



Diagnostic Testing for Zika Virus: a Postoutbreak Update

Elitza S. Theel,^a D. Jane Hata^b

^aDivision of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA

^bLaboratory Medicine and Pathology, Mayo Clinic, Jacksonville, Florida, USA

ABSTRACT Since the emergence and dissemination of Zika virus (ZIKV) in late 2015, our understanding of the biology, transmission, clinical disease, and potential sequelae associated with infection has markedly expanded. Over the past 2 years, the number of diagnostic assays for ZIKV has increased from none in 2015 to 5 serological assays and 14 molecular assays in 2017, all with emergency use authorization granted through the U.S. Food and Drug Administration. Here we provide an update on ZIKV, addressing what we have collectively learned since the outbreak began, including a summary of currently available diagnostic assays for this virus.

KEYWORDS diagnostic assays, RT-PCR, serology, Zika virus

Despite multiple outbreaks of Zika virus (ZIKV) in the Pacific Islands between 2007 and 2014, this mosquito-borne flavivirus did not garner international attention until mid-2015, when it was detected for the first time in Brazil among 24 patients presenting with a dengue virus (DENV)-like illness (1). Public alarm with respect to ZIKV heightened in late 2015, following reports of increased rates of congenital microcephaly and fetal central nervous system (CNS) defects among ZIKV-infected pregnant women in Brazil (2). This led the World Health Organization (WHO) to declare the emerging ZIKV outbreak a public health emergency of international concern in February 2016. A causal relationship between *in utero* ZIKV infection and the development of birth defects, including severe microcephaly, was officially established soon thereafter (3).

Over the past 2 years, our knowledge about ZIKV, including its biology, pathogenesis, and modes of transmission, has expanded considerably, as has our appreciation of the clinical manifestations associated with infection and the importance of accurate and timely diagnosis. Between 2015 and the writing of this article, over 3,200 articles related to ZIKV were published, compared to 175 publications between 1952 and 2014. This minireview is intended to provide an update on what we have learned regarding ZIKV and to describe the current state of diagnostic testing for this mosquito-borne public health threat (4).

UPDATE ON THE EPIDEMIOLOGY, TRANSMISSION, AND CLINICAL DISEASE ASSOCIATED WITH ZIKA VIRUS

Emergence of ZIKV in the Americas. Two lineages (i.e., African and Asian) and three genotypes (West African, East African, and Asian) of ZIKV have circulated in tropical and semitropical countries in the Eastern Hemisphere since the 1950s (5). The precise event or time frame associated with the introduction of the Asian ZIKV lineage into Brazil has been debated, although initial hypotheses focused on the 2014 World Cup soccer tournament (June and July) or the 2014 International Va'a Canoe World Sprint competition (August), both in Rio De Janeiro, Brazil (6). More recently, phylogenetic studies of multiple ZIKV genomes collected from Brazilian patients, alongside molecular clock analyses, suggest that the initial introduction of ZIKV occurred during the second half of 2013, a time frame that notably coincided with increased air travel of passengers to Brazil from regions in the Pacific Islands where ZIKV was circulating (7).

Accepted manuscript posted online 31 January 2018

Citation Theel ES, Hata DJ. 2018. Diagnostic testing for Zika virus: a postoutbreak update. *J Clin Microbiol* 56:e01972-17. <https://doi.org/10.1128/JCM.01972-17>.

Editor Colleen Suzanne Kraft, Emory University

Copyright © 2018 American Society for Microbiology. All Rights Reserved.

Address correspondence to Elitza S. Theel, theel.elitza@mayo.edu.

Locally acquired cases of ZIKV have been documented throughout the Caribbean Islands and in all but two countries (i.e., Chile and Uruguay) in North America and Latin America since the outbreak began (8). According to the Pan American Health Organization (PAHO) and the WHO, over 1 million individuals in those regions have been infected with ZIKV since 2015 and 20 cases have resulted in death, excluding those related to congenital infections or Guillain-Barré syndrome (GBS) (9). In the United States, over 5,500 symptomatic ZIKV cases have been reported to the Centers for Disease Control and Prevention (CDC) since 2015, with the height of the outbreak occurring in July and August 2016 (10). The number of cases increases to more than 37,000 when U.S. territories are included, among which Puerto Rico was most affected, with nearly 35,000 infected individuals. Notably, in 2017, ZIKV infection rates declined dramatically both in the United States and in all other countries affected by the outbreak.

Transmission. The principal route of ZIKV transmission to humans is via mosquitoes, with *Aedes aegypti* mosquitoes being considered the most competent species (11). As a result, the vast majority (>90%) of ZIKV cases in the United States have been associated with travel to regions with ongoing ZIKV circulation among mosquito populations. *Aedes* sp. mosquitoes are also endemic in the United States, being found primarily in the Southeast, Mid-Atlantic, and Central Midwest regions, ranging from Texas to New Jersey; therefore, the risk of ZIKV introduction into the continental United States continues to be a significant public health concern (12). This risk was realized in July 2016, when the first cases of autochthonous ZIKV transmission were reported in Miami, Florida, followed by confirmation of locally acquired ZIKV in Brownsville, Texas, in November 2016 (13, 14). Over 200 locally acquired cases have been confirmed in both of those states. As a result of intense mosquito control efforts, autochthonous mosquito-borne transmission of ZIKV in the continental United States has been significantly reduced, with only 4 cases presumed to be acquired via this route reported to the CDC in 2017.

Although it is less frequent, we now know that ZIKV transmission may also occur through vector-independent routes. Vertical transmission of ZIKV, from mother to fetus, has now been well documented, with over 95 live-born infants in the United States being confirmed to have ZIKV-associated congenital defects (3, 10). Transmission of ZIKV through sexual contact, a characteristic unique to ZIKV among the flaviviruses, has also been established and was the mode of acquisition for over 50 patients in the United States (10, 15). Infection with ZIKV via transfusion of infected blood products remains of significant concern, as 1.1% and 2.8% of asymptomatic blood donors in Puerto Rico and French Polynesia, respectively, were positive for ZIKV during their respective outbreaks (16–18). In August 2016, this led the U.S. Food and Drug Administration (FDA) and the American Association for Blood Banks to implement deferral of blood donation for 28 days among individuals returning from travel to regions in which ZIKV is endemic, in addition to requiring that all blood products be screened for ZIKV using a molecular assay. To date, no blood-product-transmitted infections have been reported in the United States. While ZIKV infections associated with solid organ transplants have likewise not been documented, the risk exists. There are no specific ZIKV tests recommended for donor screening, with current protocols focusing on assessments of donor travel history, epidemiological risk factors, and symptoms (19). Finally, ZIKV infection through secondary nonsexual contact has also been reported, although the specific modes of transmission have not yet been determined (20, 21). Of note, recent studies suggest prolonged viral shedding in a variety of body fluids, including tears, although the risk of transmission following contact with these fluids remains unclear (22).

Pathogenesis and clinical disease. The pathogenesis of ZIKV, particularly the neurotropic nature of the virus, continues to be characterized. ZIKV has a predilection for fetal neural progenitor and neural retinal cells, with infection resulting in marked inflammation, reduced cellular proliferation, and apoptosis (23). It is estimated that

approximately 1 of 3 infants infected with ZIKV *in utero* will develop fetal abnormalities, which can include severe sequelae such as blindness, dramatic loss of brain parenchyma, ventriculomegaly, and microcephaly (24). Mouse models have shown that ZIKV can also infect and destroy adult neural progenitor cell niches, which are important for neural plasticity and learning (25). Acute flaccid paralysis (AFP) as a result of ZIKV infection has also been reported, with some geographic regions reporting 2.5- to 40-fold increases in AFP rates during ZIKV outbreaks (26). AFP can result from either direct injury to the spinal cord (e.g., myelitis) or a postinfectious, immune-mediated, demyelinating process (e.g., GBS). While the precise mechanism of ZIKV-induced AFP continues to be defined, a recent systemic review by the WHO Zika Causality Working Group determined that sufficient evidence does exist to indicate that ZIKV can trigger GBS (24, 27).

ZIKA VIRUS DIAGNOSTIC ASSAYS

Due to the infrequency of ZIKV infections prior to 2016, there were no commercially available assays for detection of the virus; diagnostic testing for ZIKV was offered only through the CDC and select research laboratories in the United States. This changed rapidly in the United States following declaration of the ZIKV outbreak as a public health emergency by the U.S. Secretary of Health and Human Services (HHS) in August 2016. This declaration allowed assay manufacturers to apply for and receive emergency use authorization (EUA) from the FDA for unapproved assays related to ZIKV diagnostics (28). The overarching goal of the EUA process is to "... support emergency preparedness and response and foster the development and availability of medical products for use in these emergencies" (29). For EUA to be granted, test developers must provide certain device performance data (e.g., analytical sensitivity and specificity in the desired specimen type) to the FDA and are obligated to advance the product toward traditional FDA approval. The FDA is charged with periodic review of the EUA with respect to assay performance, while laboratories performing assays under EUA must be Clinical Laboratory Improvement Amendments (CLIA) certified to perform high-complexity testing and are required to follow the procedure specified by the EUA without variation (29). Although the CDC deactivated its emergency response to ZIKV on 29 September 2017, the Secretary of HHS has not yet terminated the public health emergency declaration. Currently, there are 5 serological assays and 14 molecular assays for ZIKV with FDA EUA (Tables 1 and 2). Despite the availability of those commercial assays, as well as assays without FDA EUA, diagnostic testing for ZIKV remains limited in many resource-strained regions in which ZIKV is endemic. Specimens from such regions are often sent internationally for testing, leading to delays in the receipt of results, which may negatively affect clinical care. Point-of-care assays for ZIKV that meet the WHO ASSURED (affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable) criteria are needed to help meet the ZIKV diagnostic challenges in resource-limited countries (30).

ZIKA VIRUS AND THE HUMORAL IMMUNE RESPONSE

For the majority of symptomatic patients, IgM class antibodies to ZIKV are detectable approximately 1 week following infection, although recent studies suggest that seroconversion may occur earlier for one-third of patients (31, 32). While anti-ZIKV antibody titers subsequently decrease, IgM to ZIKV may still be detectable more than 2 months following infection for over 80% of individuals (31). Such seropersistence is consistent with the antibody responses following infection with other flaviviruses, including West Nile virus (WNV) and DENV, for which modeling studies suggest that the mean time to IgM seronegativity ranges from 5 to 6 months (33, 34). IgG class neutralizing antibodies (NAs) develop soon after the IgM response and can persist for years to decades following infection. The NA response to ZIKV is specific in patients without prior exposure to flaviviruses; however, NA specificity decreases in patients for whom ZIKV infection occurs in the setting of past exposure to a closely related flavivirus, such as DENV, which shares significant homology with ZIKV at key epitopes

TABLE 1 Summary of serological assays with emergency use authorization for detection of antibodies to ZIKV and performance characteristics in serum according to instructions for use

Assay name and manufacturer	Date EUA issued ^a	Specimen source ^a	Method	ZIKV antigen(s)	Positive agreement (% [95% CI])	Negative agreement (% [95% CI])	Peer-reviewed positive agreement (%)	Peer-reviewed negative agreement (%)	Interpretive categories
ZIKV MAC-ELISA (CDC)	26 February 2016	S	MAC-ELISA (colorimetric)	Recombinant, noninfectious, ZIKV-like particles	94 (89.3–96.7) ^b	30.9 (26.7–35.5) ^b	83.3–100 ^c	47.1 ^c	Presumptive ZIKV positive, equivocal, inconclusive, or negative
ZIKV Detect IgM capture ELISA (InBios International, Inc.)	17 August 2016	S	MAC-ELISA (colorimetric)	Envelope	100 (90.6–100) ^d	92.5 (85.8–96.1) ^d	100 ^e	20–74 ^e	Presumptive ZIKV positive, possible ZIKV positive, presumptive another flavivirus positive, or negative
Liaison XL Zika capture IgM assay (DiaSorin Incorporated)	5 April 2017	S	Microparticle IgM capture immunoassay (chemiluminescence)	NS1	94.2 (88.7–97.2) ^f	99.3 (97.5–99.9) ^f	85 ^g	56 ^g	Presumptive ZIKV IgM positive, presumptive recent ZIKV positive, or negative
ADVIA Centaur Zika test (Siemens Healthcare Diagnostics Inc.)	18 September 2017	S, P	Microparticle IgM capture immunoassay (chemiluminescence)	NS1	90.2 (87.5–94) ^f	95.9 (91.6–98.2) ^f	NA	NA	Presumptive ZIKV positive or negative
DPP Zika IgM system (Chembio Diagnostic Systems, Inc.)	27 September 2017	Fingerstick or venous WB, S, P	Immunochromatographic	NS1	95.1 (83.9–98.2) ^f	98.2 (96.7–99) ^f	NA	NA	IgM reactive or nonreactive

^aS, serum; P, plasma; WB, whole blood; NA, not available.

^bAgreement determined in comparison to ZIKV PRNT.

^cPerformance compared to ZIKV PRNT and/or RT-PCR results (45, 47).

^dAgreement determined in comparison to CDC ZIKV PRNT and Trioplex RT-PCR results.

^ePerformance compared to ZIKV and DENV PRNT results (48, 50).

^fAgreement determined in comparison to unspecified comparator ZIKV IgM serological assay with FDA EUA, with specimens collected \geq 8 days PSO.

^gPerformance compared to ZIKV and DENV PRNT results (48).

TABLE 2 Summary of molecular assays with emergency use authorization for detection of ZIKV RNA and performance characteristics according to instructions for use and peer-reviewed literature (as available)

Assay name and manufacturer	Date EUA issued	Specimen source ^a	Method	Gene target(s)	Positive agreement in serum (% [95% CI])	Negative agreement in serum (% [95% CI])	Limit of detection in serum (95% CI)	Peer-reviewed positive agreement (% [95% CI])	Peer-reviewed negative agreement (% [95% CI])
Trioplex rRT-PCR (CDC) ^b	17 March 2016	WB, S, CSF, U, AF	RT-PCR (TaqMan)	Envelope (ZIKV)	100 (92.6–100)	99.1 (95.5–100)	1.93 × 10 ⁴ GCE/ml	NA	NA
Zika virus RNA qualitative real-time RT-PCR (Quest Diagnostics Infectious Disease, Inc.) ^c	28 April 2016	S, U	RT-PCR (TaqMan)	Envelope and membrane	94.6 (85.4–98.2)	100 (93.4–100)	250 copies/ml	NA	NA
RealStar Zika virus RT-PCR kit (Altona Diagnostics, GmbH)	13 May 2016	S, U	RT-PCR (TaqMan)	NS1	96.8 (89.0–99.1)	95.1 (83.9–98.7)	0.61 copies/μl (0.39–1.27 copies/μl) (56); 0.015 PFU/ml (0.004–0.982 PFU/ml) (57)	91.4–95.5 (56, 57)	91.8–97.1 (56, 57)
Aptima Zika virus assay (Hologic Inc.)	17 June 2016	S, P, U	Transcription-mediated amplification	NS1, NS4/NS5	100 (96.7–100)	97.2 (90.4–99.2)	11.5 copies/ml (7.9–20.2 copies/ml) (55)	94.7 (73.5–99.9) (55)	94.8 (88.9–97.8) (55)
Zika virus real-time RT-PCR test (Viracor Eurofins) ^d	19 July 2016	S, P, U	RT-PCR (TaqMan)	Not specified	100 (69.8–100)	96.4 (86.6–99.4)	97 copies/ml (plasma)	NA	NA
Versant Zika RNA 1.0 assay (kPCR) (Siemens Healthcare Diagnostics Inc.)	29 July 2016	S, P, U	RT-PCR	Not specified	90.6 (83.1–95.0)	85.2 (73.4–92.3)	721 GCE/ml	NA	NA
xMAP MultifLEX Zika RNA assay (Luminex Corp.)	4 August 2016	S, P, U	RT-PCR	Not specified	95.8 (85.1–99.5)	98.1 (93.4–99.5)	687 copies/ml	NA	NA
Sentosa SA ZIKV RT-PCR test (Vela Diagnostics USA, Inc.)	23 September 2016	S, P, U	RT-PCR	NS4A	94.9 (87.5–98.0)	100 (93.6–100)	6.0 × 10 ³ copies/ml	NA	NA
Zika virus detection by RT-PCR (ARUP Laboratories) ^e	28 September 2016	S, P, U	RT-PCR	Not specified	98 (89.5–99.7)	100 (79.6–100)	160 copies/ml	NA	NA
RealTime Zika (Abbott Molecular)	11 November 2016	WB, S, P, U	RT-PCR	Premembrane, NS3	92.6 (82.1–97.9)	97.0 (89.6–99.6)	30 copies/ml (12.2–30.89 copies/ml) (58)	96.5 (80.0–99) (58)	71.4 (35.2–94.4) (58)
Zika ELITE MGB kit U.S. (ELITechGroup)	9 December 2016	S, P	RT-PCR	NS3	94.7 (88.1–97.7)	90.9 (82.4–95.5)	270 copies/ml (plasma)	NA	NA
Gene-RADAR Zika virus test (Nanobiosym Diagnostics)	20 March 2017	S	RT-PCR	Not specified	100 (92.9–100)	100 (92.9–100)	200 PFU/ml	NA	NA
Cil-ARboViroPlex rRT-PCR (Columbia University)	11 July 2017	S, U	RT-PCR	3' untranslated region	97.0 (89.8–99.2)	100 (93.5–100)	5.16 × 10 ³ GEO/ml	NA	NA
TaqPath zika virus kit (Thermo Fisher Scientific)	2 August 2017	S, U	RT-PCR (TaqMan)	Not specified	94.9 (90.8–98.9)	100 (95.3–100)	50 genomic copies/ml	NA	NA

^aWB, whole blood; S, serum; P, plasma; U, urine; CSF, cerebrospinal fluid; AF, amniotic fluid; GCE, genome copy equivalents; GEO, genome copy equivalents; NA, not available.
^bMultiplex assay for ZIKV, DENV, and chikungunya virus.
^cUse limited to Quest Diagnostics Infectious Disease, Inc.
^dUse limited to Viracor Eurofins Laboratories.
^eUse limited to ARUP Laboratories.

and antigenic determinants (32). This presents a diagnostic dilemma for patients living in regions in which both ZIKV and DENV are endemic, where correct identification of the infecting virus may result in significantly different management strategies. A recent comparison of four enzyme-linked immunosorbent assay (ELISA)-based ZIKV serological assays utilizing well-characterized, sequential serum specimens collected from a Nicaraguan cohort indicated variations in assay sensitivity depending on prior exposure to DENV, compared to specimens obtained from flavivirus-naïve individuals (35). Diagnostic assays that are able to discriminate between primary and secondary ZIKV or DENV infections are needed. Toward that goal, multiple studies using different approaches, including anti-ZIKV IgG avidity testing using a novel plasmonic gold nanotechnology platform, blockade-of-binding testing, or use of multitest serological algorithms, have suggested that, with refinement, such differentiation may be possible in the future (36–38).

Similar to other flaviviruses, the ZIKV envelope (E) glycoprotein is a major antigenic determinant that is able to elicit a strong humoral immune response. Structurally, the E glycoprotein is divided into three domains (EI to EIII), among which NAs to EIII appear to provide the greatest power for discrimination between ZIKV and DENV, as they share only 29% amino acid (AA) homology at this domain, compared to 35% and 51% AA homology at EI and EII, respectively (39, 40). Among the five anti-ZIKV IgM serological assays with FDA EUA, two are based on detection of IgM reactivity to the ZIKV E glycoprotein and the remaining three were developed using ZIKV nonstructural protein 1 (NS1) as the target antigen (Table 1). ZIKV NS1, an essential viral protein released from infected cells, shares 51% to 53% AA homology with NS1 from the four DENV serotypes (40). Despite this high level of similarity, structural studies suggest significant electrostatic differences at key antigenic epitopes within the ZIKV and DENV NS1 proteins, possibly leading to decreased antibody cross-reactivity and greater specificity among ZIKV-NS1-based serological assays (41).

The reference standard for detection of NAs to flaviviruses remains the plaque reduction neutralization test (PRNT), which, although technically challenging to perform, with a turnaround time (TAT) of days to weeks and a requirement for live viral cultures, offers the highest achievable level of specificity. PRNT is performed by serially diluting patient serum and incubating aliquots with live ZIKV or other closely related viruses (e.g., DENV), followed by overlay of this mixture onto a virus-susceptible cell monolayer. Any resulting plaques, suggesting the presence of live virus and thus the absence of NAs, are quantified and compared to the number of plaques in virus-only control wells in order to establish the dilution at which a 90% reduction in plaques occurs in the patient sample (PRNT₉₀) (42). PRNT₉₀ titers are subsequently compared between ZIKV and DENV to determine the specificity of the NAs (if present), although interpretive challenges exist with this method, as discussed below. Studies to improve on the classic PRNT technique are ongoing, including most recently the use of luciferase-labeled viruses to decrease the TAT while maintaining the accuracy of the classic PRNT (43). Importantly, however, while ZIKV testing guidance established by the CDC has been fluid, with multiple revisions over the past 2 years, the recommendation that positive results from anti-ZIKV IgM screens be confirmed by supplemental testing with the PRNT has remained constant (44).

PERFORMANCE OF SEROLOGICAL ASSAYS FOR DETECTION OF ANTIBODIES TO ZIKA VIRUS

There are currently five serological assays with FDA EUA available for detection of IgM class antibodies to ZIKV. However, peer-reviewed studies independently assessing the performance characteristics of the assays are limited and are available primarily for the CDC ZIKV IgM antibody capture (MAC)-ELISA and the InBios ZIKV Detect MAC-ELISA (InBios International, Inc., Seattle, WA), both of which detect antibodies to the ZIKV E protein, and most recently for the Liaison XL Zika capture assay (DiaSorin Inc., Stillwater, MN), a ZIKV NS1-based chemiluminescent microparticle IgM capture immunoassay (32, 45–48) (Table 1). Compared to PRNT and/or real-time reverse transcriptase PCR

(rRT-PCR) for ZIKV, recent studies evaluating the CDC ZIKV MAC-ELISA show high rates of positive agreement, ranging from 83.3% to 100% for samples collected approximately 1 week to 85 days post symptom onset (PSO), which is consistent with the reported positive agreement rate of 94% in the assay instructions for use (IFU) (45, 47, 49). Negative agreement rates for this assay are low, however, ranging from 30.9% to 47.1%, with nearly 50% of serum samples confirmed for anti-DENV antibodies by PRNT also being positive by the CDC ZIKV MAC-ELISA. This underscores the importance of follow-up confirmatory testing for sera reactive by the CDC ZIKV MAC-ELISA with the ZIKV PRNT, which, according to CDC guidelines, remains a recommended follow-up test for all samples reactive by any anti-ZIKV IgM serological assay (44). The clinical interpretation of PRNT results may be challenging, however. Lanciotti and colleagues found that, while PRNT was highly specific for ZIKV in the setting of primary flavivirus infection, PRNT specificity for ZIKV diminished in patients with prior DENV exposure (32). More specifically, a ≥ 4 -fold difference in PRNT₉₀ titers for ZIKV versus DENV was observed for only three of seven patients with secondary ZIKV infections (32). As a result, for a significant number of individuals with prior DENV infections, anti-ZIKV IgM reactivity results may remain unconfirmed by PRNT, a limitation that is particularly problematic during the diagnostic assessment of pregnant women (46).

Performance of the InBios ZIKV MAC-ELISA has been evaluated by three studies, which reported performance characteristics comparable to those of the CDC ZIKV MAC-ELISA, with positive and negative agreement rates ranging from 87.5% to 100% and from 95.7% to 98.5%, respectively (46, 48, 50). When evaluated against ZIKV and DENV PRNT results, the InBios ZIKV MAC-ELISA showed excellent sensitivity, 100% across studies, but variable specificity, ranging from 20% to 74% in sera confirmed for the presence of NAs to DENV (Table 1). Notably, these specificity values differ from the 92.5% specificity reported in the InBios ZIKV MAC-ELISA IFU. To date, a single study has been published evaluating the Liaison XL Zika capture IgM assay, which showed sensitivity and specificity values of 85% and 56%, respectively, in sera confirmed by PRNT for NAs to ZIKV and DENV, values that differ notably from those reported in the assay IFU (Table 1) (48). The remaining two serological assays with FDA EUA are both based on detection of antibodies to the ZIKV NS1 antigen and include the ADVIA Centaur Zika test (Siemens Healthcare Diagnostics, Tarrytown, NY), a chemiluminescent microparticle IgM capture immunoassay, and the DPP Zika IgM assay system (ChemBio Diagnostic Systems, Medford, NY), an immunochromatographic assay read by an automated reader. Notably, the only available performance characteristics for these assays are derived from the manufacturers' package inserts. Using unspecified ZIKV IgM serological assays with FDA EUA as the comparator methods and specimens collected at least 8 days PSO, the manufacturers report high positive and negative agreement rates, ranging from 90.2% to 95.1% and from 95.9% to 98.2%, respectively (Table 1).

Finally, multiple groups have assessed the Euroimmun anti-ZIKV IgM and IgG ELISAs (Lubeck, Germany), both of which are based on the ZIKV NS1 antigen, although neither assay has received FDA EUA. All reports suggest that the Euroimmun ZIKV IgM ELISA provides limited sensitivity, ranging from 54% to 79%, in sera collected within 30 days PSO from patients with ZIKV infections confirmed by rRT-PCR or PRNT (45, 47, 48, 51, 52). Interestingly however, the majority of those studies showed that combined interpretation of results from both the Euroimmun anti-ZIKV IgM and IgG ELISAs increased sensitivity to over 88% in this same patient cohort. The specificity of the Euroimmun ZIKV ELISAs, particularly for patients with prior exposure to closely related flaviviruses such as DENV, WNV, or Japanese encephalitis virus, has repeatedly been shown to be high, over 95%, although L'Huillier and colleagues suggested more frequent cross-reactivity among patients seropositive for DENV (45, 47, 48, 53). Overall, while these emerging data are encouraging and the availability of such assays has greatly improved the accessibility to diagnostic testing for ZIKV, expanding beyond the capacity of public health laboratories and the CDC, thorough assessment of these and future diagnostic assays for ZIKV is needed to fully understand their diagnostic accuracy.

PERFORMANCE OF MOLECULAR ASSAYS WITH FDA EUA FOR DETECTION OF ZIKA VIRUS RNA

The pace of molecular test development for detection of ZIKV RNA and concomitant acquisition of FDA EUA for such assays has increased rapidly, although with limited peer-reviewed studies independently assessing their performance. Highly conserved regions of the ZIKV RNA genome, such as the flanking 5' and 3' noncoding regions (NCRs), are often used as the rRT-PCR primer and probe sequence targets; however, other regions, such as the E, NS1, NS3, NS5, membrane junction, and partial envelope regions, have also been used successfully to develop ZIKV rRT-PCR assays (4, 17).

The first molecular test to receive FDA EUA was the CDC Trioplex rRT-PCR assay, which was designed for qualitative simultaneous detection of ZIKV, DENV, and chikungunya virus in serum, whole blood (WB), urine, cerebrospinal fluid (CSF), and amniotic fluid (Table 2). This assay targets the ZIKV E gene and, according to the IFU, shows high positive and negative agreement rates in serum (100% and 98.2%, respectively), compared to a matched monoplex rRT-PCR assay targeting the ZIKV NS3 gene, and has a lower limit of detection (LLoD) of 1.93×10^4 genome copy equivalents in serum (Table 2) (54). ZIKV RNA can be detected in serum, urine, and WB as early as the day of symptom onset and throughout the first 7 days of illness, although sensitivity is highest throughout this time frame in the latter specimen source (90.9% to 96.2%). While the specificity of the CDC Trioplex rRT-PCR assay is high (>98%), false-positive results have been documented, and a cautionary statement regarding this was notably included in the most recent CDC guidance document for ZIKV (44).

The Aptima ZIKV assay (Hologic Inc., San Diego, CA) employs transcription-mediated amplification (TMA) and targets the ZIKV NS1 and NS4/NS5 genes for detection of ZIKV RNA in serum and urine. With specimens collected from travelers to and residents in regions in which ZIKV is endemic, in addition to spiked specimens, the Aptima ZIKV TMA assay demonstrated positive, negative, and overall agreement rates of 94.7%, 94.8%, and 94.8%, respectively, compared to the CDC Trioplex method (55). The LLoDs for the assay in serum and urine samples were determined to be 11.5 and 17.9 genome copy equivalents/ml, respectively, similar to the LLoD in plasma of 5.9 copies/ml reported in the assay IFU.

The Altona RealStar Zika rRT-PCR kit (Altona Diagnostics GmbH, Hamburg, Germany) targets the ZIKV NS1 gene and has EUA approval for testing of serum, plasma, and urine. Similar to the Aptima ZIKV TMA assay, the Altona RealStar ZIKV rRT-PCR demonstrated high positive and negative agreement rates (95.5% and 91.8% respectively), compared to a ZIKV rRT-PCR assay targeting the E gene, in urine and serum samples (56). These results were mirrored by a similar study performed at the Public Health Ontario Laboratory in Canada, primarily using serum samples from returning travelers, which showed a positive agreement rate of 91.4% and a negative agreement rate of 97.1%, compared to rRT-PCRs for ZIKV RNA (57). The 95% LLoD was calculated to be 0.61 copies/ μ l and 0.15 PFU/ml by these studies (57).

The final ZIKV rRT-PCR assay with current peer-reviewed literature is the Abbott RealTime ZIKV RT-PCR, which targets the *prM* and NS3 genes and can be performed with WB, plasma, serum, and urine samples, using the mSample RNA preparation system kit and automated m2000sp and m2000rt instruments (Abbott Molecular Inc., Des Plaines IL). Compared to the Altona RealStar ZIKV rRT-PCR, the Abbott ZIKV rRT-PCR demonstrated positive and negative agreement rates of 96.5% and 71.4% in serum samples and 72.4% and 71.4% in urine samples, respectively, with LLoD values of 30 copies/ml for serum samples and 40 copies/ml for urine and plasma samples, as reflected in the assay IFU (58).

Impact of specimen source on detection of ZIKV RNA. The specimen types most frequently cited for ZIKV RNA detection have been plasma or serum, with viral titers of 5.0×10^9 to 3.7×10^6 copies/ml (mean of 9.9×10^4 copies/ml) being determined with the CDC Trioplex ZIKV rRT-PCR for serum samples collected within the first 7 days PSO (59). A 2016 longitudinal study of travelers returning from the Caribbean region or

Central or South America showed prolonged detection of ZIKV in WB samples, compared to plasma or serum samples, with median durations of ZIKV viremia of 22 days versus 10 days. Comparison of the last ZIKV-rRT-PCR-positive samples for those patients showed that viral loads were 2.7 to 3.9 log copies/ml in WB and 2.2 to 2.8 log copies/ml in plasma, suggesting that WB may be a more sensitive ZIKV source than other blood fractions (60). While WB appears to be the optimal specimen type for ZIKV rRT-PCR, challenges associated with its long-term storage and extraction have limited the widespread validation of this sample type for molecular applications.

Multiple studies have shown that, due to the longevity of ZIKV shedding and the ease of specimen collection, urine is an advantageous specimen type for ZIKV RNA detection (61). ZIKV RNA was reliably detected 7 to 20 days PSO for 93% of patients in one study, with viral loads ranging from 3.8×10^3 to 2.2×10^8 copies/ml using rRT-PCR assays targeting the ZIKV *prM* and E genes (17, 62). Notably, recent studies have shown more frequent detection and significantly longer persistence of ZIKV RNA in serum versus urine, with median times to clearance of 14 days (95% confidence interval [CI], 11 to 17 days) for serum and 8 days (95% CI, 6 to 10 days) for urine (31, 63). The stability of ZIKV RNA in urine samples stored under different conditions (e.g., maintained at room temperature, refrigerated at 4°C, or frozen at -80°C) was recently evaluated by Tan and colleagues, with results indicating significant degradation of RNA even during storage at -80°C (64). On the basis of those findings, the authors suggest optimal specimen storage at 4°C, with the addition of a nucleic acid stabilizer to minimize the risk of RNA degradation and false-negative results. Notably, testing for ZIKV RNA in urine, with a paired serum sample, is a part of the ZIKV testing guidelines outlined by the CDC, and urine is an approved specimen source for all but two of the current ZIKV molecular assays with FDA EUA (44).

The use of saliva as a specimen source for detection of ZIKV RNA has also been evaluated, due to the ease of collection, particularly for neonates and young children. One study, conducted through the Florida Department of Health, showed that ZIKV RNA could be detected in saliva as early as 1 day PSO and up to 19 days later, although sensitivity was highest within 5 days PSO (62). Importantly, however, despite the ease of sample acquisition, the inconsistencies in sample collection methods and possible challenges associated with specimen processing, as well as the finding that all patients positive for ZIKV in saliva were also positive in other specimen types (e.g., serum or urine), negate the use of this specimen type for routine ZIKV diagnostic assays (65).

Detection of ZIKV RNA in semen has been considered during assessment of fetal infection risks. Among 23 male patients reporting a self-limited, mild illness consistent with ZIKV infection, 56.5% had detectable ZIKV at high copy numbers (threshold cycle of <30 cycles) in semen specimens collected within 28 days PSO (66, 67). Other reports indicated a maximum period of RNA detection in semen of 4 to 6 months, with propagation in culture up to 69 days PSO (31, 68). Importantly however, as RNA from noninfectious virus may be detected by molecular techniques, routine evaluation of semen for ZIKV as a means to determine preconception risk is not recommended. Rather, couples are encouraged to delay conception until 6 months after the last possible exposure of the male partner to ZIKV, until there is a better understanding of viral clearance from this source (44).

Evaluation of products of conception (e.g., amniotic fluid, placenta, and fetal tissue) can be invaluable for identification of neonatal ZIKV infection; however, the performance of molecular assays with these specimen types has not been fully characterized. A prospective study of eight pregnant women on the island of Martinique found that ZIKV RNA was detectable in five of eight postmortem fetal brain tissue specimens but was variably detected in placental tissue, amniotic fluid, fetal blood, or maternofetal circulation at the time of delivery or upon termination of pregnancy, possibly due to the transient nature of ZIKV viremia in these specimen types (69). Although one meta-analysis established that ZIKV RNA could be detected in breast milk and in the blood of two of three infants, evidence could not fully support ZIKV transmission solely

via breastfeeding, and none of the three infants suffered any long-term complications of ZIKV infection (70).

CONCLUSIONS

Significant strides have been made over the past 2 years with respect to our understanding of ZIKV, including the biology of the virus, the multiple and unique modes of transmission, the clinical disease, and the potentially devastating sequelae associated with neonatal infection. The rapidity with which diagnostic assays were developed and made available via FDA EUA to public health and clinical laboratories was a testament to the dedication, collaborative efforts, and ingenuity of both researchers and assay developers. A variety of monoplex and multiplex ZIKV assays, utilizing multiple amplification methods and unique detection chemistries, were recently reviewed (61). There remains, however, a need for further peer-reviewed studies independently assessing the clinical performance of ZIKV diagnostic assays (both molecular and serological) in clinical settings. This need will continue as new diagnostic assays continue to be developed, including novel methods such as paper-based sensors for detection of ZIKV RNA using a chromatographic output, loop-mediated amplification (LAMP) assays performed on a microchip with results visualized using smartphone optical imaging systems, and CRISPR-Cas13b fluorescence detection (61, 71, 72). Although they were not reviewed in detail here, the CDC guidelines and algorithms for the diagnosis of ZIKV, including patient testing criteria, selection of diagnostic method (i.e., molecular versus serological), timing of specimen collection, specimen source, and frequency of testing, have been fluid over the past year and may be amended again as we learn more about this virus. Despite this, these recommendations have been invaluable to clinicians, epidemiologists, and laboratorians, providing guidance for the diagnosis, management, and surveillance of this infectious agent, which was entirely unknown to the vast majority of medical professionals prior to 2015. As the ZIKV pandemic continues to evolve, continuing collaborations between clinicians, researchers, laboratorians, and assay manufacturers will be essential in order to optimize the detection of ZIKV and the management of infected patients.

REFERENCES

- Campos GS, Bandeira AC, Sardi SI. 2015. Zika virus outbreak, Bahia, Brazil. *Emerg Infect Dis* 21:1885–1886. <https://doi.org/10.3201/eid2110.150847>.
- Carvalho FH, Cordeiro KM, Peixoto AB, Tonni G, Moron AF, Feitosa FE, Feitosa HN, Araujo Junior E. 2016. Associated ultrasonographic findings in fetuses with microcephaly because of suspected Zika virus (ZIKV) infection during pregnancy. *Prenat Diagn* 36:882–887. <https://doi.org/10.1002/pd.4882>.
- Rasmussen SA, Jamieson DJ, Honein MA, Petersen LR. 2016. Zika virus and birth defects: reviewing the evidence for causality. *N Engl J Med* 374:1981–1987. <https://doi.org/10.1056/NEJMs1604338>.
- Waggoner JJ, Pinsky BA. 2016. Zika virus: diagnostics for an emerging pandemic threat. *J Clin Microbiol* 54:860–867. <https://doi.org/10.1128/JCM.00279-16>.
- Haddow AD, Schuh AJ, Yasuda CY, Kasper MR, Heang V, Huy R, Guzman H, Tesh RB, Weaver SC. 2012. Genetic characterization of Zika virus strains: geographic expansion of the Asian lineage. *PLoS Negl Trop Dis* 6:e1477. <https://doi.org/10.1371/journal.pntd.0001477>.
- Musso D. 2015. Zika virus transmission from French Polynesia to Brazil. *Emerg Infect Dis* 21:1887. <https://doi.org/10.3201/eid2110.151125>.
- Faria NR, Azevedo R, Kraemer MUG, Souza R, Cunha MS, Hill SC, Theze J, Bonsall MB, Bowden TA, Rissanen I, Rocco IM, Nogueira JS, Maeda AY, Vasami F, Macedo FLL, Suzuki A, Rodrigues SG, Cruz ACR, Nunes BT, Medeiros DBA, Rodrigues DSG, Queiroz ALN, da Silva EVP, Henriques DF, da Rosa EST, de Oliveira CS, Martins LC, Vasconcelos HB, Casseb LMN, Smith DB, Messina JP, Abade L, Lourenco J, Alcantara LCJ, de Lima MM, Giovanetti M, Hay SI, de Oliveira RS, Lemos PDS, de Oliveira LF, de Lima CPS, da Silva SP, de Vasconcelos JM, Franco L, Cardoso JF, Vianez-Junior J, Mir D, Bello G, Delatorre E, Khan K, Creatore M, Coelho GE, de Oliveira WK, Tesh R, Pybus OG, Nunes MRT, Vasconcelos PFC. 2016. Zika virus in the Americas: early epidemiological and genetic findings. *Science* 352:345–349. <https://doi.org/10.1126/science.aaf5036>.
- Centers for Disease Control and Prevention. 2017. World map of areas with risk of Zika. <https://wwwnc.cdc.gov/travel/page/world-map-areas-with-zika>. Accessed 26 September 2017.
- Pan American Health Organization/World Health Organization. 2017. Zika cases and congenital syndrome associated with Zika virus reported by countries and territories in the Americas, 2015–2017: cumulative cases: data as of 21 September 2017. Pan American Health Organization/World Health Organization, Washington, DC. http://www.paho.org/hq/index.php?option=com_content&view=article&id=12390&Itemid=42090&lang=en.
- Centers for Disease Control and Prevention. 2017. Zika cases in the United States. <https://www.cdc.gov/zika/reporting/case-counts.html>. Accessed 10 October 2017.
- Hayes EB. 2009. Zika virus outside Africa. *Emerg Infect Dis* 15:1347–1350. <https://doi.org/10.3201/eid1509.090442>.
- Johnson TL, Haque U, Monaghan AJ, Eisen L, Hahn MB, Hayden MH, Savage HM, McAllister J, Mutebi JP, Eisen RJ. 2017. Modeling the environmental suitability for *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus* (Diptera: Culicidae) in the contiguous United States. *J Med Entomol* 54:1605–1614. <https://doi.org/10.1093/jme/tjx163>.
- Centers for Disease Control and Prevention. 2016. CDC guidance for travel and testing of pregnant women and women of reproductive age for Zika virus infection related to the investigation for local mosquito-borne Zika virus transmission in Brownsville, Cameron County, Texas. CDCHAN-00399. Centers for Disease Control and Prevention, Atlanta, GA. <https://emergency.cdc.gov/han/han00399.asp>.
- Walker WL, Lindsey NP, Lehman JA, Krow-Lucal ER, Rabe IB, Hills SL, Martin SW, Fischer M, Staples JE. 2016. Zika virus disease cases: 50 states and the

- District of Columbia, January 1–July 31, 2016. *MMWR Morb Mortal Wkly Rep* 65:983–986. <https://doi.org/10.15585/mmwr.mm6536e5>.
15. D’Ortenzio E, Matheron S, Yazdanpanah Y, de Lamballerie X, Hubert B, Piorkowski G, Maquart M, Descamps D, Damond F, Leparac-Goffart I. 2016. Evidence of sexual transmission of Zika virus. *N Engl J Med* 374:2195–2198. <https://doi.org/10.1056/NEJMc1604449>.
 16. Musso D, Nhan T, Robin E, Roche C, Bierlaire D, Zisou K, Shan Yan A, Cao-Lormeau VM, Broutet J. 2014. Potential for Zika virus transmission through blood transfusion demonstrated during an outbreak in French Polynesia, November 2013 to February 2014. *Euro Surveill* 19:20761. <https://doi.org/10.2807/1560-7917.ES2014.19.14.20761>.
 17. Musso D, Gubler DJ. 2016. Zika virus. *Clin Microbiol Rev* 29:487–524. <https://doi.org/10.1128/CMR.00072-15>.
 18. Kuehnert MJ, Basavaraju SV, Moseley RR, Pate LL, Gale SA, Williamson PC, Busch MP, Alsina JO, Climent-Peris C, Marks PW, Epstein JS, Nakhasi HL, Hobson JP, Leib DA, Akolkar PN, Petersen LR, Rivera-Garcia B. 2016. Screening of blood donations for Zika virus infection: Puerto Rico, April 3–June 11, 2016. *MMWR Morb Mortal Wkly Rep* 65:627–628. <https://doi.org/10.15585/mmwr.mm6524e2>.
 19. Silveira FP, Campos SV. 2016. The Zika epidemics and transplantation. *J Heart Lung Transplant* 35:560–563. <https://doi.org/10.1016/j.healun.2016.03.010>.
 20. Swaminathan S, Schlager R, Lewis J, Hanson KE, Couturier MR. 2016. Fatal Zika virus infection with secondary nonsexual transmission. *N Engl J Med* 375:1907–1909. <https://doi.org/10.1056/NEJMc1610613>.
 21. Grischott F, Puhan M, Hatz C, Schlagenhauf P. 2016. Non-vector-borne transmission of Zika virus: a systematic review. *Travel Med Infect Dis* 14:313–330. <https://doi.org/10.1016/j.tmaid.2016.07.002>.
 22. Tan JLL, Balne PK, Leo YS, Tong L, Ng LFP, Agrawal R. 2017. Persistence of Zika virus in conjunctival fluid of convalescence patients. *Sci Rep* 7:11194. <https://doi.org/10.1038/s41598-017-09479-5>.
 23. Tang H, Hammack C, Ogden SC, Wen Z, Qian X, Li Y, Yao B, Shin J, Zhang F, Lee EM, Christian KM, Didier RA, Jin P, Song H, Ming GL. 2016. Zika virus infects human cortical neural progenitors and attenuates their growth. *Cell Stem Cell* 18:587–590. <https://doi.org/10.1016/j.stem.2016.02.016>.
 24. Li H, Saucedo-Cuevas L, Shresta S, Gleeson JG. 2016. The neurobiology of Zika virus. *Neuron* 92:949–958. <https://doi.org/10.1016/j.neuron.2016.11.031>.
 25. Li H, Saucedo-Cuevas L, Regla-Nava JA, Chai G, Sheets N, Tang W, Tersikh AV, Shresta S, Gleeson JG. 2016. Zika virus infects neural progenitors in the adult mouse brain and alters proliferation. *Cell Stem Cell* 19:593–598. <https://doi.org/10.1016/j.stem.2016.08.005>.
 26. Cao-Lormeau VM, Blake A, Mons S, Lastere S, Roche C, Vanhomwegen J, Dub T, Baudouin L, Teissier A, Larre P, Vial AL, Decam C, Choumet V, Halstead SK, Willison HJ, Musset L, Manuguerra JC, Despres P, Fournier E, Mallet HP, Musso D, Fontanet A, Neil J, Ghawche F. 2016. Guillain-Barre syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. *Lancet* 387:1531–1539. [https://doi.org/10.1016/S0140-6736\(16\)00562-6](https://doi.org/10.1016/S0140-6736(16)00562-6).
 27. Krauer F, Riesen M, Reveiz L, Oladapo OT, Martinez-Vega R, Porgo TV, Haefliger A, Broutet NJ, Low N. 2017. Zika virus infection as a cause of congenital brain abnormalities and Guillain-Barre syndrome: systematic review. *PLoS Med* 14:e1002203. <https://doi.org/10.1371/journal.pmed.1002203>.
 28. Assistant Secretary for Preparedness and Response. 2016. Determination that a public health emergency exists in Puerto Rico as a consequence of the Zika virus outbreak. <https://www.phe.gov/emergency/news/healthactions/phe/Pages/zika-pr.aspx>. Accessed 15 November 2017.
 29. Food and Drug Administration. 2017. Emergency use authorization of medical products and related authorities. Food and Drug Administration, Silver Spring, MD. <https://www.fda.gov/RegulatoryInformation/Guidances/ucm125127.htm#intro>.
 30. Drain PK, Hyle EP, Noubary F, Freedberg KA, Wilson D, Bishai WR, Rodriguez W, Bassett IV. 2014. Diagnostic point-of-care tests in resource-limited settings. *Lancet Infect Dis* 14:239–249. [https://doi.org/10.1016/S1473-3099\(13\)70250-0](https://doi.org/10.1016/S1473-3099(13)70250-0).
 31. Paz-Bailey G, Rosenberg ES, Doyle K, Munoz-Jordan J, Santiago GA, Klein L, Perez-Padilla J, Medina FA, Waterman SH, Gubern CG, Alvarado LI, Sharp TM. 2017. Persistence of Zika virus in body fluids: preliminary report. *N Engl J Med* <https://doi.org/10.1056/NEJMoa1613108>.
 32. Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, Stanfield SM, Duffy MR. 2008. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerg Infect Dis* 14:1232–1239. <https://doi.org/10.3201/eid1408.080287>.
 33. Prince HE, Matud JL. 2011. Estimation of dengue virus IgM persistence using regression analysis. *Clin Vaccine Immunol* 18:2183–2185. <https://doi.org/10.1128/CI.05425-11>.
 34. Busch MP, Kleinman SH, Tobler LH, Kamel HT, Norris PJ, Walsh I, Matud JL, Prince HE, Lanciotti RS, Wright DJ, Linnen JM, Caglioti S. 2008. Virus and antibody dynamics in acute West Nile virus infection. *J Infect Dis* 198:984–993. <https://doi.org/10.1086/591467>.
 35. Balmaseda A, Zambrana JV, Collado D, Garcia N, Saborio S, Elizondo D, Mercado JC, Gonzalez K, Cerpas C, Nunez A, Corti D, Waggoner JJ, Kuan G, Burger-Calderon R, Harris E. 2018. Comparison of four serological methods and two RT-PCR assays for diagnosis and surveillance of Zika. *J Clin Microbiol* <https://doi.org/10.1128/JCM.01785-17>.
 36. Balmaseda A, Stettler K, Medialdea-Carrera R, Collado D, Jin X, Zambrana JV, Jaconi S, Cameroni E, Saborio S, Rovida F, Percivalle E, Ijaz S, Dicks S, Ushiro-Lumb I, Barzon L, Siqueira P, Brown DWG, Baldanti F, Tedder R, Zamboni M, de Filippis AMB, Harris E, Corti D. 2017. Antibody-based assay discriminates Zika virus infection from other flaviviruses. *Proc Natl Acad Sci U S A* 114:8384–8389. <https://doi.org/10.1073/pnas.1704984114>.
 37. Zhang B, Pinsky BA, Ananta JS, Zhao S, Arulkumar S, Wan H, Sahoo MK, Abeynayake J, Waggoner JJ, Hopes C, Tang M, Dai H. 2017. Diagnosis of Zika virus infection on a nanotechnology platform. *Nat Med* 23:548–550. <https://doi.org/10.1038/nm.4302>.
 38. Tsai WY, Youn HH, Brites C, Tsai JJ, Tyson J, Pedrosa C, Drexler JF, Stone M, Simmons G, Busch MP, Lanteri M, Stramer SL, Balmaseda A, Harris E, Wang WK. 2017. Distinguishing secondary dengue virus infection from Zika virus infection with previous dengue by a combination of 3 simple serological tests. *Clin Infect Dis* 65:1829–1836. <https://doi.org/10.1093/cid/cix672>.
 39. Screaton G, Mongkolsapaya J, Yacoub S, Roberts C. 2015. New insights into the immunopathology and control of dengue virus infection. *Nat Rev Immunol* 15:745–759. <https://doi.org/10.1038/nri3916>.
 40. Stettler K, Beltramello M, Espinosa DA, Graham V, Cassotta A, Bianchi S, Vanzetta F, Minola A, Jaconi S, Mele F, Foglierini M, Pedotti M, Simonelli L, Dowall S, Atkinson B, Percivalle E, Simmons CP, Varani L, Blum J, Baldanti F, Cameroni E, Hewson R, Harris E, Lanzavecchia A, Sallusto F, Corti D. 2016. Specificity, cross-reactivity, and function of antibodies elicited by Zika virus infection. *Science* 353:823–826. <https://doi.org/10.1126/science.aaf8505>.
 41. Song H, Qi J, Hayward J, Shi Y, Gao GF. 2016. Zika virus NS1 structure reveals diversity of electrostatic surfaces among flaviviruses. *Nat Struct Mol Biol* 23:456–458. <https://doi.org/10.1038/nsmb.3213>.
 42. Roehrig JT, Hombach J, Barrett AD. 2008. Guidelines for plaque-reduction neutralization testing of human antibodies to dengue viruses. *Viral Immunol* 21:123–132. <https://doi.org/10.1089/vim.2008.0007>.
 43. Shan C, Ortiz DA, Yang Y, Wong SJ, Kramer LD, Shi PY, Loeffelholz MJ, Ren P. 2017. Evaluation of a novel reporter virus neutralization test for serological diagnosis of Zika and dengue virus infection. *J Clin Microbiol* 55:3028–3036. <https://doi.org/10.1128/JCM.00975-17>.
 44. Oduyebo T, Polen KD, Walke HT, Reagan-Steiner S, Lathrop E, Rabe IB, Kuhnert-Tallman WL, Martin SW, Walker AT, Gregory CJ, Ades EW, Carroll DS, Rivera M, Perez-Padilla J, Gould C, Nemhauser JB, Ben Beard C, Harcourt JL, Viens L, Johansson M, Ellington SR, Petersen E, Smith LA, Reichard J, Munoz-Jordan J, Beach MJ, Rose DA, Barzilay E, Noonan-Smith M, Jamieson DJ, Zaki SR, Petersen LR, Honein MA, Meaney-Delman D. 2017. Update: interim guidance for health care providers caring for pregnant women with possible Zika virus exposure: United States (including U.S. territories), July 2017. *MMWR Morb Mortal Wkly Rep* 66:781–793. <https://doi.org/10.15585/mmwr.mm6629e1>.
 45. L’Huillier AG, Hamid-Allie A, Kristjanson E, Papageorgiou L, Hung S, Wong CF, Stein DR, Olsha R, Goneau LW, Dimitrova K, Drobot M, Saffronetz D, Gubbay JB. 2017. Evaluation of Euroimmun anti-Zika virus IgM and IgG enzyme-linked immunosorbent assays for Zika virus serologic testing. *J Clin Microbiol* 55:2462–2471. <https://doi.org/10.1128/JCM.00442-17>.
 46. Granger D, Hilgart H, Misner L, Christensen J, Bistodeau S, Palm J, Strain AK, Konstantinovskii M, Liu D, Tran A, Theel ES. 2017. Serologic testing for Zika virus: comparison of three Zika virus IgM-screening enzyme-linked immunosorbent assays and initial laboratory experiences. *J Clin Microbiol* 55:2127–2136. <https://doi.org/10.1128/JCM.00580-17>.
 47. Kadkhoda K, Gretchen A, Racano A. 2017. Evaluation of a commercially available Zika virus IgM ELISA: specificity in focus. *Diagn Microbiol Infect Dis* 88:233–235. <https://doi.org/10.1016/j.diagmicrobio.2017.04.002>.

48. Sloan A, Safronetz D, Makowski K, Barairo N, Ranadheera C, Dimitrova K, Holloway K, Mendoza E, Wood H, Drebot M, Gretchen A, Kadkhoda K. 2017. Evaluation of the Diasorin Liaison XL Zika Capture IgM CMIA for Zika virus serological testing. *Diagn Microbiol Infect Dis* <https://doi.org/10.1016/j.diagmicrobio.2017.11.018>.
49. Centers for Disease Control and Prevention. 2017. Zika MAC-ELISA, for use under an emergency use authorization only: instructions for use. Centers for Disease Control and Prevention, Atlanta, GA. <https://www.fda.gov/downloads/MedicalDevices/Safety/EmergencySituations/UCM488044.pdf>.
50. Safronetz D, Sloan A, Stein DR, Mendoza E, Barairo N, Ranadheera C, Scharikow L, Holloway K, Robinson A, Traykova-Andonova M, Makowski K, Dimitrova K, Giles E, Hiebert J, Mogk R, Beddome S, Drebot M. 2017. Evaluation of 5 commercially available Zika virus immunoassays. *Emerg Infect Dis* 23:1577–1580. <https://doi.org/10.3201/eid2309.162043>.
51. Steinhagen K, Probst C, Radzinski C, Schmidt-Chanasit J, Emmerich P, van Esbroeck M, Schinkel J, Grobusch MP, Goorhuis A, Warnecke JM, Lattwein E, Komorowski L, Deerberg A, Saschenbrecker S, Stocker W, Schlumberger W. 2016. Serodiagnosis of Zika virus (ZIKV) infections by a novel NS1-based ELISA devoid of cross-reactivity with dengue virus antibodies: a multicohort study of assay performance, 2015 to 2016. *Euro Surveill* 21:30426. <https://doi.org/10.2807/1560-7917.ES.2016.21.50.30426>.
52. Lustig Y, Zelena H, Venturi G, Van Esbroeck M, Rothe C, Perret C, Koren R, Katz-Likovnik S, Mendelson E, Schwartz E. 2017. Sensitivity and kinetics of an NS1-based Zika virus enzyme-linked immunosorbent assay in Zika virus-infected travelers from Israel, the Czech Republic, Italy, Belgium, Germany, and Chile. *J Clin Microbiol* 55:1894–1901. <https://doi.org/10.1128/JCM.00346-17>.
53. Huzly D, Hanselmann I, Schmidt-Chanasit J, Panning M. 2016. High specificity of a novel Zika virus ELISA in European patients after exposure to different flaviviruses. *Euro Surveill* 21:30203. <https://doi.org/10.2807/1560-7917.ES.2016.21.16.30203>.
54. Centers for Disease Control and Prevention. 2016. Triplex real-time RT-PCR assay, for use under an emergency use authorization only: instructions for use. Centers for Disease Control and Prevention, Atlanta, GA. <https://www.fda.gov/downloads/MedicalDevices/Safety/EmergencySituations/UCM491592.pdf>.
55. Ren P, Ortiz DA, Terzian ACB, Colombo TE, Nogueira ML, Vasilakis N, Loeffelholz MJ. 2017. Evaluation of Aptima Zika virus assay. *J Clin Microbiol* 55:2198–2203. <https://doi.org/10.1128/JCM.00603-17>.
56. Olschlager S, Enfissi A, Zaruba M, Kazanji M, Rousset D. 2017. Diagnostic validation of the RealStar Zika virus reverse transcription polymerase chain reaction kit for detection of Zika virus RNA in urine and serum specimens. *Am J Trop Med Hyg* 97:1070–1071. <https://doi.org/10.4269/ajtmh.17-0268>.
57. L'Huillier AG, Lombos E, Tang E, Perusini S, Eshaghi A, Nagra S, Frantz C, Olsha R, Kristjanson E, Dimitrova K, Safronetz D, Drebot M, Gubbay JB. 2017. Evaluation of Altona Diagnostics RealStar Zika virus reverse transcription-PCR test kit for Zika virus PCR testing. *J Clin Microbiol* 55:1576–1584. <https://doi.org/10.1128/JCM.02153-16>.
58. Frankel MB, Pandya K, Gersch J, Siddiqui S, Schneider GJ. 2017. Development of the Abbott RealTime Zika assay for the qualitative detection of Zika virus RNA from serum, plasma, urine, and whole blood specimens using the m2000 system. *J Virol Methods* 246:117–124. <https://doi.org/10.1016/j.jviromet.2017.05.002>.
59. Musso D, Rouault E, Teissier A, Lanteri MC, Zisou K, Brout J, Grange E, Nhan T, Aubry M. 2017. Molecular detection of Zika virus in blood and RNA load determination during the French Polynesian outbreak. *J Med Virol* 89:1505–1510. <https://doi.org/10.1002/jmv.24735>.
60. Mansuy JM, Mengelle C, Pasquier C, Chapuy-Regaud S, Delobel P, Martin-Blondel G, Izopet J. 2017. Zika virus infection and prolonged viremia in whole-blood specimens. *Emerg Infect Dis* 23:863–865. <https://doi.org/10.3201/eid2305.161631>.
61. St. George K, Pinsky BA. 2018. Molecular diagnosis of Zika virus infections. *Rev Med Microbiol* 29:8–16.
62. Bingham AM, Cone M, Mock V, Heberlein-Larson L, Stanek D, Blackmore C, Likos A. 2016. Comparison of test results for Zika virus RNA in urine, serum, and saliva specimens from persons with travel-associated Zika virus disease: Florida, 2016. *MMWR Morb Mortal Wkly Rep* 65:475–478. <https://doi.org/10.15585/mmwr.mm6518e2>.
63. Brasil P, Pereira JP, Jr, Moreira ME, Ribeiro Nogueira RM, Damasceno L, Wakimoto M, Rabello RS, Valderramos SG, Halai UA, Salles TS, Zin AA, Horovitz D, Daltro P, Boechat M, Raja Gabaglia C, Carvalho de Sequeira P, Pilotto JH, Medialdea-Carrera R, Cotrim da Cunha D, Abreu de Carvalho LM, Pone M, Machado Siqueira A, Calvet GA, Rodrigues Baiao AE, Neves ES, Nassar de Carvalho PR, Hasue RH, Marschik PB, Einspieler C, Janzen C, Cherry JD, Bispo de Filippis AM, Nielsen-Saines K. 2016. Zika virus infection in pregnant women in Rio de Janeiro. *N Engl J Med* 375:2321–2334. <https://doi.org/10.1056/NEJMoa1602412>.
64. Tan SK, Sahoo MK, Milligan SB, Taylor N, Pinsky BA. 2017. Stability of Zika virus in urine: specimen processing considerations and implications for the detection of RNA targets in urine. *J Virol Methods* 248:66–70. <https://doi.org/10.1016/j.jviromet.2017.04.018>.
65. Landry ML, St. George K. 2017. Laboratory diagnosis of Zika virus infection. *Arch Pathol Lab Med* 141:60–67. <https://doi.org/10.5858/arpa.2016-0406-SA>.
66. Atkinson B, Thorburn F, Petridou C, Bailey D, Hewson R, Simpson AJ, Brooks TJ, Aarons EJ. 2017. Presence and persistence of Zika virus RNA in semen, United Kingdom, 2016. *Emerg Infect Dis* 23:611–615. <https://doi.org/10.3201/eid2304.161692>.
67. Pyke AT, Daly MT, Cameron JN, Moore PR, Taylor CT, Hewitson GR, Humphreys JL, Gair R. 2014. Imported Zika virus infection from the Cook Islands into Australia, 2014. *PLoS Curr* <https://doi.org/10.1371/currents.outbreaks.4635a54dbffa2156fb2fd76dc49f65e>.
68. Garcia-Bujalance S, Gutierrez-Arroyo A, De la Calle F, Diaz-Mendez M, Arribas JR, Garcia-Rodriguez J, Arsuaga M. 2017. Persistence and infectivity of Zika virus in semen after returning from endemic areas: report of 5 cases. *J Clin Virol* 96:110–115. <https://doi.org/10.1016/j.jcv.2017.10.006>.
69. Schaub B, Vouga M, Najjioullah F, Gueneret M, Montheux A, Harte C, Muller F, Jolivet E, Adenet C, Dreux S, Leparac-Goffart I, Cesaire R, Volumentie JL, Baud D. 2017. Analysis of blood from Zika virus-infected fetuses: a prospective case series. *Lancet Infect Dis* 17:520–527. [https://doi.org/10.1016/S1473-3099\(17\)30102-0](https://doi.org/10.1016/S1473-3099(17)30102-0).
70. Colt S, Garcia-Casal MN, Pena-Rosas JP, Finkelstein JL, Rayco-Solon P, Weise Prinzo ZC, Mehta S. 2017. Transmission of Zika virus through breast milk and other breastfeeding-related bodily-fluids: a systematic review. *PLoS Negl Trop Dis* 11:e0005528. <https://doi.org/10.1371/journal.pntd.0005528>.
71. Shukla S, Hong SY, Chung SH, Kim M. 2016. Rapid detection strategies for the global threat of Zika virus: current state, new hypotheses, and limitations. *Front Microbiol* 7:1685. <https://doi.org/10.3389/fmicb.2016.01685>.
72. Ganguli A, Ornob A, Yu H, Damhorst GL, Chen W, Sun F, Bhuiya A, Cunningham BT, Bashir R. 2017. Hands-free smartphone-based diagnostics for simultaneous detection of Zika, Chikungunya, and Dengue at point-of-care. *Biomed Microdevices* 19:73. <https://doi.org/10.1007/s10544-017-0209-9>.