THE SECRETARY OF HEALTH AND HUMAN SERVICES
WASHINGTON, D.C. 20201

SEP 0 2 2016

The Honorable Carolyn N. Lerner
Special Counsel
Office of the Special Counsel
1730 M Street, N.W., Suite 300
Washington, D.C. 20036-4505

Re: OSC File No. DI-16-3709

Dear Ms. Lerner:

I received your July 1, 2016 correspondence, which concludes that allegations raised by Dr. Robert S. Lanciotti, an employee of the United States Department of Health and Human Services, Centers for Disease Control and Prevention (CDC), may constitute a substantial and specific danger to public health. The Office of Special Counsel requested an investigation and report on Dr. Lanciotti's allegations. At my request, the CDC Associate Director of Laboratory Science and Safety conducted an investigation into the allegations and drafted a report on the investigation's findings.

The investigation found that the evidence does not support the allegations or that the CDC's actions presented a substantial and specific danger to public health. Findings from the investigations are included in the enclosed report, which I am submitting for your review. I have reviewed the enclosed report in accordance with 5 U.S.C. § 1213(d).

Sincerely,

Sylvia M. Burwell

Sylvia M. Burwell

Enclosure

United States Department of Health and Human Services

Report of Investigation

OSC File Number DI-16-3709

Contents

I. Executive Summary

In a letter dated July 1, 2016, the U.S. Office of Special Counsel tasked the Secretary of the Department of Health and Human Services (HHS) to conduct an investigation into the whistleblower disclosure of Dr. Robert Lanciotti, Chief of the Diagnostic and Reference Activity in the Arboviral Diseases Branch at the Centers for Disease Control and Prevention (CDC). The HHS Secretary delegated authority to conduct the investigation to the CDC Associate Director for Laboratory Science and Safety (ADLSS), Dr. Stephan Monroe. The investigative team led by Dr. Monroe interviewed Dr. Lanciotti and 10 other witnesses, reviewed extensive documents, and collected and evaluated all available data on the issue presented. The investigative team included scientists with expertise in microbiology, virology, and biostatistics, and no member of the investigative team was involved in the events mentioned in the whistleblower disclosure, in the management reporting structure associated with any of the allegations, or worked in CDC's National Center for Emerging and Zoonotic Infectious Diseases or the Emergency Operations Center (EOC) at the time of the events that are the subject of the whistleblower disclosure.

Dr. Lanciotti alleged that CDC's EOC created a substantial and specific danger to public health when it failed to disclose that a CDC assay used to detect Zika virus—called the Trioplex Real-time RT-PCR Assay (Trioplex)—was substantially less sensitive (i.e., detected Zika virus infections less reliably) than an assay called the Singleplex that is used in Dr. Lanciotti's CDC laboratory and in a number of public health laboratories across the country.

The Singleplex tests only for the Zika virus while the Trioplex is designed to detect Zika virus and two other mosquito-borne viruses—dengue virus and chikungunya virus. This feature of the Trioplex assay provides an important clinical benefit because testing for all three viruses helps clinicians distinguish between the viruses, which produce similar initial symptoms, and helps inform patient care. Because of this clinical utility and the added efficiency that the Trioplex offered to public health laboratories, CDC submitted the Trioplex to the Food and Drug Administration (FDA) for emergency use authorization, which FDA granted in March 2016. This authorization allows CDC to provide quality-controlled assay components and standardized support to public health laboratories to set up and run the Trioplex. Following FDA's emergency use authorization of the Trioplex, Dr. Lanciotti performed comparisons of the sensitivity of the two assays, which he alleges show that the Trioplex is substantially less sensitive than the Singleplex.

After reviewing the available evidence and performing an independent evaluation of all available data on the sensitivity of the Trioplex and Singleplex assays, the investigative team concludes that Dr. Lanciotti's allegations are not substantiated. With regard to the specific allegations in the whistleblower disclosure:

- **Allegation 1: Use of the Trioplex in place of the Singleplex in a clinical setting will result in an additional 39 percent of Zika infections in their acute phase going undetected.**

  This allegation is not substantiated. There is insufficient, statistically robust, definitive data to reach an evidence-based conclusion that use of the Trioplex assay over the Singleplex in clinical practice will result in 39 percent of Zika virus infections being missed. The investigative team evaluated three different comparisons of the Trioplex and Singleplex assays; two comparisons, including the one performed by Dr. Lanciotti, found that the Singleplex was more sensitive than the Trioplex; a third comparison, performed by a CDC laboratory in San Juan, Puerto Rico, which produced the clearest, most complete, and most reproducible data available to the investigative team, found no difference in sensitivity. Ultimately the comparison data were limited and inconclusive; inconsistencies in how the assays were performed and in data reporting precluded making a statistically-valid conclusion about the relative performance of the assays.

- **Allegation 2: The EOC is aware of information indicating that the Trioplex is less sensitive in detecting Zika virus RNA than the Singleplex but is withholding this information from public health laboratories.**

  This allegation is not substantiated. Part of this allegation is accurate insofar as the EOC was aware of Dr. Lanciotti's concerns about the Trioplex's sensitivity relative to the Singleplex and did not share his

findings—or the contradictory findings from the San Juan comparison—with public health laboratories. However, the EOC did not have reliable information that the Trioplex was less sensitive than the Singleplex; instead it had only inconclusive data showing conflicting results between two CDC laboratories. The EOC's decision to keep these conflicting data internal was reasonable under the circumstances. Sharing inconclusive performance data that showed a conflict between CDC laboratories would have provided little actionable information to external laboratories. It had the potential to create considerable confusion during an ongoing emergency response and could have caused states to abandon the Trioplex and forfeit its practical and clinical benefits despite the absence of available evidence to support such an action. The decision by the EOC to recommend the Trioplex while not prohibiting the use of other validated laboratory diagnostic tests like the Singleplex and also working to improve the Trioplex's diagnostic sensitivity was a reasonable and appropriate course of action.

- **Allegation 3: The EOC's promotion of the Trioplex may have led public health laboratories that were approved[1] to use the more sensitive Singleplex to run the Trioplex preferentially, believing it to be the superior method for detecting Zika virus RNA.**

  The key premise of this allegation—that the EOC knowingly promoted an inferior assay—is not substantiated by the available evidence. This allegation may be correct to the extent that the EOC's promotion of the Trioplex—and more importantly the material support CDC provided to laboratories running the Trioplex—likely led some laboratories to preferentially run the Trioplex over the Singleplex, though seven laboratories continue to use the Singleplex. However, there are no statistically significant data to demonstrate that the Trioplex was less sensitive than the Singleplex and therefore the allegation that the EOC knowingly promoted an inferior assay cannot be substantiated. Further, there is no evidence that CDC ever instructed external laboratories to discontinue their use of the Singleplex.

In sum, the investigative team concludes that the available evidence does not substantiate that the EOC's actions presented a substantial and specific danger to public health. The EOC was presented with conflicting and inconclusive data about the Trioplex's sensitivity relative to the Singleplex and the clearest, most complete, and most reproducible of these data indicated there was in fact no meaningful difference between the sensitivity of the two assays. Virtually all witnesses, including Dr. Lanciotti, agreed that there was extensive discussion within CDC to determine the validity and reliability of these data and that the EOC took Dr. Lanciotti's concerns seriously. The EOC did not ultimately adopt Dr. Lanciotti's proposed course of action to share conflicting and inconclusive information with states and recommend use of Singleplex, which was not reviewed or authorized by FDA, over the Trioplex. The EOC reasonably assessed that such an action had the potential to create confusion during an ongoing emergency response and could have caused public health laboratories to discontinue use of the Trioplex and forfeit its practical and clinical benefits despite the lack of evidence to support such an action. Instead, the EOC chose to continue working to improve the Trioplex while not actively discouraging the continued use of the Singleplex. Efforts to improve the Trioplex include the August 22, 2016 submission of a major amendment to change the Instructions for Use that will include multiple substantive changes that have promise for improving the Trioplex's diagnostic sensitivity, namely increasing the sample input volume and adding whole blood as a specimen type. The EOC's actions were reasonable given the circumstances, available data, and the benefits offered by the Trioplex.

It is recommended that CDC continue its ongoing efforts to improve the sensitivity of the Trioplex, such as the August 22, 2016 amendment to the Trioplex's Instructions for Use that includes changes that hold promise for enhancing the Trioplex's diagnostic sensitivity.

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[1] This is the language from the whistleblower disclosure, but as explained in the report, public health laboratories were not "approved" to perform the Singleplex by CDC or FDA. The allegation appears to refer to laboratories that went through a validation of the Singleplex test as performed in their laboratory under the Clinical Laboratory Improvement Amendments, or CLIA.

II. Summary of the Information with Respect to which the Investigation Was Initiated

This investigation was initiated based on a whistleblower disclosure from Robert S. Lanciotti, PhD, Chief of the Diagnostics and Reference Activity in the Division of Vector-Borne Infectious Diseases (DVBD), National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), at the Centers for Disease Control and Prevention (CDC). Dr. Lanciotti alleges that CDC's Emergency Operations Center (EOC), which is managing CDC's response to a multi-country outbreak of Zika virus, caused a substantial and specific danger to public health and safety by promoting a type of Zika virus diagnostic test called the Trioplex Real-time RT-PCR Assay (Trioplex) and that this test is less sensitive in detecting Zika virus ribonucleic acid (RNA) and consequently less diagnostically sensitive (i.e., will identify positive cases less reliably) than an alternative test developed by Dr. Lanciotti, the Singleplex real-time RT-PCR assay (Singleplex). Both tests are designed to detect Zika virus RNA in clinical samples. The Singleplex detects RNA only for the Zika virus; the Trioplex detects RNA for Zika virus and two additional mosquito-borne viruses: dengue virus and chikungunya virus.

Specifically, Dr. Lanciotti alleges:

1. Use of the Trioplex in place of the Singleplex in a clinical setting will result in an additional 39 percent of Zika infections in their acute phase going undetected;
2. The EOC is aware of information indicating that the Trioplex is less sensitive in detecting Zika virus RNA than the Singleplex but is withholding this information from public health laboratories; and
3. The EOC's promotion of the Trioplex may have led public health laboratories that were approved to use the more sensitive Singleplex to run the Trioplex preferentially, believing it to be the superior method for detecting Zika virus RNA.

In a letter dated July 1, 2016, the U.S. Office of Special Counsel tasked the Secretary of the Department of Health and Human Services (HHS) to conduct a statutorily-required investigation of Dr. Lanciotti's whistleblower disclosure and submit a written report signed by the Secretary or her delegate. In a letter dated July 20, 2016, the HHS Secretary delegated authority to conduct the investigation to the CDC Associate Director for Laboratory Science and Safety (ADLSS), Stephan Monroe, PhD.

III. Conduct of the Investigation

The CDC ADLSS, Dr. Monroe, led the investigation into Dr. Lanciotti's whistleblower disclosure. The ADLSS serves as the key point of oversight and accountability for laboratory science and safety at all CDC campuses. The Office of the Associate Director for Laboratory Science and Safety (OADLSS) is charged with overseeing and monitoring the development, implementation, and evaluation of laboratory safety and quality programs across CDC and leads responses to laboratory incidents at the agency.[2] Dr. Monroe has 29 years of experience as a virologist at CDC and has been the co-author of more than 130 scientific manuscripts and book chapters. He has expertise in the development and implementation of real-time polymerase chain reaction assays, which is the type of assay at issue in this case, and has held a number of leadership positions in the agency, including Deputy Director of NCEZID, Director of the Office of Advanced Molecular Detection, and Director of the Division of High-Consequence Pathogens and Pathology.

The investigative team also included Conrad Quinn, PhD; Samuel Posner, PhD; and Noah Aleshire, JD. Dr. Quinn is the Director of the Office of Laboratory Science in CDC's OADLSS. In that role, he provides oversight and coordination for CDC laboratory quality programs across the agency. He has 27 years of experience as a microbiologist with nearly 100 peer-reviewed publications and served as the Chief of the Meningitis and Vaccine Preventable Diseases Branch at CDC prior to his role in OADLSS. Dr. Posner is the Associate Director for

[2] 81 Fed. Reg. 46677 (July 18, 2016).

Epidemiologic Science in CDC's National Center for Immunization and Respiratory Diseases. He has more than 100 peer-reviewed publications and expertise in biostatistics. Mr. Aleshire is the Senior Advisor for Policy in OADLSS.

No member of the investigative team was involved in the events mentioned in the whistleblower disclosure, in the management reporting structure associated with any of the allegations, or worked in the CDC EOC or NCEZID at the time of the events that are the subject of the whistleblower disclosure.

A. Methodology and Scope

The scope of the investigative team's inquiry centered on the core allegations of Dr. Lanciotti's whistleblower complaint, namely the sensitivity of the Trioplex and Singleplex tests, the decision making within the EOC around the promotion of the Trioplex, and whether the EOC's actions around the Trioplex presented a substantial and specific danger to public health and safety.

The investigative team conducted interviews with all witnesses named in the whistleblower disclosure and other witnesses relevant to the investigation, and reviewed extensive documents including emails, meeting summaries, internal memoranda, and other relevant documents.

The investigative team collected all available data on the sensitivity of the Trioplex and Singleplex assays, including data and analyses by Dr. Lanciotti, Dr. Jorge Muñoz, and researchers external to CDC. The investigative team performed an independent evaluation of these data to assess the strengths and weaknesses in the different datasets, compare the performance of the two tests, and review the basis for the decisions made in the EOC.

B. Witnesses Interviews

The investigative team conducted 11 interviews during the course of the investigation. All witness interviews except for one—with Dr. Muñoz in CDC's Dengue Branch located in San Juan, Puerto Rico—were conducted in person by Dr. Monroe. Other members of the investigative team participated in person or, for those interviews conducted outside of Atlanta, via teleconference. Dr. Lanciotti was interviewed at the outset of the investigation in Fort Collins, Colorado on July 7, 2016.

Witnesses were asked about their role in the events discussed in the whistleblower disclosure and, when applicable, their knowledge about the scientific decision making around the promotion of the Trioplex and Singleplex assays. The investigative team asked witnesses, including Dr. Lanciotti, for other potentially relevant witnesses and interviewed those witnesses when deemed pertinent to the scope of the investigation.

Witnesses interviewed by the investigative team included the following CDC staff:

- Robert S. Lanciotti, PhD
- Amy J. Lambert, PhD
- Ronald M. Rosenberg, ScD
- Ann M. Powers, PhD
- Julie M. Villanueva, PhD
- Jorge L. Muñoz-Jordán, PhD
- Kimberly B. Hummel, PhD
- Lyle R. Petersen, MD, MPH
- Toby L. Merlin, MD
- Laura E. Rose, MTS
- Jennifer D. Thomas, PhD

IV. Summary of the Evidence Obtained from the Investigation

A. Zika, Chikungunya, and Dengue Viruses

Zika virus is a mosquito-borne virus of the flavivirus genus, which includes other mosquito-borne viruses like dengue, yellow fever, and West Nile. Zika is predominantly spread through the bite of an infected *Aedes* species mosquito, but other routes of transmission (e.g., sexual transmission, blood transfusion, and maternal-fetal) are possible. Approximately 80 percent of people who become infected with Zika do not develop symptoms. The 20 percent of infected people who do become ill have generally mild symptoms, including rash, conjunctivitis, joint pain, and fever. Infection with Zika virus has the most severe implications for pregnant women; Zika virus infections during pregnancy can cause severe birth defects, including microcephaly, in which a baby is born with an abnormally small head and brain. Zika virus is also linked to miscarriage and stillbirth. It is also very likely that Zika virus infections trigger a serious autoimmune disorder called Guillain-Barré syndrome in a small proportion of people.

First identified in 1947 in Uganda, sporadic human Zika infections have been reported across Asia, Africa, and the Pacific Islands in the decades that followed. In February 2015, public health authorities identified a Zika virus outbreak in Brazil, the first known outbreak of Zika virus in the Americas. The virus then spread rapidly through South and Central America and the Caribbean. The World Health Organization (WHO) declared the clusters of microcephaly and other neurological disorders and their possible association with Zika virus a Public Health Emergency of International Concern on February 1, 2016. As of August 2016, more than 50 countries and territories across the Americas, Pacific Islands, and Africa reported active Zika transmission. This includes widespread transmission in the Commonwealth of Puerto Rico, sustained transmission in American Samoa and the U.S. Virgin Islands, and limited local transmission in certain areas within Miami, Florida.

Dengue virus is a member of the flavivirus genus, like Zika, and is also primarily transmitted by *Aedes* mosquitoes. There are four types of dengue virus that can cause disease, called dengue fever. Dengue fever symptoms typically include fever; severe headache; pain behind the eyes; joint, bone, and muscle pain; nausea and vomiting; and rash. Infections with these viruses can also cause dengue hemorrhagic fever and dengue shock syndrome, more serious manifestations of disease. Dengue virus is widespread. WHO estimates that there are 50 to 100 million dengue infections annually and 22,000 deaths, primarily among children.[3] While there is no specific treatment, early detection and appropriate supportive care, including avoiding the use of non-steroidal anti-inflammatory agents, can lower fatality rates.

Chikungunya virus is a member of the alphavirus genus, and like Zika is primarily transmitted by *Aedes* mosquitoes. WHO reported the first cases of chikungunya virus transmission in the Western Hemisphere in 2013. Since then, local transmission of the virus has been reported in 45 countries, with more than 1.7 million suspected cases reported to the Pan American Health Organization.[4] Most people infected with chikungunya virus develop symptomatic disease, which most commonly includes fever and joint pain that can be severe and debilitating.[5] Death from the disease is rare.

Efforts to understand, address, and control Zika, dengue, chikungunya viruses are interrelated. All three viruses are primarily transmitted by the same mosquitoes, *Aedes aegypti* and *Aedes albopictus*. There is local transmission of all three viruses throughout South and Central America and the Caribbean, and clinical presentation of Zika virus infection can be similar to infections of chikungunya or dengue, especially dengue. A February 7, 2016 memorandum on diagnostic testing from CDC's Division of Vector-Borne Diseases (DVBD) (where Dr. Lanciotti works) and available on CDC's website, highlights the importance of testing for all three

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[3] CDC, Dengue Epidemiology, http://www.cdc.gov/dengue/epidemiology/index.html (last visited Aug. 15, 2016).
[4] CDC, Chikungunya Virus: Geographic Distribution, https://www.cdc.gov/chikungunya/geo/index.html (last visited Aug. 15, 2016).
[5] Marc Fisher & Erin Staples. *Notes from the Field: Chikungunya Virus Spreads in the Americas—Caribbean and South America, 2013-2014*. 63 MORBIDITY & MORTALITY WKLY. RPT. 500, 500 (2014), *available at* http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6322a5.htm?s_cid=mm6322a5_w.

viruses, given their similar clinical presentation of symptoms and overlapping geographic distribution.[6] Distinguishing the three viruses would help health care providers make better clinical decisions for patients potentially infected with one of these viruses and assist public health authorities trying to identify and control outbreaks of these viruses.

B. CDC's Zika Work Prior to the 2015 Outbreak

CDC, headquartered in Atlanta, Georgia, is comprised of a number of different centers, institutes, and offices.[7] Prior to the 2015 Zika outbreak in South America, CDC's work on Zika virus was based primarily in DVBD, which is one of seven divisions in CDC's National Center for Emerging and Zoonotic Infectious Diseases (NCEZID).[8] DVBD is charged with the prevention and control of vector-borne bacterial and viral diseases—that is, those transmitted by mosquitoes, ticks, and fleas, including Zika virus, chikungunya virus, and dengue virus.

The bulk of the Division, including DVBD leadership, is based in Fort Collins, Colorado. This includes the Arboviral Diseases Branch, which conducts surveillance, field investigations, and laboratory studies to prevent and control arboviruses (i.e., viruses carried by mosquitoes, ticks, and other arthropods), including Zika and chikungunya. DVBD's Dengue Branch is based in San Juan, Puerto Rico and focuses on dengue virus studies and prevention, although Zika virus studies have become a growing part of the Dengue Branch's work since 2015, as the risk of an outbreak there increased (the island reported its first Zika virus cases in December 2015). DVBD includes two additional branches, Bacterial Diseases Branch (based in Fort Collins) and Rickettsial Zoonoses Branch (based in Atlanta), which were not involved in this investigation.

The Arboviral Diseases Branch is comprised of four teams or "activities:" Diagnostics and Reference, Surveillance and Epidemiology, Ecology and Entomology, and Virology. Dr. Robert Lanciotti is Chief of the Diagnostics and Reference Activity and has been with DVBD since 1989. In this role he leads the Division's efforts in developing diagnostic tests for arboviruses and providing diagnostic reference consultation to external partners, including state and local health departments. He was moved from that position by DVBD leadership on May 17, 2016, and resumed his role as Chief in July 2016. He is one of the leading experts on Zika virus at CDC, with an extensive publication record, and he actively participated in CDC's response to previous Zika outbreaks.

C. Activation of the CDC Emergency Operations Center for Zika Virus Outbreak

CDC's framework for emergency preparedness, called the CDC All-Hazards Plan, lays out the overall structure, organization, and responsibilities of the agency during an emergency response. The Plan is based on the National Incident Management System (NIMS), a unified set of principles and organizational processes for incident management administered by the Department of Homeland Security. Both NIMS and the CDC All-Hazards Plan emphasize the importance of a unified chain of command for incident management during an emergency.

As described in the All-Hazards Plan, CDC's Emergency Operations Center (EOC) on the CDC Roybal campus in Atlanta, serves as the central incident management and control facility for CDC during emergency responses to serious public health threats.[9] The EOC operates under an Incident Management System (IMS) model to organize and manage the response. Under the IMS, an Incident Manager, who reports to the CDC Director, leads the agency's emergency response. Upon activation of the EOC, incident management functions are centralized in the EOC.

The CDC All-Hazards Plan highlights the importance of centralizing the flow of information from the agency during the response. The Plan notes that the "CDC EOC through the CDC IMS is the center of information flow to and from partners," and that information is "directed during the event or incident by the Incident Manager with

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[6] Memorandum from CDC, Division of Vector Borne Diseases (Feb. 7, 2016), Revised Diagnostic Testing for Zika, Chikungunya, and Dengue Viruses in U.S. Public Health Laboratories, *available at* https://www.cdc.gov/zika/pdfs/denvchikvzikv-testing-algorithm.pdf.

[7] The CDC organizational chart is available at http://www.cdc.gov/about/pdf/organization/cdc-photo-org-chart.pdf.

[8] The NCEZID organizational chart is available at http://www.cdc.gov/ncezid/pdf/ncezid-org-chart.pdf.

[9] CDC. CDC ALL-HAZARDS PLAN (2013).

support from the Command staff."[10] The All-Hazards Plan highlights the need to "coordinate CDC emergency communication across all programs and channels to ensure consistency and that CDC speaks with *one voice*" (emphasis in original).[11] The Plan also outlines the role of the "Laboratory Outreach Desk" within the EOC, tasking that group to "standardize messaging to clinical laboratory organizations" and "facilitate the exchange of laboratory-related information between CDC and others in the laboratory community."[12]

On January 22, 2016, CDC activated the EOC to respond to the ongoing Zika virus outbreaks in the Americas. On February 3, 2016, the EOC moved to a Level 1 activation, the highest level of emergency response.[13] Dr. Lyle Petersen, the Director of DVBD in Fort Collins, was selected as the Incident Manager and temporarily relocated to Atlanta to lead the response efforts.

The activation of the EOC marked a fundamental change in how the agency coordinated and managed its work on Zika virus. It moved the core management of the response to the Zika virus outbreak from DVBD to the EOC in Atlanta, although arbovirus subject matter experts, like Drs. Lanciotti, Lambert, and Muñoz, continued to play a major role in the response and worked closely with the EOC. This included the creation of an EOC laboratory team that reported directly to the Incident Manager. Because the core laboratory subject matter expertise on Zika virus resided in Fort Collins, rather than Atlanta, the EOC laboratory team had a unique structure that included two co-leads—one located in Atlanta and one located in Fort Collins. This was an effort to enhance coordination between activities in Fort Collins and those in Atlanta. Dr. Ann Powers, currently the acting Virology Chief in the Arboviral Diseases Branch and also the former acting Branch Chief of the Arboviral Diseases Branch, served as the EOC laboratory team co-lead from Fort Collins during the time period that is the subject of the whistleblower complaint. Dr. Julie Villanueva was the Atlanta co-lead of the EOC laboratory team during much of this period, with Dr. Kimberly Hummel serving in this role from April 11 to June 10, 2016.

D. Development, Emergency Use Authorization, and Dissemination of the Trioplex Assay

i. Overview of Diagnostic Testing for Zika virus

Diagnosing current or recent Zika virus infection in a patient relies on two different approaches: (1) detecting the virus's genetic material (RNA) in an appropriate clinical specimen, and (2) detecting antibodies in a serum sample that indicate a patient's immune system has responded to a Zika virus infection.

To detect Zika viral RNA, laboratories use a technique called real-time reverse transcription-polymerase chain reaction (real-time RT-PCR). PCR is a method for amplifying small amounts of a specific segment of double stranded genetic material (DNA) in a sample so that it can be easily detected. The specificity of the assay results from the use of short, synthetic DNA primers that are designed to uniquely bind to the DNA target of interest.

Reverse transcription-PCR (RT-PCR) is a variation of the PCR technique used for detecting specific regions of RNA. It includes an additional step to first transcribe an RNA sequence into a complementary strand of DNA. This is necessary because PCR can only be performed on DNA sequences, not RNA (Zika, dengue, and chikungunya are all RNA viruses).

Finally, "real-time" RT-PCR is a refinement of the PCR technique that incorporates the use of synthetic DNA probes labelled with fluorescent dyes and specialized instrumentation that allows scientists running the test to monitor the amplification process in real time.

The other available approach to diagnosing Zika virus infection is by detecting virus-specific immunoglobulin M (IgM) antibodies produced by the patient in response to infection. While IgM testing is a critical tool for diagnosing recent Zika virus infections, interpretation of the results from currently-available assays is complicated

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[10] *Id.* at 65.
[11] *Id.* at 139.
[12] *Id.* at 150.
[13] Press Release, CDC, CDC Emergency Operations Center Moves to the Highest Level of Activation for Zika Response (Feb. 3, 2016), *available at* http://www.cdc.gov/media/releases/2016/s0208-zika-eoca-activation.html.

by cross-reactivity between antibodies produced in response to infection with Zika virus and those generated by infection with dengue, or other flaviviruses. That is, infection with dengue virus, or related flaviviruses, can induce an antibody response in a patient that cross reacts in Zika virus antibody detection assays. As discussed further below, the CDC IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA) is the main FDA-authorized IgM testing assay currently in use to diagnose recent Zika virus infection.

Given limitations on both RT-PCR and IgM testing, current best practice for diagnosing Zika virus infection typically requires using both of these approaches on appropriate clinical samples. The steps laboratories should follow to conduct the diagnostic testing (e.g., when they test, what tests they use, how to interpret the results, and when to re-test) are referred to as the diagnostic testing algorithm.

ii. The Singleplex Assay

The "Singleplex" assay is a laboratory developed test (i.e., in vitro diagnostic device that is intended for clinical use and designed, manufactured, and used within a single laboratory)[14] designed in Dr. Lanciotti's laboratory during a 2007 Zika virus outbreak in Micronesia and manufactured and used in his laboratory in DVBD thereafter. It is a real-time RT-PCR assay and is designed to detect Zika virus RNA only; it does not detect genetic material from any other viruses. The assay is not cleared, approved, or authorized by the Food and Drug Administration (FDA).[15]

While laboratory developed tests like the Singleplex are devices subject to FDA regulation, historically FDA has exercised enforcement discretion for laboratory developed tests by generally not enforcing premarket review and other applicable regulatory requirements.[16] However, this enforcement discretion is not guaranteed, and indeed, FDA does not believe its policy of enforcement discretion is appropriate for laboratory developed tests offered in public health emergencies, including the public health challenge involving the Zika virus. In the case of an emerging infectious disease with serious public health implications, FDA expects developers of laboratory developed tests to submit information about their tests for FDA authorization. Hospitals, laboratories, and providers that market medical products, which are subject to but do not have FDA authorization, have run afoul of FDA laws and rules. In the current Zika virus outbreak, for instance, FDA sent "It Has Come to Our Attention" letters alerting several entities that were offering Zika virus diagnostic tests that FDA believed their tests were subject to FDA authorization.[17]

As the Diagnostics and Reference Activity Chief, Dr. Lanciotti worked with partners in state and local health departments so that they could run a version of the Singleplex assay in their laboratories. Dr. Lanciotti provided external laboratories interested in the assay with a basic, two-page protocol that provided information on deploying a version of the Singleplex in their laboratories. Dr. Lanciotti's laboratory also provided positive control material to laboratories running the Singleplex and technical assistance to help them run the test. These public health laboratories demonstrated their ability to run the assay by successfully analyzing a proficiency panel using their local version of the Singleplex.

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[14] Although FDA defines a laboratory developed test as an in vitro diagnostic device that is intended for clinical use and designed, manufactured, and used within a single laboratory, the term "laboratory developed test" has been used more broadly by CDC, laboratories, and others.

[15] For the purposes of this document, products that are "authorized" by FDA refers to products that are cleared, approved, granted *de novo* classification, or received EUA authorization, by FDA.

[16] FDA, Laboratory Developed Tests, http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm407296.htm (last visited Aug. 15, 2016).

[17] *See, e.g.*, Letter from FDA to Texas Children's Hospital and Houston Methodist Hospital (Mar. 2, 2016), *available at* http://www.fda.gov/downloads/MedicalDevices/ResourcesforYou/Industry/UCM490410.pdf; Letter from FDA to MD Biosciences, Inc. (Mar. 4, 2016), *available at* http://www.fda.gov/downloads/MedicalDevices/ResourcesforYou/Industry/UCM490077.pdf; Letter from FDA to First Diagnostic Corporation (Mar. 10, 2016), *available at* http://www.fda.gov/downloads/MedicalDevices/ResourcesforYou/Industry/UCM490404.pdf.

Laboratories running the Singleplex were also expected to demonstrate compliance with the Clinical Laboratory Improvement Amendments (CLIA). CLIA is a regulatory program administered by the Centers for Medicare and Medicaid Services (CMS) that requires laboratories to demonstrate that the tests they run find what they are supposed to find (i.e., are "analytically valid"). CLIA does *not* look at "clinical" validity—that is, the accuracy with which the test identifies, measures, or predicts the presence or absence of a clinical condition or predisposition in a patient. In short, CLIA compliance assures that a laboratory is running a test reliably and according to local protocol, not whether it is a clinically accurate test. Rather, it is FDA that assesses individual test performance. CLIA-compliant validation of a laboratory developed test is on a lab-by-lab basis. So CLIA-compliant validation of the Singleplex in state health department Lab A would have no bearing on whether the assay was analytically valid in Lab B. CMS review of analytical validation data only occurs during a laboratory's CLIA inspection, conducted at least biennially, after the laboratory has started running the test.[18]

iii. Origins of the Trioplex Assay

The Trioplex assay is a real-time RT-PCR assay that is designed to detect Zika virus, all four dengue viruses, and chikungunya virus in a single clinical sample. This has an important implication for laboratory efficiency; by integrating all three tests into a single assay, laboratories would not have to run multiple tests and could work from one clinical sample. It also has important clinical implications. Since Zika, dengue, and chikungunya viruses circulate in overlapping regions and symptoms of one viral infection can be confused with symptoms of another viral infection, testing for all three viruses provides valuable information to clinicians to distinguish the viruses and guide patient care.

The Trioplex was initially conceived in the fall of 2015 at CDC. Dr. Muñoz, the lead of the Diagnostics and Research Laboratory Activity in the DVBD Dengue Branch in San Juan, Puerto Rico and Dr. Lanciotti were initially co-principal investigators on a project to develop a multiplex assay that tested for Zika, dengue, and chikungunya viruses.[19] The original goal of the project was to develop the assay and generate the extensive validation data to have the Trioplex assay submitted for FDA review through a premarket notification to FDA, called a 510(k) submission. A 510(k) submission must demonstrate to FDA that the device is substantially equivalent to (including as safe and effective as), a legally marketed predicate device which would allow for marketing of the device. By the winter of 2015, however, the worsening Zika virus outbreak increased the urgency to quickly develop an assay and to seek expedited regulatory review to allow distribution to state, local, and territorial public health laboratories. CDC turned to FDA's emergency use authorization process to obtain this regulatory review.

iv. Emergency Use Authorization of the MAC-ELISA and Trioplex Assays

The Federal Food, Drug, and Cosmetic Act empowers the FDA to authorize the emergency use of an unapproved medical product (or unapproved use of an approved medical product) during an emergency, called an Emergency Use Authorization (EUA).[20] This authority allows FDA to facilitate the timely, legal distribution of medical countermeasures (like a diagnostic assay) to respond to certain types of emergencies that might otherwise not be available under the traditional regulatory process.[21] An EUA does not signify that a medical product is safe and effective and does not indicate FDA approval of the product; rather, it indicates that the FDA has authorized the emergency use of the device during a declared emergency based on, among other things, a determination that it is reasonable to believe that the product "may be effective" in diagnosing, treating or preventing the disease/condition at issue.

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[18] CMS, LDT and CLIA FAQ, https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/Downloads/LDT-and-CLIA_FAQs.pdf (last visited Aug. 15, 2016).

[19] The initial conception of the project did not include Zika virus, but Zika was added to the proposal as the Zika virus outbreak in Brazil emerged.

[20] 21 U.S.C. § 360bbb–3.

[21] FDA. DRAFT GUIDANCE: EMERGENCY USE AUTHORIZATION OF MEDICAL PRODUCTS AND RELATED AUTHORITIES. (2016), *available at* http://www.fda.gov/downloads/EmergencyPreparedness/Counterterrorism/MedicalCountermeasures/MCMLegalRegulatoryandPolicyFramework/UCM493627.pdf.

FDA is authorized to issue an EUA if a number of statutory requirements are met. First, there must be a determination of an emergency or potential emergency by the Secretary of Homeland Security, Secretary of Defense, or HHS Secretary under 21 USC 360bbb-3(b). Second, there must be a declaration by HHS that circumstances exist to justify issuance of EUAs. Third, the declared emergency must pertain to a serious or life-threatening disease or condition. Fourth, FDA must determine that based on the totality of the available scientific evidence it is reasonable to believe that the product "may be effective" to diagnose, treat, or prevent the condition. Fifth, the known and potential benefits of the medical product must outweigh the known and potential risks, which includes consideration of the threat that the disease or condition presents. Finally, there must be no adequate, approved, and available alternatives to the product.[22]

EUAs can be updated and amended. Certain minor updates, such as an update to the Instructions for Use, do not generally require a formal reauthorization of the EUA and can be submitted through a relatively straightforward process. More substantial amendments may require reauthorization from FDA.

With the spread of the Zika virus in the Americas and increasing evidence of a relationship between Zika and birth defects, by late 2015 it was clear to scientists at CDC that an EUA for diagnostic assays would be necessary to support widespread Zika virus diagnostic testing. CDC eventually submitted two diagnostic assays for EUA: a real-time RT-PCR assay and an IgM assay. FDA has also authorized other non-CDC Zika virus diagnostic tests for emergency use.[23]

The MAC-ELISA assay was the first Zika assay CDC submitted for an EUA, which FDA issued on February 26, 2016.[24] As noted above, the MAC-ELISA is an IgM assay. It is a difficult test to run and its results can be ambiguous. Because of these difficulties, the MAC-ELISA was intended for use in conjunction with additional diagnostic testing. As the Instructions for Use that accompany the MAC-ELISA EUA explain:

> Positive and equivocal [MAC-ELISA] results are not definitive for diagnosis of Zika virus infection. . . . Confirmation of the presence of anti-Zika IgM antibodies in equivocal or presumptive positive specimens requires additional testing using the CDC-issued algorithm. Positive or equivocal results must be considered in conjunction with additional testing using the CDC-issued algorithm and/or considered alongside test results for other patient matched specimens using the CDC-issued algorithm.[25]

In short, while the MAC-ELISA is an important diagnostic tool for detecting Zika virus infections, it is intended to be used as part of a clinical diagnostic algorithm that includes additional diagnostic tests, specifically real-time RT-PCR and plaque reduction neutralization test (PRNT).

There were discussions within CDC about which Zika virus RT-PCR assay should be considered for EUA submission. Based on witness interviews, the Trioplex was ultimately selected because (1) there was added clinical utility of testing for three different viruses, (2) testing for all three viruses in a single test would be more efficient and reduce the testing burden on laboratories, (3) the preliminary work that had already been done to

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[22] 21 U.S.C. § 360bbb–3(b), (c).

[23] As of August 30, 2016 FDA had authorized the Roche Molecular Systems, Inc.'s *LightMix®* Zika rRT-PCR Test (authorized August 26, 2016); InBios International, Inc.'s ZIKV Detect™ IgM Capture ELISA (authorized August 17, 2016); Luminx Corporation's xMAP® MultiFlex™ Zika RNA Assay (authorized August 4, 2016); Siemens Healthcare Diagnostics Inc.'s VERSANT® Zika RNA 1.0 Assay (kPCR) Kit (authorized July 29, 2016); Viracor-IBT Laboratories, Inc.'s Zika Virus Real-time RT-PCR test (authorized on July 19, 2016); Hologic, Inc.'s Aptima® Zika Virus assay (authorized June 17, 2016); altona Diagnostics RealStar® Zika Virus RT-PCR Kit U.S. (authorized May 13, 2016); and Focus Diagnostics, Inc.'s, Zika Virus RNA Qualitative Real-Time RT-PCR test (authorized April 28, 2016).

[24] On the same date, the HHS Secretary determined that there was a "significant potential for a public health emergency" involving Zika virus. On June 29, 2016, FDA reissued the Zika MAC-ELISA EUA to incorporate amendments requested by CDC.

[25] CDC, Zika MAC-ELISA: Instructions for Use, *available at* http://www.fda.gov/downloads/MedicalDevices/Safety/EmergencySituations/UCM488044.pdf.

prepare the assay for 510(k) submission had laid much of the groundwork for submitting an EUA, and (4) Dr. Muñoz was willing to guide the test through the submission process.

CDC submitted the request for FDA's emergency authorization of the Trioplex real-time RT-PCR assay on March 16, 2016. As is recommended practice by FDA, CDC had been working with FDA in the weeks leading up to the EUA request, sharing pieces of the submission package as they became available to facilitate FDA review prior to approval of the final package. FDA authorized the Trioplex for emergency use on March 17, 2016.

As authorized by FDA, Trioplex tests for Zika virus, dengue viruses, and chikungunya virus in human serum (a blood component) and cerebrospinal fluid (fluid in the brain and spine).[26] It can also be used to detect Zika virus only (not dengue or chikungunya viruses) in urine and amniotic fluid. The authorization for testing urine is particularly important since data from the Florida Department of Health published on May 13, 2016 indicates that Zika virus RNA may be detectable in urine for up to 14 days after onset of symptoms. The assay is authorized for use in patients who meet CDC clinical and/or epidemiological (e.g., travel to affected countries or territories) criteria for Zika virus infection during the acute phase of infection.[27]

The EUA is accompanied by detailed and prescriptive Instructions for Use. The 41-page document provides specific instructions on each step of the process: nucleic acid extraction, preparation of primers and probes, equipment preparation, master mix and plate set-up, reaction mixture volumes, running the PCR, and interpretation of results. It includes a full-page flow chart to visualize the steps laboratories should follow when testing a clinical specimen and explains how to understand test validity and interpret results.

The instructions set strict controls on how the test is performed, which limits opportunities for possible variation in how different laboratories can run the assay. The instructions give CDC tight control over the assay and its performance. The assay is authorized for use only by "qualified laboratories designated by [CDC]." Laboratories can use only primers and probes provided by CDC, ensuring CDC oversight of the manufacture and quality control of the materials. Similarly, laboratories performing the Trioplex must use CDC-provided positive controls for the test.[28] The instructions dictate the commercially-available components required to run the assay (e.g., RNA extraction kits and the real-time RT-PCR master mix kits), and the specific type of real-time RT-PCR instrument that laboratories can use to run the test. The instructions prohibit modification of the assay and bar distribution of the assay without explicit consent from CDC.

The instructions also lay out the limitations of the Trioplex. They explain that negative results do not rule out dengue, chikungunya, or Zika virus infections and that the test "should not be used as the sole basis for patient management decisions." The results, the instructions explain, "should be interpreted by a trained professional in conjunction with review of the patient's history and clinical signs and symptoms."

In addition to the Instructions for Use, FDA required that CDC develop and distribute factsheets for providers and patients (including one specifically for pregnant women) to explain the details of the assay and its implications. The factsheet for providers describes how and when the test should be used. It explains that a negative result indicates that the RNA from the tested viruses "is not present in the specimen at the detection level of the assay" and reiterates a negative result does not rule out potential infection and should not be the sole basis of treatment

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[26] CDC, Trioplex Real-time RT-PCR Assay: Instructions for Use, *available at* http://www.fda.gov/downloads/MedicalDevices/Safety/EmergencySituations/UCM491592.pdf.

[27] The EUA Instructions for Use describe the acute phase of infection as "approximately 7 days following onset of symptoms, if present." However, CDC guidance on the window for Zika viral RNA detection continues to evolve as more information about Zika viral infections come to light. For instance, the recent guidance from CDC on caring for pregnant women with potential Zika virus exposure indicates that RNA can persist in serum for longer than originally thought—potentially up to 10 weeks—and recommends expanded real-time RT-PCR testing of pregnant women, which is reflected in revisions to CDC's diagnostic testing algorithm.

[28] Positive controls are samples with inactivated virus that should show up as positive for Zika virus, dengue virus, and chikungunya virus if the assay is run correctly. Use of positive controls is a vital step to ensure that the assay is working as it should.

decision making.[29] The patient factsheets similarly state that "it is possible for [the Trioplex] to give a negative result that is incorrect (false negative) in some people."[30]

There are significant differences in the level of detail and supporting information provided to laboratories for implementing the two tests. Distribution of the Trioplex includes a detailed 41-page Instructions for Use guidance document that has been reviewed and authorized by FDA. In contrast, the two-page protocol that Dr. Lanciotti distributed to public health laboratories for the Singleplex (which was not reviewed or authorized by FDA) provided only basic information about the design of the assay. Dr. Lanciotti's protocol does not include information on the Singleplex assay's intended use, appropriate specimen types (e.g., whether urine is an acceptable specimen), quality control guidance, detailed instructions on performing the assay, interpretation of results, or performance characteristics. The Trioplex EUA Instructions for Use includes information on all these aspects. While the Trioplex requires states to use primers and probes distributed by CDC, the Singleplex protocol directs laboratories to independently obtain two Zika virus primer and probe sets and to conduct their own quality control of these reagents. Unlike the Trioplex, the Singleplex does not prescribe what instrument laboratories should use to perform the testing. While this does allow for flexibility in how laboratories implement the assay, it limits the ability to evaluate performance of the assay or troubleshoot problems across multiple laboratories. The lack of detail in the protocol provided by Dr. Lanciotti allows for wide variability in how external laboratories can configure and perform the Singleplex. Finally, the Singleplex does not include any information for providers or patients on how to interpret its results, while the Trioplex includes factsheets for healthcare providers, patients, and pregnant women.

v. CDC Dissemination of the Trioplex

Following FDA authorization of the Trioplex, CDC recommended to partners in state and local health departments that they use the Trioplex and clearly supported its use and dissemination. However, at no time did CDC instruct states to stop running the Singleplex or any other Zika virus real-time RT-PCR assays.

Upon FDA authorization of the Trioplex, CDC provided information and support to public health laboratories so they could use the new assay. On March 18, 2016, the CDC distributed a communication to the directors of public health laboratories across the U.S. through the Laboratory Response Network (LRN), a national network of more than 150 federal, state, and local public health laboratories that is administered by CDC. The March 18 communication provided guidance and information on the Trioplex test and the terms of the FDA EUA. In the communication, CDC stated that it would begin shipping the assay (which included the primers, probes, positive controls, and verification test panel) starting on March 21. The communication did not mention the Singleplex or instruct states to stop using other RT-PCR assays. Dr. Lanciotti states that approximately 20 to 25 states were running the Singleplex, or some version of it, when FDA authorized emergency use of the Trioplex in mid-March. CDC held a technical conference call for LRN members on the use of the new assay on March 23. By March 29, 2016, CDC had deployed the assay to 79 LRN laboratories. None of these LRN communications in the rollout of the Trioplex mentioned other Zika virus RT-PCR assays, including the Singleplex.

E. Sensitivity of the Trioplex Relative to the Singleplex

i. Sensitivity vs. Limit of Detection

The issue at the core of the whistleblower disclosure is the sensitivity of the Trioplex relative to the Singleplex assay, but the term *sensitivity* requires some unpacking in this context. In the rigorous use of the term to describe diagnostic assay performance, the "sensitivity" of an assay would be the number of positive results the test

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[29] CDC, Fact Sheet for Health Care Providers: Interpreting Trioplex Real-Time RT-PCR Assay Results, *available at* http://www.fda.gov/downloads/MedicalDevices/Safety/EmergencySituations/UCM491588.pdf.

[30] CDC, Fact Sheet for Pregnant Women: Understanding Results from the Trioplex Real-Time RT-PCR Assay, *available at* http://www.fda.gov/downloads/MedicalDevices/Safety/EmergencySituations/UCM491591.pdf; CDC, Fact Sheet for Patients: Understanding Results from the Trioplex Real-Time RT-PCR Assay (Trioplex rRT-PCR), *available at* http://www.fda.gov/downloads/MedicalDevices/Safety/EmergencySituations/UCM491590.pdf.

produces relative to the number of true positives. So for instance, if there are 100 clinical samples and 20 of them are from patients infected with virus X (i.e., there are 20 true positives), an assay for virus X is 100 percent sensitive when all 20 of the samples from infected patients test positive. However, if the assay correctly identified only 10 of the 20 true positives, it would be only 50 percent sensitive. In short, "sensitivity" measures the performance of an assay with unknown performance characteristics against a gold standard—an assumption that a true positive is always detectable.

In the absence of a gold standard, a better description of an assay's performance is its *limit of detection*, also called analytic sensitivity. In the context of an assay for Zika virus, a limit of detection describes the smallest amount of viral RNA that can be distinguished from the absence of the RNA. Limit of detection does not compare an assay's performance to the number of true positives (a gold standard); it looks at how much RNA needs to be in a sample for the assay to accurately detect it. A specimen from an infected patient with an amount of virus lower than what the assay could detect would be *outside* of the assay's limit of detection; a sample with an amount of virus that the assay could detect would *within* the limit of detection.

For the purpose of this analysis, the term *limit of detection* is preferred to describe and compare the performance of the two assays because the available comparisons of the Trioplex and the Singleplex assays do not measure their performance against a defined set of true positive clinical samples, but rather assess the limits of their ability to detect low concentrations of viral RNA.

ii. Discussion within CDC Regarding Trioplex Limit of Detection

In April and May 2016, there was extensive communication, debate, analysis, and discussion within CDC about the limit of detection for the Trioplex assay relative to the Singleplex, what could be done to improve the limit of detection for the Trioplex assay, and what should be communicated to states about the issue. Dr. Lanciotti stated that over the course of these discussions he felt that his colleagues listened to his concerns and his suggestions about how to improve the limit of detection for the Trioplex. Dr. Lanciotti's colleagues in the EOC did not, however, ultimately agree with Dr. Lanciotti's proposed solution to the problem: that CDC should tell laboratories who were running a CLIA-validated version of the Singleplex that the Trioplex had an inferior limit of detection relative to the Singleplex and that they should not to switch to the Trioplex.

Dr. Lanciotti first started to examine the relative limits of detection between the two tests on March 25, 2016. Over the next approximately two weeks, he conducted six experiments comparing the performance of the Trioplex, the Singleplex assay, and another assay kit that included tests for Zika, dengue, and chikungunya viruses. On April 4, 2016, he emailed Dr. Muñoz a brief summary of his non-final comparisons. He reported that the Trioplex "worked reasonably well" for Zika and chikungunya viruses and poorly for dengue and found that the Trioplex performed the worst of the three assays in terms of relative limits of detection. He also emailed the raw data to Dr. Powers the next day.

On April 12, 2016, Dr. Lanciotti communicated specific concerns with the Trioplex's limit of detection based on his finalized comparisons. On that date, he distributed the results of an experiment he ran examining Trioplex and Singleplex limits of detection to his acting division director, Dr. Rosenberg; the acting Virology activity chief and co-lead of the EOC Laboratory Team, Dr. Powers; and Dr. Lambert, his subordinate in the Diagnostics and Reference activity. Dr. Lanciotti stated that this one experiment showed that the Trioplex assay failed to detect 30-39 percent of samples that were positive by the Singleplex assay, depending on how the data were interpreted. Dr. Lanciotti noted that while his findings showed "a very minor difference in analytical sensitivity" between the two tests, the typically low level of Zika virus in a patient's serum "greatly amplifie[d]" the impact of this small difference, causing the Trioplex to miss 13 of the 33 total Zika-positive samples in his analysis. Drs. Rosenberg and Powers agreed that the findings highlighted a potentially serious issue with the Trioplex assay and that further examination was needed.

On April 13, 2016, an official from the Blood Systems Research Institute (BSRI) emailed Dr. Petersen and other staff at CDC and FDA, stating that BSRI had generated Trioplex clinical sensitivity data that were "disturbing." His initial findings echoed Dr. Lanciotti's analysis, indicating that the Trioplex assay missed approximately one

third of Zika virus infections in persons presenting for testing one to three days after the onset of symptoms. He noted that additional comparisons needed to be done to more fully understand the issue.

The discussion of the limit of detection issue arose again on April 18, 2016, when Dr. Lambert emailed Drs. Lanciotti, Rosenberg, Powers, and Petersen asking for a recommended course of action, given Dr. Lanciotti's findings. She explained that state laboratories she worked with were running the Trioplex preferentially over the Singleplex and that she was at a loss about what to say to them about the potential limit of detection issue with the Trioplex. Dr. Lanciotti emailed the group (adding Dr. Villanueva, the co-lead of the EOC laboratory team with Dr. Powers), reiterating that his comparative testing showed the Trioplex to be "slightly less sensitive (2-10 fold) than Singleplex." He also shared data from a separate analysis from BSRI that Dr. Lanciotti described as showing the same thing as his own findings—a small but impactful difference in the Trioplex's limit of detection for Zika virus that resulted in the Trioplex missing approximately one-third of positive samples.

In the same April 18 email thread, Dr. Rosenberg recommended that the "next best step" was for Drs. Lambert, Lanciotti, and Muñoz speak with Dr. Petersen about the issue. In a subsequent email in the same thread, Dr. Rosenberg also wrote: "The simplest resolution might be to convey this information to the states and let them decide. But whatever they decide as their routine, it might be unwise to abandon the Singleplex."[31] Dr. Lanciotti responded to the group, "I agree; the States should decide" and noted that many of the states were "under the impression that they *have* to switch to the Trioplex, and that they are no longer *allowed* to use the Singleplex—as mandated by LRN, FDA, or some other regulatory group?" While states may have had this belief, none of the communications from CDC to the public health laboratories at that date indicated that states were required to abandon the Singleplex or use the Trioplex.

On April 19, 2016, Drs. Petersen, Lanciotti, Villanueva, Powers, Muñoz, and several other CDC staff held a meeting on the limit of detection issue. According to notes from this meeting, they discussed three different data analyses that yielded inconsistent findings. The first, from Dr. Lanciotti, showed that the Zika Singleplex detected approximately one-third more positive Zika cases than Trioplex. The second analysis, from the BSRI data, showed similar results as Dr. Lanciotti's analysis. The third analysis, from Dr. Muñoz's laboratory in San Juan, found that the Trioplex and Singleplex had essentially the same limit of detection. Ultimately the discussion at the meeting about the difference between the limits of detection of the two assays was not conclusive, but the participants agreed to additional experiments to investigate the limits of detection between the two assays and that steps should be taken to improve Trioplex's diagnostic sensitivity by changing how the test is performed (e.g., increasing RNA input and using urine as a specimen in the assay).

The next day, on April 20, Dr. Lanciotti emailed Dr. Petersen (cc'ing 11 different staff in DVBD and the EOC laboratory team), stating that he planned to contact states that had validated the Singleplex to encourage them to keep using the Singleplex and to not use the Trioplex until CDC revised it. He said he planned to contact the states that day. In a separate email thread, Drs. Petersen and Villanueva expressed that they did not agree with Dr. Lanciotti's plan to reach out to states, given the ongoing uncertainty about the limit of detection issue. Dr. Lanciotti was not included on these emails and it does not appear he received a response to his email.

On April 21, Dr. Lanciotti sent an email to approximately 30 external laboratory contacts, primarily in state public health laboratories. The email had no subject heading and included no members of DVBD leadership or the EOC; Dr. Lambert, who worked in his laboratory, was the only CDC employee included on the email. The email read:

> We would like to provide an update on RT-PCR testing for Zika virus. It is our understanding that your laboratory has passed the Zika Singleplex proficiency evaluation through the application of this Singleplex assay to a Zika validation panel that was generated and distributed by our Division in advance of the Trioplex FDA/EUA. Subsequently, many laboratories have validated the Trioplex EUA test (distributed by CDC LRN) in their laboratories. We want to inform you

[31] Dr. Rosenberg insists that his April 18 email was not permission for Dr. Lanciotti to reach out to states but rather an option for discussion. Further, he noted that such a decision would have to come from the EOC, not DVBD. Dr. Lanciotti interpreted Dr. Rosenberg's statement as tacit endorsement of reaching out to states, if not explicit permission.

> that in the Fort Collins laboratory we are continuing to use the Zika Singleplex due to its greater relative sensitivity (that we have just established/become aware of through comparative analyses in several laboratories).
>
> Accordingly, if you require additional proficiency samples to satisfy internal regulatory or validation requirements for the Zika Singleplex assay, we can work with you to supply these as soon as possible.

This email caused confusion among state laboratories because it appeared that CDC was promoting a non-FDA authorized assay over the Trioplex. Some members of the EOC found out about the email not from Dr. Lanciotti but from contacts in state laboratories or the Association of Public Health Laboratories, a national organization of public health laboratories. Dr. Powers, as co-lead of the EOC laboratory team, wrote Drs. Lanciotti and Lambert on April 25 stating that Dr. Lanciotti's email "created more trouble and confusion than it clarified." She highlighted the need for CDC to provide consistent information during an emergency and that broad communications to states needed to be reviewed by the EOC before being distributed. Drs. Lanciotti and Lambert both responded, stating they believed they were operating with permission from the EOC.

To address the confusion among public health laboratories, CDC sent a message to all state and LRN laboratories on April 26, stating that there were only two assays authorized by FDA for distribution (MAC-ELISA and the Trioplex) and that these two tests "are distributed by CDC to select qualified laboratories and are recommended for use in the current Zika response." The communication acknowledged that some laboratories were using laboratory developed tests including "some employing sequences published by CDC scientists," an apparent reference to the Singleplex. It noted that these tests were not covered by an EUA and needed to meet CLIA requirements. It did not, however, instruct states to abandon these other laboratory developed tests:

> We acknowledge that some laboratories may have created in-house laboratory developed tests (LDTs) to detect arbovirus infections, some employing sequences published by CDC scientists. Laboratories should understand that these LDTs are not covered by the FDA EUAs for the CDC Zika MAC-ELISA and Trioplex Real-Time RT-PCR assays. Laboratories utilizing assays other than the EUA CDC Zika MAC-ELISA and CDC Trioplex Real-time RT-PCR assays will need to perform in-house validations to adequately characterize the performance of their assay and ensure that CLIA requirements are met. Per CMS guidance, when a laboratory develops a test system such as an LDT in-house without receiving FDA clearance or approval, CLIA prohibits the release of any test results prior to the laboratory establishing certain performance characteristics relating to analytical validity for the use of that test system in the laboratory's own environment, see 42 CFR 493.1253(b) (2) (establishment of performance specifications).

The message did not mention the Trioplex's limit of detection relative to the Singleplex, as mentioned in Dr. Lanciotti's April 21 email. The email read:

> We recognize the low level of viremia observed in some Zika cases can pose a challenge for molecular testing. CDC continues to evaluate the performance of both the CDC Zika MAC-ELISA and CDC Trioplex Real-time RT-PCR assays as additional data become available. As we learn more, any updated recommendations regarding the use and interpretation of results of these assays are the responsibility of CDC's Emergency Operations Center (EOC) and will be communicated via updates to CDC's website (http://www.cdc.gov/zika/state-labs/index.html) and through the LRN.

Dr. Powers shared the email with Dr. Lanciotti and others, asking that staff "adhere to this information when providing guidance to state partners" to avoid future confusion. Dr. Rosenberg, who had seen Dr. Lanciotti's comparison data but had not reviewed the data from BSRI or Dr. Muñoz, asked Dr. Powers: "Shouldn't CDC officially communicate the [Trioplex sensitivity] limitation to users?" In an April 28 email to Drs. Rosenberg and Lambert, Dr. Lanciotti expressed frustration with the response and its "vague and confusing" treatment of potential sensitivity in Zika diagnostic testing.

Discussions between Dr. Lanciotti, Dr. Muñoz, and the others about Trioplex's sensitivity continued through the end of April and into May. On April 27, Dr. Muñoz shared an analysis with the EOC laboratory team comparing Trioplex and Singleplex using Zika virus controls in the Trioplex kit, and found no demonstrable difference between the two assays. On April 28, Dr. Muñoz shared additional data with the EOC laboratory team, again finding no demonstrable difference between the two assays. On a May 2 conference call, Drs. Lanciotti, Powers, Muñoz, Villanueva, and others from the EOC discussed Dr. Muñoz's data and potential enhancements to the Trioplex. The issue was discussed during a May 4 Zika virus daily update call with the CDC Director, and Dr. Muñoz discussed the ongoing limit of detection issues and steps to enhance the Trioplex.

Dr. Lanciotti remained deeply concerned with what he viewed as CDC's promotion of an inferior assay. He viewed BSRI's data as supportive of these concerns and disagreed with Dr. Muñoz's analyses. On May 4, he emailed Drs. Petersen and Powers expressing "deep concern about how this Trioplex/single assay situation has been handled."[32] On May 12, Dr. Lanciotti emailed Drs. Petersen and Powers again, stating that the EOC's recommendation of the Trioplex in spite of the comparison data from his laboratory and BSRI failed to support state partners. He accused the EOC of deliberately withholding information on the Trioplex's sensitivity issues and stated that the EOC appeared interested in "promoting the Trioplex in spite of the observed facts" and was making decisions that were not based on data.

As of late July 2016, 87 laboratories had received reagents for the Trioplex assay (including all states and Washington, DC, and Puerto Rico) and 70 laboratories had completed the Trioplex verification panel (which includes 46 states, Washington, D.C., and Puerto Rico). Seven laboratories have chosen not to conduct the Trioplex. During this time period, the EOC was aware of seven state or local laboratories that continued to perform a version of the Singleplex (sometimes in addition to the Trioplex), though this list may not be exhaustive.[33]

During the course of the investigation, Dr. Lanciotti shared anecdotal reports he received in July and August 2016 of some discordant testing results between the Trioplex and another FDA-authorized assay, Focus Diagnostics, Inc.'s (formerly part of Quest Diagnostics, Inc.) Zika Virus RNA Qualitative Real-Time RT-PCR assay (Focus assay). The Focus assay, like the Trioplex and unlike the Singleplex, is authorized for emergency use by FDA. While the primer and probe sequences for the Focus assay are based on those used in Dr. Lanciotti's Singleplex, its EUA Instructions for Use differs substantially in content and specificity from Dr. Lanciotti's Singleplex protocol (e.g., the Focus assay uses higher input volumes, a different extraction platform and two separate PCR reaction mixes). The Focus assay cannot be described as the same assay as the Singleplex. Further, a careful examination of the EUA Instructions for Use for the Focus assay indicates that during Focus's evaluation of the assay, they identified three discrepancies where the Trioplex assay detected Zika virus RNA in a sample that the Focus assay reported as negative (15 percent false negatives in 20 samples tested). The discordant results between the two assays warrant further examination by CDC and have some potentially important implications, especially whether use of a higher input volume for the Trioplex (as discussed further below) would improve the Trioplex's diagnostic sensitivity. However, the discordant results between the Trioplex and the Focus assay do not illuminate the core issue of the whistleblower complaint—the relative limits of detection between Dr. Lanciotti's Singleplex and the Trioplex—because the Focus assay is a distinct, separate assay than Dr. Lanciotti's Singleplex.

iii. Efforts to Improve the Trioplex

As the Zika virus outbreak progressed, more data accumulated to indicate that most patients had a much lower viremia (level of virus in the blood) than is typical of infection with dengue or chikungunya viruses. Thus, despite disagreements about the Trioplex's limit of detection relative to the Singleplex assay, virtually all witnesses agreed that improving the sensitivity of the Trioplex should be a priority. Improvements to the

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[32] Dr. Lanciotti also mentioned that Dr. Muñoz had stated that the data from the BSRI comparison had not been run in the San Juan laboratory, which Dr. Lanciotti believed to be incorrect. In an interview with the investigative team, Dr. Muñoz confirmed that the panel used in the BSRI study was run in San Juan, Puerto Rico.

[33] The seven include Tampa, Florida; Jacksonville, Florida; Maryland; Massachusetts; New Jersey; New York State; and Ohio.

Trioplex's limit of detection were discussed throughout the period of April and May 2016 and were major topics of deliberation on the various conference calls, meetings, and discussions on the Trioplex limit of detection issues. Dr. Lanciotti was involved in many of these discussions and reported that he felt that the EOC had listened to his input on how to improve the Trioplex.

On August 22, 2016, CDC submitted a substantial amendment to the Trioplex EUA, which includes several changes with important implications for the assay's diagnostic sensitivity (i.e., the ability of the assay to detect Zika virus). One such change is the authorization of the use of larger sample volumes in the assay. This means that laboratories could test a larger amount of patient serum or urine than is currently authorized under the EUA. This could potentially improve the assay's diagnostic sensitivity because a larger sample could contain more virus for the assay to detect. CDC laboratories in San Juan and Atlanta have been running experiments to examine the impact of larger sample volumes and understand the impact these higher volume inputs have on the assay's sensitivity relative to the standard input volume.

Another key change to be included in the EUA amendment is the addition of whole blood (i.e., blood that has not had the serum separated from the white and red blood cells) as a specimen type. The EUA currently does not authorize whole blood as a specimen type. However, recent studies indicate that Zika virus RNA may be detected at much higher levels and persist for as long as two months after onset of symptoms in whole blood. Adding whole blood as a specimen type has promise for improving the assay's diagnostic utility because of the higher levels of virus present in whole blood compared to serum. As part of its amendment submission, CDC is conducting its own experiments on the use of whole blood in the Trioplex, including determining the Trioplex's limit of detection using whole blood relative to other specimen types. CDC is working on additional potential changes to improve the performance of the Trioplex that may be included in future amendments, depending on available data. For instance, CDC is assessing the use of a more concentrated master mix (a ready-to-use solution with chemicals needed for PCR). This would allow laboratories to use a smaller volume of master mix, freeing up room for a larger sample volume and thereby potentially increasing the assay's limit of detection.

These planned and potential amendments would be in addition to CDC's ongoing efforts to improve diagnostic testing in the field through regular updates to CDC's testing algorithm. The Trioplex EUA Instructions for Use refer users to the CDC website for an up-to-date testing algorithm. CDC regularly updates this algorithm to ensure that laboratories are employing the best available methods when running and interpreting clinical testing results. For instance, CDC updated its testing algorithm to allow for a longer window of testing for urine when new studies showed that Zika virus could be detected in urine longer than originally believed.

F. Investigative Team's Analysis of Available Data

The investigative team collected and analyzed all available data to understand the main scientific issue in the whistleblower disclosure: the limit of detection of the Trioplex assay relative to the Singleplex assay. The available data are limited, inconsistent, and inconclusive in supporting a definitive conclusion that the Trioplex has an inferior limit of detection than the Singleplex.

i. Definitions

In its analysis of the data, the investigative team considered three types of sensitivity, defined as follows:

1) **Analytic sensitivity (limit of detection)** is a measure of how much viral RNA needs to be in a sample for the assay to detect the presence of the virus. Comparisons of the assays' analytic limit of detection would measure each assay's ability to detect viral RNA in samples with successively lower virus concentrations.
2) **Diagnostic sensitivity** is a measure of a specific assay to detect a true positive sample collected in a clinical setting. That is, diagnostic sensitivity describes how often the assay alone correctly identifies a positive Zika infection in a patient.
3) **Clinical sensitivity** is a measure of a diagnostic algorithm to detect a positive case. In the case of Zika virus, the full clinical algorithm involves both real time RT-PCR and IgM tests to make a clinical

diagnosis. That is, a clinical sensitivity describes how often the entire clinical algorithm (RT-PCR + IgM testing) correctly identifies a positive Zika infection in a patient.

The differences between these characteristics are important to understand an assay's effectiveness. For example, if Assay X is capable of detecting very low viral concentrations while Assay Z is only able to detect high viral concentrations, they have very different analytical sensitivity (limits of detection). But if they are assays for a virus where infections almost always feature high levels of viral load in infected patients (e.g., Ebola), then the different analytical limits of detection may be insignificant for diagnostic sensitivity—i.e., both Assay X and Assay Z would correctly identify most infections. Thus while the two assays have very different analytical sensitivity (limits of detection), they have nearly identical diagnostic sensitivity (ability to correctly identify a true positive specimen). Conversely, if the assays were for a virus with a typically low level of viral load in infected patients (e.g., Zika), then they would have different diagnostic sensitivities because Assay Z would miss many infections. But if Assay Z was used as part of a clinical algorithm that included use of an additional test that would correctly identify most of the cases that the assay itself would miss, then it may still have a similar clinical sensitivity as Assay X even though it has different analytic sensitivity (limit of detection) and diagnostic sensitivity.

ii. Data Examined

The investigative team acquired and analyzed data on assay performance from Dr. Lanciotti's laboratory in Fort Collins (Fort Collins data) and Dr. Muñoz's laboratory in San Juan (San Juan data). BSRI provided data on the assays' analytic sensitivity (limits of detection) compiled from both Fort Collins and San Juan (BSRI data).

The Fort Collins data included results on 79 clinical samples. The data did not include a date of specimen collection or symptom onset. In his comparative study, Dr. Lanciotti used the Singleplex per the standard Fort Collins protocol. However, it appears that the Fort Collins laboratory departed from the specified protocol, using a different cycle threshold (Ct) cutoff value (37.5) than is specified in the protocol (38.5).[34] Dr. Lanciotti did not precisely follow the EUA protocol for the Trioplex assay, using different RNA extraction and amplification instrumentation than is specified in the EUA (the Fort Collins laboratory does not have the EUA-specified instruments).

The San Juan data included results on 129 clinical specimens collected between 2007 and 2016 and included dates of specimen collection. The specimen data did not include date of symptoms onset. Dr. Muñoz used the Singleplex protocol with the same RNA extraction and amplification instrumentation as used in the Trioplex (not the same as Dr. Lanciotti used in Fort Collins). Dr. Muñoz followed the Trioplex EUA protocol correctly.

The BSRI data were summary data from multiple sites (including Fort Collins and San Juan) using standard analytic samples. The data included each specimen three separate times over a series of different dilutions. The BSRI data included results using standard input volumes and high input volumes for both assays, although the Trioplex EUA currently only authorizes standard input volumes (pending review of the August 22 submission that addresses increased sample input volume).. Comparing results across sites was difficult because of differences in how the tests were performed; the sites used different standard input volumes (i.e., they put different amounts of serum in the assays), different RNA extraction and amplification instrumentation, and different Ct cutoff values.

iii. Analysis of Data

Inconsistencies in assay processes and data reporting preclude making a statistically valid conclusion that the Trioplex has a significantly lower analytical limit of detection compared to the Singleplex. Of the three available datasets, the San Juan data—which showed no significant difference in the limit of detection between the

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[34] Ct (cycle threshold) refers to the number of cycles of rapid heating and cooling that a sample needs to run through during the DNA amplification process for the fluorescent signal in the probes to accumulate to a level that indicates the presence of the target DNA sequence. The lower the Ct level, the greater the amount of the target genetic material. The Ct cutoff value is the number of cycles after which identification of the target DNA sequence cannot be reliably obtained because of the degradation of fluorescent probes.

Singleplex and the Trioplex—is the clearest, most complete, and most reproducible. While issues of data quality and completeness prohibit a robust statistical analysis between the three datasets, weaknesses in the Fort Collins and BSRI data undermine their findings of a statistically significant difference between the Singleplex and Trioplex's relative limits of detection.

The Fort Collins data (which served as the basis for Dr. Lanciotti's findings that the Trioplex had an inferior limit of detection than the Singleplex) are insufficient to serve as a basis for a valid comparison between the two assays. The protocol that Dr. Lanciotti provided and the conditions of the Singleplex assay were inconsistent. The Fort Collins data for the Trioplex did not follow the EUA Trioplex protocol, making the results of the Trioplex not directly comparable to a test using the FDA-authorized protocol. In addition the Fort Collins data included an instance in which the Trioplex found one specimen to be positive that was not detected by the Singleplex.

The data compiled and provided by BSRI, which echoed the Fort Collins data in showing that the Trioplex had a less sensitive analytic limit of detection, also have inconsistencies that prevent a robust and definitive interpretation. The BSRI data compare the Fort Collins laboratory's testing of the Singleplex with the San Juan testing of the Trioplex on analytic limits of detection (i.e., how much virus needs to be in the sample for the assay to detect it) and finds that the Fort Collins Singleplex was able to detect a 10-fold smaller amount of viral RNA than the San Juan Trioplex. However, this finding is based on a comparison of the Fort Collins *high input volume* Singleplex to the *standard input volume* San Juan Trioplex. This is not an appropriate comparison. Available data indicate that standard input volume Singleplex is able to detect a three-fold smaller amount of viral RNA compared to the standard input volume Trioplex, not 10-fold. A comparison of high input volume Fort Collins Singleplex and high input volume Trioplex yields an opposite conclusion than the standard input comparison; the high input Trioplex is able to detect an approximately three-fold smaller amount of viral RNA compared to the high input Singleplex. The absence of data on the assays' reproducibility at the lower limits of detection preclude a rigorous analysis of whether these detection levels are statistically significantly different.

The San Juan data provide the highest quality comparison of the available datasets and shows no difference in the diagnostic sensitivity or analytical limits of detection between the two assays. The comparison in San Juan ran the Trioplex according to the EUA protocol and ran the Singleplex using the same RNA and extraction protocols as used in the Trioplex EUA. This means that the Singleplex as run in San Juan is not comparable to the Singleplex as run in Fort Collins because they used different instruments. However, unlike the Trioplex EUA protocol, Dr. Lanciotti's Singleplex protocol does not prescribe what extraction methods laboratories use to conduct the Singleplex, so the San Juan laboratory did run the Singleplex correctly under its protocol. The San Juan data also had the clearest and most complete presentation of data and provided more information on how the data were tested.

While none of the available comparisons had sufficiently complete data to demonstrate the day-to-day reproducibility of either assay, the San Juan data came closest in this regard. Reproducibility is expressed as a measure of an assay's "variance" under normal use; that is, the ability to reproduce the analytical method in different laboratories or under different circumstances without unexpected differences in results. Variance data should include three types of information: (1) how the assay performs when running a sample multiple times in the same laboratory and under the same conditions (called "precision"); (2) how it performs when running the assay in different laboratories with different scientists under similar but not identical conditions (called "intermediate precision"); and (3) how it performs when run in "real world" conditions, that is, in widely different laboratories and circumstances (called "reproducibility"). None of the comparisons included information on intermediate precision or reproducibility. All of the comparisons included some information on precision, and the San Juan data had the strongest data as it had the highest number of replicates (i.e., it ran each sample more times than the other two comparisons) and had the clearest information on how its tests were conducted. The lack of complete reproducibility data appreciably undermines any conclusion that the Singleplex is statistically significantly more sensitive than the Trioplex.

G. Conclusions on the Allegations Based on Available Evidence

The investigative team concludes that the allegations in the whistleblower disclosure are not substantiated based on the available evidence.

i. Allegation 1: Use of the Trioplex in place of the Singleplex in a clinical setting will result in an additional 39 percent of Zika infections in their acute phase going undetected.

This allegation is not substantiated by the available evidence.

The data from the available comparisons are contradictory, inconclusive, and from small sample size evaluations; they do not verify that the Trioplex will fail to detect a third of all Zika virus infections. The Fort Collins and BSRI data rely on limited and inadequate comparisons between the Trioplex and Singleplex tests. In the Fort Collins data, the Trioplex is not run according to its required, FDA-authorized protocol. The BSRI data relies on a comparison of high input Singleplex and standard input Trioplex, an inappropriate comparison. The Fort Collins data also shows that the Trioplex detected a Zika virus infection that the Singleplex failed to detect. Further, the Fort Collins and BSRI data are contradicted by the San Juan data, which found no significant difference in the performance of the two assays. The San Juan data, while still having data quality and completeness limitations shared by the Fort Collins and BSRI data, is methodologically the strongest available comparison between the two tests. The San Juan data also have the clearest and most complete presentation of data and the most extensive precision data of the three comparisons. The lack of consistency in the Fort Collins and San Juan comparisons suggests that the differences in protocols and how they were implemented may account for some of the difference in the findings.

It should be further noted that even if the Fort Collins data did reliably show that the Trioplex failed to detect 39 percent of Zika virus infections (which it does not), this would only describe the *diagnostic sensitivity* of the assay (i.e., how often the assay alone identifies an infection) not the *clinical sensitivity* (i.e., how often the complete clinical algorithm correctly identifies an infection). As acknowledged by Dr. Lanciotti in the whistleblower disclosure, the risk of false negative tests is not as large as 39% because the results from the Trioplex are not used solely to finalize a diagnosis in clinical practice. Even if the Fort Collins data presented a complete, reproducible, and statistically valid comparison of the two assays, the most favorable interpretation of its findings would result in a maximum of 12%, not 39%, of missed cases using the clinical diagnostic algorithm. Discussion of a 39 percent rate of missed cases overstates the potential impact of the differences between the assays, even under an interpretation most favorable to the Fort Collins data. However, as discussed above, even the finding of a 12% rate of missed cases relies on data from limited and inadequate comparisons between the Trioplex and Singleplex and is contradicted by the San Juan data, which found no significant difference between the two assays' performance.

While there remain overall issues with the data quality and completeness of available comparisons between the assays, there is insufficient evidence to demonstrate that the Trioplex has a statistically significantly less sensitive limit of detection than the Singleplex. Of the available datasets, the one that finds no significant difference between the two assays—the San Juan data—is the strongest, clearest, and most reproducible. In short, there is insufficient, statistically robust, definitive data provided to reach an evidence-based conclusion that use of the Trioplex assay over the Singleplex in clinical practice will result in 39 percent of Zika virus infections being missed.

ii. Allegation 2: The EOC is aware of information indicating that the Trioplex is less sensitive in detecting Zika virus RNA than the Singleplex but is withholding this information from public health laboratories.

The available evidence does not substantiate this allegation. The data available to the EOC comparing the Singleplex and Trioplex were inconclusive and contradictory and it was reasonable to not share this information with external public health laboratories, as it did not provide any meaningful information for laboratories to act upon.

Part of this allegation is accurate insofar as the EOC was aware of Dr. Lanciotti's concerns about the Trioplex's limit of detection relative to the Singleplex and did not share his findings—or the contradictory findings from the

San Juan comparison—with public health laboratories. The message from the EOC to public health laboratories on April 26 expressly recommended the Trioplex and MAC-ELISA and was intended by the EOC to amend Dr. Lanciotti's April 21 email to states. The EOC message acknowledged that the low level of viremia associated with Zika virus infections presented diagnostic challenges and ensured that CDC would continue to evaluate the Trioplex's performance and update its recommendations on its use as needed, but did not question the Trioplex's limit of detection relative to the Singleplex. The communication also acknowledged that some laboratories were using laboratory developed tests, like the Singleplex, and did not prohibit laboratories from using them or otherwise instruct them to abandon these tests.

As explained with regard to Allegation 1, the evidence that the Trioplex had a significantly inferior limit of detection than the Singleplex was contradictory and inconclusive. The data from Fort Collins, BSRI, and San Juan all had flaws that made it difficult to draw a reliable comparison between the two assays. Further, the analysis from San Juan contradicted the findings from Fort Collins and BSRI. In April and May 2016 the EOC had, at best, contradictory and inconclusive information that the FDA-authorized Trioplex may have less analytic sensitivity compared to a version of the Singleplex.

The issue then, is whether it was reasonable for the EOC to withhold conflicting and inconclusive data questioning the Trioplex's performance from public health laboratories. Based on the available information, the EOC's decision to withhold these data and instead focus on improving the Trioplex's diagnostic sensitivity was reasonable.

Sharing the inconclusive and conflicting data about the Trioplex's performance relative to the Singleplex would likely have caused confusion among the public health laboratories and negated the positive attributes of the Trioplex (e.g., FDA authorization, standardization of approach, and ability to detect three viruses) for uncertain benefit. First, EOC issuance of contradictory information (i.e., that one CDC laboratory found that the Singleplex had a superior limit of detection while another CDC laboratory did not) would cause confusion among the public health laboratories. Given the communication among state laboratories, any message to the 20 to 25 states running the Singleplex would also inevitably be shared with states not running the Singleplex, creating broad uncertainty across public health laboratories about which diagnostic test they should use.

Second, CDC's public abandonment of the Trioplex would forfeit the considerable benefits of the Trioplex. Under the EUA, CDC is able to provide a much higher level of assistance to help laboratories set up, conduct quality control, and troubleshoot the Trioplex and quickly initiate clinical testing in a shorter timeframe. The Trioplex allows for streamlined testing of Zika, dengue, and chikungunya viruses, easing the testing burden, conserving precious clinical specimens, and ensuring providers have clinical information that could help in differentiating between other potential viral infections. While none of these benefits would justify CDC's promotion of an assay it knew to be inferior, they do indicate that abandonment of the Trioplex should be based on reliable data about its performance. As indicated above, no such data were provided in this case, only conflicting information that would have given states little clear direction on how they should proceed.

The decision by the EOC to recommend the Trioplex while not prohibiting the use of other validated laboratory diagnostic tests like the Singleplex and working to improve the Trioplex's diagnostic sensitivity was a reasonable and appropriate course of action. While the data questioning the Trioplex's limit of detection were inconclusive, they did warrant further investigation, which the EOC did initiate. Virtually all witnesses stated that there were concerted and ongoing discussions about the Trioplex limit of detection issue. Further, the EOC did not instruct states to stop running the Singleplex, and as of July 19 at least seven public health laboratories continued to run a version of the Singleplex.

Witnesses also broadly agreed that the EOC is working to improve the Trioplex's diagnostic sensitivity, regardless of its performance relative to the Singleplex. On August 22, 2016 CDC submitted a substantial revision of the Trioplex EUA, which includes improvements that will likely have an impact on the assay's diagnostic sensitivity, namely increasing the sample input volume and adding whole blood as a specimen type. This is in addition to CDC's ongoing efforts to provide up-to-date information on diagnostic testing best practices through updates to its testing algorithm, which is specifically referenced in the Trioplex EUA Instructions for Use. These

efforts indicate that the EOC appears to be taking potential sensitivity issues seriously and is taking substantive action to address the issue.

iii. Allegation 3: The EOC's promotion of the Trioplex may have led public health laboratories that were approved to use the more sensitive Singleplex to run the Trioplex preferentially, believing it to be the superior method for detecting Zika virus RNA.

The key premise of this allegation—that the EOC knowingly promoted an inferior assay—is not substantiated by the available evidence. This allegation may be correct to the extent that the EOC's promotion of the Trioplex—and more importantly the material support CDC provided to laboratories running the Trioplex—likely led some laboratories to preferentially run the Trioplex over the Singleplex. However, there are no statistically significant data to demonstrate that the Trioplex was less sensitive than the Singleplex and therefore no evidence that the EOC knowingly promoted an inferior assay. Importantly, while CDC clearly encouraged use of the Trioplex, the agency did not instruct public health laboratories to abandon the Singleplex, and seven laboratories continue to run it. While CDC's promotion and support of the FDA-authorized assay likely encouraged other laboratories to adopt the test, laboratories were never required to do so and could choose to continue to perform their CLIA-compliant versions of the Singleplex.

iv. Primary allegation: The EOC's decision making presented a substantial and specific danger to public health and safety

The available evidence does not substantiate that the EOC's decision making on the promotion of the Trioplex presented a substantial and specific danger to public health and safety. EOC's actions were based on a reasonable assessment of the available data and appeared to be the best option to maximize public health benefit during an ongoing emergency.

A key issue in this case is the tension between the EOC's decision making and the established practices of subject matter experts in Dr. Lanciotti's laboratory in Fort Collins. Decision making and information flow in an emergency response is centralized in the Incident Manager and the EOC to ensure that CDC provides timely, consistent, and clear information to partners and the public. This hierarchal command structure, while essential in an emergency response, was unsettling to the subject matter experts in Dr. Lanciotti's laboratory, who had been working with external laboratories for years and had well-established ways of working with these partners. This included providing assistance to laboratories to set up versions of laboratory developed tests, like the Singleplex. The EOC's perceived interference in these established relationships and insistence on standardized, FDA-authorized assays appeared to frustrate Dr. Lanciotti and others in DVBD.

However, the evidence indicates that the EOC's actions were a reasonable and justifiable response that appeared to be the best available course of action to protect the public's health. Operating in an emergency response requires making decisions with incomplete or imperfect information, which was the circumstance faced by EOC leadership in this situation. The EOC was presented with conflicting and inconclusive data about the Trioplex's limit of detection relative to the Singleplex. Virtually all witnesses, including Dr. Lanciotti, agree that there was extensive discussion to determine the validity and reliability of these data and that the EOC took Dr. Lanciotti's concerns seriously. The EOC did not ultimately adopt Dr. Lanciotti's proposed course of action to share conflicting and inconclusive information with states and recommend use of a non-FDA authorized assay over the Trioplex. Instead, the EOC chose to keep the conflicting datasets internal and continue working to improve the Trioplex while not actively discouraging the continued use of the Singleplex. This was a reasonable course of action. Sharing inconclusive performance data that showed a conflict between two different CDC laboratories would have provided little actionable information to external laboratories. It had the potential to create considerable confusion during an ongoing emergency response and could have caused states to abandon the Trioplex and forfeit its practical and clinical benefits despite the absence of available evidence to support such an action.

The EOC's actions were also reasonable given the regulatory environment around laboratory diagnostic testing. With the FDA-authorized Trioplex, CDC could offer a detailed, standardized protocol that provided greater control and standardization than the substantially more limited Singleplex protocol. The EUA gave CDC clear

authorization to manufacture and distribute reagents for the Trioplex and provide direct, substantive assistance to states to get the assay up and running. With CDC oversight of the materials used in the assay it could exercise stronger quality control over the assay's components and could troubleshoot problems occurring among laboratories with greater efficiency and ease, an important factor when coordinating a national emergency response. In contrast, because the Singleplex was not submitted for EUA and has never undergone FDA's EUA review process CDC could not exercise the kind of oversight and quality control over the reagents for the Singleplex or provide the level of assistance that it could for the Trioplex. The two-page protocol for the Singleplex provided much less detail and specificity than the Trioplex's 41-page Instructions for Use and invited greater potential variation in how external laboratories implemented the Singleplex.

Given the totality of the circumstances, the EOC's decision to continue recommending the Trioplex did not present a substantial and specific danger to public health. Its decision to not share conflicting information on the Trioplex's performance and instead work on improving the assay while not discouraging use of other validated laboratory developed assays was reasonable, based on the information available.

V. Listing of Any Violation or Apparent Violation of Any Law, Rule, or Regulation

The investigative team has concluded that there were no violations or apparent violations of laws, rules, or regulations relevant to the whistleblower's allegations.

VI. Description of Any Actions Taken or Planned as a Result of the Investigation

While the allegation that the Trioplex has an inferior limit of detection relative to the Singleplex is not substantiated by the available evidence, it is clear that efforts to improve the Trioplex's diagnostic sensitivity should continue. CDC's revision of the Trioplex EUA to increase the sample input volume and authorize the use of whole blood as a specimen type is an important step in this direction. It is recommended that CDC continue to prioritize efforts like this amendment that have promise for improving the Trioplex's diagnostic sensitivity. It is further recommended that the agency continue to ensure that the Zika diagnostic algorithm incorporates up-to-date information on best testing practices.

As the number of Zika virus RNA detection assays granted EUAs by FDA increases, it is expected that there will be examples of discrepancies between the results obtained on the same sample tested by multiple assays within the same or different laboratories. In these cases, it will be important to consider the results from antibody testing and the patient's clinical presentation in making a determination of their infection status. CDC should continue to monitor developments in Zika diagnostic testing and ensure that the agency's recommendations to the field reflect the best available evidence.