose potency for which it was seeking approval and that a new clinical trial was necessary to support its sBLA: "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."[267] Therefore, Merck's original licensing studies are not relevant to and provide no clinical support for M-M-R II at potencies below 4.3 $\log_{10}$ $TCID_{50}$/dose. They likewise are not relevant to and provide no support for ProQuad or M-M-R II formulated with rHA.

2. <u>The WT ELISA Assays Also Do Not Provide Any Clinical Data Demonstrating M-M-R II or ProQuad Protection From Mumps</u>

For all the reasons I already discussed, the WT ELISA test developed in Protocol 007 also provides no clinical support for M-M-R II or ProQuad. *See* Sec. V.B. Beyond the scientific and clinical failings I have described at length, my opinion is supported by Merck's witnesses who have acknowledged that the WT ELISA test and the 10Ab cutoff it used had no relevance to clinical protection. Dr. Antonello, who calculated the serostatus cutoff and conducted the AIGENT/WT ELISA correlation, was especially clear on this point:

> I'm not aware of [10Ab] being identified as protective level.[268]

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[266] MRK-CHA00615152, at '157. *See also* MRK-CHA00019225, at '228 (same).

[267] MRK-CHA00095320, at '320. *See also* MRK-CHA00017605, at '607 ("No data exists for mumps at the expiry potency Merck has selected. A clinical immunogenicity trial is necessary to provide these data.").

[268] Antonello Dep. 33:21-22.

> We don't really know what a clinically protective level is in either [AIGENT or WT ELISA] assay.[269]

> Q: So in your work in calculating [WT ELISA cutoff], did you take into account in any way the level of seroprotection that would be measured by the particular serostatus cutoff that you were calculating? A: I don't believe I did.[270]

> I don't know what a protective level is. So for me I can't say that 10 is the correct protective level . . . So [AIGENT/WT ELISA correlation] doesn't give me greater confidence in that sense that 10 is a protective level.[271]

> While we have compared the mumps WT ELISA to Dave Krah's Mumps AIGENT neutralization assay, I am not aware of any clinical evaluations where 10Ab units has been established as a 'protective' level.[272]

> [T]here is no clinical history/expectation/meaning that can be attached to the 90% response level in the PRN assay.[273]

Dr. Barbara Kuter, who I understand Merck selected as the corporate representative to answer questions regarding, among other things, how well Merck's mumps vaccine protects against disease, testified similarly.

> Q. Do Merck's serology results reflect the protection afforded by the mumps component of Merck's mumps vaccines in any way? A. It's not a direct correlate. Q. Does it have any relationship to protection from disease? A. I really can't answer that.[274]

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[269] MRK-CHA00791315, at '319.

[270] Antonello Dep. 66:25-67:21. *See also* Antonello Dep. 66:25-67:21 ("Q: But in your work in calculating what you thought was the appropriate serostatus cutoff level for the mumps wild type ELISA assay, did seroprotection play any role in that exercise? A: Not that I'm aware of. . . ."); Antonello Dep. 233:7-12 (Antonello affirming "that the serostatus cutoff of 10Ab used for the wild type mumps ELISA did not in any way relate to seroprotection.").

[271] Antonello Dep. 240:9-15. *See also* Antonello Dep. 236:3-14 ("Q. As far as your performing the calculation, did it give you any comfort that the 10Ab serostatus cutoff that you calculated was relevant to seroprotection? . . . A. Yeah, I don't know what is protection. I did the comparison that was requested and showed how the two assays relate. What that means beyond that, that's not my area of expertise, how to interpret the results in that sense."); Antonello Dep. 235:11-236:1 ("Q: Did the correlation that you performed between the wild type mumps ELISA assay and the AIGENT assay results provide, in your opinion, any support that the 10Ab serostatus cutoff was relevant to seroprotection? . . . A: I don't know what a protective level is. So . . . I can't address what a protective level is and whether that's protective or not protective.").

[272] MRK-CHA00649638, at '638.

[273] MRK-CHA00759061, at '061.

[274] Kuter Feb. 9, 2017 Dep. 40:14-21.

Dr. Krah, who developed and ran the AIGENT test to which the WT ELISA was correlated, similarly testified he had no understanding of whether the assay had any relevance to clinical protection:

> I would not be able to say that the AIGENT assay is the most accurate measure of mumps antibody. It's an assay that's intended as an imperfect model for looking at immune response in terms of antibody response to the vaccine. Whether it's accurate or not, that's beyond my expertise.[275]
>
> I have an opinion that the assay was reliable in measuring antibodies to mumps. As far as the impact on – or the conclusion about whether it was reliable assessment to immunogenicity, I can't say.[276]
>
> I do not agree that the data were designed to indicate whether they were protected or not. They're looking at immunogenicity and antibody responses, not – to the best of my understanding, not correlating it with protection.[277]
>
> I would say that the AIGENT assay was developed to meet [] a specific requirement . . . have the capability of measuring antibody responses. [] I don't have an expectation of what the correlation of that assay would be with protection.[278]
>
> So I don't think [the AIGENT serostatus cutoff] was ever considered a protective level.[279]
>
> No. I don't think we knew – you know, it's known what a protective level, antibody level is. . . . So, no, [the AIGENT serostatus cutoff is] not indicative of protection against the virus. . . . It just means that if you're above that, your response is likely above the variability in the assay. And it's likely due to having been vaccinated, but it doesn't reflect protection.[280]

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[275] Krah Dep. 754:17-23.

[276] Krah Dep. 412:3-9.

[277] Krah Dep. 754:2-7.

[278] Krah Dep. 772:13-20. *See also* Krah Dep. 726:9-17 ("Our – my goal and my understanding for developing the assay was to have an assay that would allow us to have the capability of measuring 95 percent seroconversion and have a pre-positivity rate of approximately 10 percent without – from my personal perspective, without considering the impact on accuracy."); Krah Dep. 599:15-21 ("My objective and our lab's objective was to develop an assay that would be capable of measuring 95 percent seroconversion. The clinical application is something that's beyond my responsibility of assigning.").

[279] Antonello Dep. 32:25-33:1.

[280] Antonello Dep. 151:5-22. *See also* MRK-CHA00791315, at '319 ("We don't really know what a clinically protective level is in either [AIGENT or ELISA] assay."); MRK-CHA00759061, at '061 ("[T]here is no clinical history/expectation/meaning that can be attached to the 90% response level in the PRN assay."); MRK-

> Q. Do you have an opinion as to whether or not the AIGENT assay was a reliable measure of how well the mumps component of MMR II protects vaccine recipients from getting the mumps disease? A. I don't have any opinion on that.[281]
>
> Q. And all the clinical testing that you did while at Merck on the mumps component of MMR II has given you no indication one way or another as to how well the vaccine works at protecting vaccine recipients from contracting mumps? . . . [A.] That's correct, none of the work – the work that I did was involved in the assay development and using the assay, not in connecting those results to project on how well the mumps component works.[282]
>
> I don't know what this [AIGENT validation] experiment means in relation to truth.[283]

This testimony supports my analysis, as I have detailed above, and leads me to the firm conclusion that the WT ELISA tests Merck used to support its rHA and end-expiry sBLAs for M-M-R II and BLA for ProQuad provide no support for M-M-R II or ProQuad claims concerning clinical protection. The studies Merck has conducted since Protocol 007 also provide no clinically relevant data since they all involved the same falsely correlated WT ELISA using the same unsupported 10Ab serostatus cutoff.[284]

3. Merck Has Conducted No Clinical Studies Demonstrating How Well M-M-R II and ProQuad Protect Against Mumps

The only efficacy studies Merck has performed on its mumps vaccine were the ones Merck conducted in the 1960's in connection with Merck's original licensing of its monovalent mumps vaccine Mumpsvax.[285] CBER rejected the relevance of these studies to Merck's current

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CHA00791315, at '315 (Merck's then Director of Clinical Vaccine Research Florian Schodel, stating "Agree with Joe [Antonello]- could not overemphasize the weakness of the PRN (50% specificity!!!!!!).").

[281] Krah Dep. 412:11-17.

[282] Krah Dep. 413:8-21.

[283] Antonello Dep. 122:16-17.

[284] *See* Antonello Dep. 282:6-9 ("So, to my knowledge, from the period it was developed to 2000 to now, it has not changed but now we are looking to change . . . ."); Musey Dep. 362:15-19 ("Q. So you know for a fact that after 2001 every ELISA clinical trial on the immunogenicity of MMR II used the 10Ab serostatus cutoff. Correct? A. Yes, correct."). *See also* Kuter BJ1, Brown M, Wiedmann RT, Hartzel J, Musey L. (2016). "Safety and Immunogenicity of M-M-RII (Combination Measles-Mumps-Rubella Vaccine) in Clinical Trials of Healthy Children Conducted Between 1988 and 2009,".Pediatr Infect Dis J. 35(9):1011-20.

[285] *See, e.g.,* MRK-CHA01449243, at '244 (M-M-R II package insert citing original licensing studies as support for product efficacy).

mumps vaccine formulations because the studies were too old, too small and conducted on the monovalent vaccine. The only mumps immunogenicity clinical trials Merck has conducted since then using a functional assay are Protocols 006 and 007.[286] Protocol 006 measured a wide range of M-M-R II seroconversion rates, some as low as 53%. In Protocol 007, CBER precluded Merck from using the AIGENT test because, among other things, the control arm failed the primary study objective and the study lacked sufficient power. Furthermore, Merck failed to provide any bridging studies to assess the correlation between any modern mumps immunogenicity assay and the functional immunogenicity test methods employed during the original licensing studies.

As a result, the only clinical data Merck has used to support the more current formulations of the mumps component of M-M-R II (since 2005) and ProQuad come from the WT ELISA assay Merck developed and used in Protocol 007. For all the reasons I have given above, none of this clinical data is reliable or relevant to clinical protection since they all derive from the same falsely correlated WT ELISA using the same unsupported 10Ab serostatus cutoff. Therefore, Merck has conducted no clinical studies that demonstrate or support how well M-M-R II (as of 2005) or ProQuad protect against mumps.

My conclusion is consistent with what I have seen in the record in this case which reflects Merck's own lack of understanding of how well its mumps vaccine works. Dr. Krah, the architect of the AIGENT test and the virologist Merck has put forward to work with the government in its efforts to better understand the causes of the recent outbreaks in the U.S.,[287] testified that he has no opinion on how well Merck's mumps vaccine protects against the disease:

> Q. Do you have an opinion on how well the mumps component of MMR II works today in protecting vaccine recipients from contracting mumps? A. I don't have an opinion on that.[288]

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[286] Musey Dep. 275:14-21.

[287] *See, e.g,,* Krah Dep. 756-767 (discussing working with CDC on mumps outbreaks).

[288] Krah Dep. 412:18-22. *See also* Kuter Feb. 9, 2018 Dep. 37:18-38:8 ("18 Q. Is there an average level of protection that Merck understands is what the mumps component of Merck's mumps vaccines affords to vaccine recipients? . . . A. I am not able to answer that question. Again, I don't have that expertise. Q. Does Merck know, does anyone at Merck know the answer to that question? . . . THE WITNESS: I don't know."); 39:1-9 ("Q. Has Merck done any studies of its own to establish whether the level of protection afforded by the mumps component of Merck's mumps vaccine has changed over time? . . . THE WITNESS: Not that I'm aware of.").

Dr. Krah's testimony is consistent with internal Merck communications during the development of the AIGENT assay questioning whether the low seroconversion rates measured against wild type strains reflected the "true efficacy" of the vaccine.[289]

E. 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

Since at least 1995, Merck's package insert for M-M-R II has contained the following statement:

> Clinical studies of [284] triple seronegative children, 11 months to 7 years of age, demonstrated that . . . a single injection of the vaccine [M-M-R II] induced . . . mumps neutralizing antibodies in 96% . . . of susceptible persons.[290]

In my opinion, this statement is inaccurate, false and misleading because the clinical study it references was not conducted on the M-M-R II vaccine that Merck has sold since it switched (in 2005) to its rHA formulation of the product and changed (in 2007) its mumps end-expiry potency specification to 4.1 $\log_{10}$ $TCID_{50}$/dose.[291] Merck has conducted no neutralization studies for M-M-R II with rHA, let alone any that demonstrated "mumps neutralizing antibodies in 96% . . . of susceptible persons." For M-M-R II at potencies as low as 4.1 $\log_{10}$, the only neutralization study Merck conducted was the AIGENT which as discussed above was not

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[289] *See* MRK-CHA00020421, at '421 ("Dorothy [Margolskee] referred to the Swiss study in which protective efficacy associated with JL was 70 some % and pointed out that if this study and similar data are reflective of true efficacy in the field then the PRN and CPE may be telling us what the neutralization against wild truly is."). *See also* 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. If we had more confidence that there was little likelihood of a drop in SC rate at expiry, of course the whole discussion above becomes moot. Are there any clinical data that you are aware of that provide some reassurance that lower mumps potency will not be problem?"); 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%"); at '869-870 ("Additional concerns: . . . vaccine doesn't work - fails neut. assay????").

[290] *See* MRK-CHA00757060; MRK-CHA01449260; M-M-R II package insert (Available at https://www.merck.com/product/usa/pi_circulars/m/mmr_ii/mmr_ii_pi.pdf). The only difference in this statement between these two package inserts is the number of test subjects in the study. In more recent versions of the M-M-R II package insert "the number of triple seronegative children was revised from 279 to 284." MRK-CHA00137876, at 876.

[291] While the M-M-R II package insert does not cite to the specific clinical study it references, it appears to be the study reported in the 1980 article Weibel, Robert E., "Clinical and Laboratory Studies of Combined Live Measles, Mumps, and Rubella Vaccines Using the RA 27/3 Rubella Virus (40979)," *Proceedings of the Society for Experimental Biology and Medicine* 165, 323-326 (1980).

specific to mumps and did not reliably measure neutralizing antibodies. *See* Sec. V.A. Moreover, in Merck's developmental testing for [Protocol 007], Merck's standard PRN measured mumps neutralization responses in the 66% - 75% range when testing against currently circulating wild-type mumps strains.[292]

Since at least 1999, Merck's package insert for M-M-R II has also stated:

> Following vaccination, antibodies associated with protection can be measured by neutralization assays . . . or ELISA [] tests.

This statement is also inaccurate, false and misleading as it relates to the mumps component of M-M-R II formulated with rHA or at potencies as low as 4.1 $\log_{10}$. As I discussed above, Merck has not conducted any clinical studies (by neutralization test, ELISA or otherwise) that measured "antibodies associated with protection" for this product or at this potency. The only clinical studies Merck has performed were with the AIGENT or ELISA (both WT and legacy) and for the reasons I set forth above in detail, none of them had any relevance to protection. *See* Sec. V.A, V.B.

Merck's package insert for ProQuad is also inaccurate, false and misleading in its statements on how well the vaccine protects against mumps. Since ProQuad's original licensure in 2005, the package insert has provided:

> ProQuad has been shown to induce measles-, mumps-, rubella-, and varicella-specific immunity, which is thought to be the mechanism by which it protects against these four childhood diseases.
>
> The efficacy of ProQuad was established through the use of immunological correlates for protection against . . . mumps.

As I explained above, the WT ELISA studies Merck conducted in support of its ProQuad BLA (which comprised the bulk of the testing) had no relevance to demonstrating mumps-specific immunity, protection or efficacy. Nor was the efficacy of ProQuad "established through the use of immunological correlates of protection" against mumps. The only correlation Merck used to

[292] *See* Sec. IV.C.1. My opinion on the false and misleading nature of this statement on the package insert is further corroborated by internal Merck documents that reflect Merck's concern about having to lower the 96% neutralization claim on the package insert, or remove the reference to "neutralizing antibodies," if Merck were unable to achieve its 95% seroconversion objective in Protocol 007. *See* n.39.

support the relevance of the WT ELISA assay was to the AIGENT, which also had no relevance to efficacy or protection.

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March 13, 2018