Robert S Lanciotti, September 15, 2016

Response to investigative team explanation concerning Allegation #1.

The investigative team alleges that there was insufficient statistically robust evidence to reach a conclusion concerning the comparative sensitivity of the Trioplex and the Singleplex assays. However, there were enough clear and convincing data, generated in particular from the multicenter comparison study conducted by Blood Systems Research Institute (in which each participating laboratory performed their own test under ideal conditions), that demonstrated a lower sensitivity of the Trioplex. This data should have influenced decision making and subsequent communications by the EOC. At a minimum, there should have been a comprehensive study of this sensitivity issue prior to the continued promotion of the Trioplex by the EOC. Further, the investigative team neglects to mention the findings that the Trioplex was significantly less sensitive for the detection of the four dengue viruses, and that this issue was also not addressed by the EOC. Taken together, there was clearly enough data to warrant a "pause" in the recommendation of the Trioplex until an extensive comparison could be performed.

Response to investigative team explanation concerning Allegation #2.

It was suggested to the EOC on at least two occasions (by Dr. Rosenberg and myself) that this sensitivity issue needed to be resolved prior to the continued recommendation of the assay. There were sufficient data from two independent laboratories (other than the developer of the Trioplex) that should have generated a pause in the promotion of the assay. At a minimum, the State Public Health Labs that were already approved for using the Singleplex should have been encouraged to continue with this format until additional data were generated with both Zika and dengue viruses, and the sensitivity issue was adequately resolved. This in fact was my recommendation. I never, in any email exchange, promoted the Singleplex over the Trioplex; my request, based upon the available data, was that laboratories using the more sensitive Singleplex should retain this test until the sensitivity issue were resolved.

Response to investigative team explanation concerning Allegation #3.

The email sent to all State Public Health Laboratories (April 27) in which the Trioplex was described as "recommended for use in the current Zika response," with no mention of the sensitivity issues with dengue and Zika viruses, clearly led these labs to believe that the Trioplex was the superior test. In fact there were several labs that subsequently switched to the Trioplex as a result of this communication. The sending of this email by the EOC with no mention of the sensitivity issues under investigation was misleading and irresponsible. The EOC had in fact held three meetings to discuss this sensitivity issue, and the issue was far from resolved at the time this email was sent. The EOC should have demonstrated greater transparency, and at a minimum shared the multicenter BSRI data. The communication also failed to mention that the CDC Fort Collins Arbovirus Diagnostic Laboratory, the reference laboratory with the greatest experience in Zika virus testing, was in fact not using the Trioplex because of concerns of its reduced sensitivity. At a minimum, State Public Health labs should have been made aware of the test actually being used by the CDC Arbovirus Laboratory.

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Comments concerning individual statements.

Page 4. "The EOC was presented with conflicting and inconclusive data about the Trioplex sensitivity..." This statement implies that the EOC evaluated the three sources of data (data generated in Fort Collins, the Blood Systems Research Institute (BSRI) data, and the Trioplex developer data) and then concluded that there "was no meaningful difference between the sensitivity of the two assays." First it is important to note that the Fort Collins data was a multi-test comparison, not merely a comparison of the Trioplex to the Singleplex, since the Fort Collins data included data generated by using a pre-release commercial kit from Applied Biosystems Inc. In all experiments both the ABI kit and the Fort Collins Singleplex assay demonstrated greater sensitivity in detecting both Zika and the four dengue viruses. With respect to detecting the four dengue viruses, the data showed a greater than 100-fold reduced sensitivity of the Trioplex when compared to both the Singleplex and the commercial ABI test. The email record clearly demonstrates that these two sources of data showing the reduced sensitivity of the Trioplex were deemed credible to such an extent that meetings were held to discuss improvements to the Trioplex to resolve the issue. At these meetings this conclusion that there was "no meaningful difference between the two assays" was never reached; in fact the actions taken by the EOC (to continue to hold meetings to discuss improvements to the Trioplex) indicated that there was great concern and that the sensitivity issue remained unresolved.

Page 18. The investigative team mentions correctly that samples were encountered that tested positive by the FOCUS Singleplex assay and negative by the Trioplex. However, they stated that this does not "illuminate the core of the whistleblower complaint" because the FOCUS Singleplex and the Fort Collins Singleplex "cannot be described as the same assay." The FOCUS assay and the Singleplex assay are essentially the same with minor differences involving the instrumentation and reagents used-see the discussion below (page 20) concerning this important topic. In addition, several of these discordant samples were eventually sent to the Fort Collins laboratory where they tested positive using the Singleplex.

Page 20. The investigative team asserts that the comparative data generated in Fort Collins is not reliable because the "Fort Collins Laboratory departed from the specified protocol" by using a different instrument. The assertion made here (and in other places in the investigative team response) is that the instrumentation and reagents used will affect the qualitative results of the assay. Data generated here (see below) and in other labs do not support this assertion. With respect to assay sensitivity, it needs to be stated up front that there is universal agreement among test developers that the most critical components of any real time RT-PCR assay are the primers utilized and the format in which these primers are applied. Studies have demonstrated that when primers directed against multiple pathogens are combined into a single assay (as is the case in the Trioplex) the sensitivity of the assay is reduced. On the other hand, it has been well established that the use of different instruments to extract nucleic acid and/or to detect fluorescence has a no effect on the qualitative outcome of the RT-PCR assay. For reference, the RT-PCR instrument is a heating and cooling instrument that also measures fluorescence. Stating that using different instruments will make a difference in the results is equivalent to saying that you can only bake a cake by using one particular brand and one particular model oven! The Fort Collins laboratory has 16 years of accumulated data from proficiency studies involving data generated by all state public health laboratories in which each laboratory utilized their own instrumentation and reagents to conduct the assay. The data are clear that there are no qualitative differences in the results-all the labs agreed on their results-and in fact the quantitative differences are minimal; demonstrating that the variety of

instruments and reagents used makes no difference. In this particular case in the Trioplex Singleplex comparison mentioned here by the investigative team, the instrument utilized by the Fort Collins Laboratory (ABI QuantStudio) is merely a newer model of the recommended EUA instrument. The statement by the investigative team concerning the use of different cutoff values is also irrelevant. The Fort Collins data provided to the EOC was the actual numerical raw data so that the EOC could apply whatever cutoff they chose. Using any cutoff value, the Trioplex still missed Zika cases. In fact, using the EUA recommended cutoff value of 38.5 actually raises the percentage of Zika samples missed by the Trioplex to 40%.

Page 20. The investigative team concludes that the BSRI data is less reliable because "comparing results across sites was difficult because of how the tests were performed." While this may be partially true when determining the theoretical analytical sensitivity, the BSRI study is actually the most accurate method to evaluate the clinical sensitivity (how the test will perform under real world conditions) of individual assays. In the BSRI study, each lab is provided with the identical panel of blind-coded specimens which are tested using their own methodology. This type of study more accurately represents what will occur in the real world of laboratory testing and is a more accurate measure of test performance. In this multi-center comparison it is important to note that the Trioplex was in fact the least sensitive of all evaluated tests. This is the data that is referred to on page 15 of the investigative team report, in which an official at BSRI stated that the sensitivity data concerning the Trioplex were "disturbing."

Pages 13 & 25. The investigative team raises a very important issue with respect to the foundational differences in approach between the Trioplex and the Singleplex tests. As mentioned by the team, the Trioplex is much less flexible than the Singleplex due to the detailed protocol which requires the use of particular instrumentation and even consumable reagents (all of which are irrelevant). As pointed out by the team "the instructions prohibited modification" of all aspects of the test. However, this lack of flexibility is more than merely a weakness of this approach; it is in fact a fatal flaw. There are many common laboratory issues that would easily render the Trioplex completely unusable. For example, if an instrument requires repair, or an instrument is discontinued, or a consumable is either backordered or unavailable, or if the Zika virus develops a mutation in which the kit no longer detects Zika virus RNA; the end user of this Trioplex assay will be unable to perform any testing. In the history of the ADB Diagnostic laboratory, with extensive experience during multiple epidemics, all of these issues mentioned above have in fact occurred. The approach of providing a minimal and flexible protocol, which has been the approach of this laboratory, allows for the rapid essential adaptability required during public health emergencies. It is important to note that this rigid method using a complete kit concept (Trioplex) represents a completely new approach and a departure from the long standing method of test dissemination by the CDC. The CDC ADB laboratory has accumulated 15 years of data demonstrating the effectiveness of this flexible approach (mentioned above); test dissemination to Public Health labs by this method have reproducibly demonstrated a high degree of accuracy and precision in external proficiency testing. The approach taken by the Trioplex represents a yet untested theory, and time will tell how effective it is and what unforeseen problems arise. In this regard, it is noteworthy that one change in the Trioplex protocol designed to address the sensitivity problem noted in April of 2016 took until August of 2016 to modify and disseminate.

Page 24. The investigative team correctly points out that the EOC becoming the primary decision making body during the Zika virus outbreak "was unsettling" to the subject matter experts at the Fort Collins laboratory. Unsettling for good reason since the EOC consisted primarily of individuals with little or no experience with arbovirus (in particular flavivirus) epidemics. The Fort Collins laboratory possesses the world subject matter experts in this area, with particular and extensive experience in epidemics, and more importantly in test development and dissemination. The EOC approach of having relatively inexperienced individuals making critical decisions may in fact represent the "root cause" of all the ensuing problems related to the recommendation of the Trioplex. The entire EOC concept as an approach to epidemic response by CDC needs to be reevaluated.

Overall Conclusion.

The investigative team's primary line of reasoning is that the two comparative analysis studies that demonstrate the lower sensitivity of the Trioplex are flawed. While this reasoning (i.e. each lab conducts their test under slightly different conditions leading to invalid results) may have some merit with respect to determining the theoretical limit of detection, there was sufficient credible data generated from multiple sources indicating that the Trioplex would in the "real world" miss cases of Zika virus and all four dengue viruses. The apparent lack of concern by the EOC in investigating the issue of the reduced sensitivity of the Trioplex to detect the dengue viruses is of great concern, as these viruses are known to co-circulate with Zika virus. In addition, the accurate diagnosis of dengue cases is critical since pregnant women with confirmed dengue infections are not clinically managed the same as Zika. The clinical case management of confirmed Zika infections among pregnant women is unique and important. As a result, it is noteworthy that the EOC has modified the overall testing algorithm on several occasions with the intent of not missing a single Zika infection-even going to the extreme of recommending tests that would not be applied under normal circumstances (i.e. PCR testing of non-acute specimens). It is ironic and inconsistent therefore that the EOC would recommend the Trioplex, which has been documented to miss clinical cases. The EOC was fully aware of this data yet did not factor it in their communications. To err on the side of caution and to maintain the highest ethical standards of Public Health policy, these data should have generated a pause in the promotion of the Trioplex and a comprehensive analysis to resolve the issue. Instead the EOC continued to promote a questionable assay with misleading communications that led laboratories to believe that it was in fact the best available test for the detection of Zika virus. Not recommending the best available test during an epidemic of a novel pathogen, in which the potential to miss cases is evident with implications for proper clinical case management, is in fact a threat to public health.