October 14, 2016

Michele Owen, PhD
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Centers for Disease Control and Prevention
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RE: Concerns regarding molecular testing for Zika virus

Dear Dr. Owen,

The American Society for Microbiology (ASM), Association for Molecular Pathology (AMP) and Pan American Society for Clinical Virology (PASCV) are committed to providing education and assistance to their members regarding laboratory testing during public health emergencies. In light of recent concerns regarding test performance characteristics of the Trioplex rRT-PCR (Trioplex) assay for the testing of Zika, dengue and chikungunya viruses, we are writing to ask that data regarding the development and verification of this assay be made available to the scientific and medical communities. In order to provide accurate and updated information to our clinicians and provide best patient care, transparency regarding the Trioplex assay and release of the data evaluating the test performance characteristics is necessary. We respectfully request specific actions as outlined below to clarify these concerns.

The issues are as follows:

1. Questions regarding the sensitivity of the Trioplex assay necessitate more extensive investigation into the performance characteristics of this test. Comparative studies of the Trioplex and Singleplex assays by the Arboviral laboratory at CDC Fort Collins and the Blood Systems Research Institute in San Francisco, CA suggest that the Trioplex is significantly less sensitive than the Singleplex assay[1]. These data conflict with findings by a CDC laboratory in Puerto Rico which detected no difference in sensitivity between the two tests[1]. However, these data are not publically available or published. Further, data are lacking regarding the performance of commercially-available EUA tests compared to the CDC tests. The lack of access to all data regarding test performance of these assays prevents laboratory professionals from making informed decisions about which test to adopt or recommend. Access to these data would provide transparency and allow for optimal patient care.

   Requested Action: Increase transparency and communication about the Trioplex assay, including the release of data from studies comparing the performance of both CDC tests and any available data regarding the comparative performance of other molecular EUA tests for Zika virus detection

2. The Department of Health and Human Services (DHHS) conducted an investigation of the Trioplex assay following concerns of decreased sensitivity. Reportedly, the investigational team concluded there was no difference in sensitivity between the Singleplex and Trioplex based on the study conducted by CDC Puerto Rico[1,2]. However, it is unclear what comparative studies were investigated by DHHS and what additional information was obtained during the investigation regarding the Trioplex development, verification and EUA approval. According to the Trioplex EUA approval document released by the FDA, it states, "…based on the totality of scientific evidence available to

[1] Cohen, J. Documents reveal intense battle over CDC Zika tests. 2016 www.sciencemag.org/news doi: 10.1126/science.aah7361

the FDA, that it is reasonable to believe that the authorized Trioplex rRT-PCR may be effective in the detection of Zika virus and diagnosis of Zika virus infection…."[3] However, the document does not state what scientific evidence was available to the FDA for review and does not discuss any potential technical limitations of the approved assay. Also, it does not disclose the scientific rationale for choosing Trioplex, or discuss whether the Singleplex was considered but determined to be unacceptable.

Requested Action: Release details of the DHHS investigation and EUA approval of the Trioplex assay, including any analysis of the Singleplex assay.

3. The Singleplex protocol was provided to public health laboratories for Zika virus testing. Regardless of CDC distribution, the assay is a laboratory-developed test and additional efforts were needed by testing laboratories to develop the test protocol and perform verification studies. However, material needed to perform proper verification was not readily available and was insufficiently comprehensive. At the time, it was necessary to obtain such materials from CDC but now there are commercially available resources. As a result, many laboratories that might have opted for the Singleplex test due to its performance characteristics[1,2] instead chose the expedient route of verifying and implementing Trioplex, which included a verification panel.

   Requested Action: If the Singleplex Zika assay is superior to or equally sensitive as the Trioplex assay, we ask that CDC consider evaluating this assay for EUA approval and provide verification and implementation support to laboratories that adopt the Singleplex assay.

We appreciate your time and attention to this important matter. We hope that the CDC will provide more complete information on the performance of Trioplex and Singleplex Zika virus assays as outlined here. Our organizations represent thousands of professionals in clinical laboratory medicine and public health. Providing rapid, thorough and correct information on the testing of novel, emerging infectious disease pathogens, such as Zika virus, is critical for clinical laboratory professionals in order to select optimal methods and provide efficient and accurate testing for our patients and our communities.

Please send any requests for clarification or additional comments to Kimberly Walker (kwalker@asmusa.org) for immediate attention.

Sincerely,

Susan E. Sharp, Ph.D., D(ABMM)
President, American Society for Microbiology

Charles E. Hill, M.D., Ph.D.
President, Association for Molecular Pathology

Alexandra Valsamakis, M.D., Ph.D.
President, Pan American Society for Clinical Virology

[2] OSC Calls for Further Review of Whistleblower Disclosures on Zika Testing. September 27, 2016. U.S. Office of Special Counsel, Washington, D.C.
[3] Trioplex Real-time RT-PCR Assay (CDC). www.fda.gov/MedicalDevices/Safety/EmergencySituations Updated: 10/4/2016