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Laboratory Diagnosis of Chikungunya Virus Infections and Commercial Sources for Diagnostic Assays

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Abstract

Detection of chikungunya virus (CHIKV) or viral RNA is the primary laboratory test used to diagnose infection in serum collected <6 days after onset of illness. Two real-time reverse transcription–polymerase chain reaction (RT-PCR) kits are available commercially, but validity data are limited. There are 2 commercial sources of inactivated positive-control CHIKV RNA to be used with purchased primers. The Centers for Disease Control and Prevention provides viral RNA–positive controls and primer and probe nucleotide sequences for real-time RT-PCR testing. Detection of CHIKV-specific immunoglobulin M (IgM) antibody becomes a sensitive test for samples collected approximately >5 days of illness. Commercially available CHIKV IgM–detection assays include lateral flow rapid tests, IgM antibody capture enzyme-linked immunosorbent assays (MAC-ELISAs), and indirect immunofluorescence tests. Nine commercial CHIKV IgM detection assays were evaluated at 3 reference laboratories to provide guidance to public health diagnostic laboratories on their performance parameters. Sensitivity of the rapid tests and 3MAC-ELISAs was <50%, and thus these assays are not recommended. Three of the MAC-ELISA kits and 1 indirect immunofluorescence kit had comparable performance to the reference assays. In summary, commercial assays with performance comparable to reference assays are available for molecular and serological diagnosis of CHIKV infections.

Keywords

Chikungunya virus; arbovirus diagnostic testing; real-time RT-PCR; IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA)

The introduction of Chikungunya virus (CHIKV) to the Caribbean in 2013 resulted in large outbreaks throughout the Americas [1–3]. The clinical similarity of CHIKV to other arbovirus infections makes diagnosis difficult on the basis of clinical symptoms alone, particularly as arboviruses such as dengue virus and now Zika virus may cocirculate in the

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same geographical regions and are transmitted by the same mosquito species [4–9]. Expansion and endemicity of CHIKV is likely, and large outbreaks of CHIKV infection may continue for the foreseeable future. CHIKV infection was classified as a reportable disease in the United States in January 2015. Public health laboratories throughout the Americas will need to build and maintain high-volume diagnostic testing capacity and will need validated and reliable commercial CHIKV diagnostic assays to respond to these increased diagnostic testing responsibilities.

Laboratory diagnosis of CHIKV infection is accomplished by serologic methods, virus isolation, and viral RNA detection by reverse transcription–polymerase chain reaction (RT-PCR). The testing algorithm developed at the Center for Disease Control and Prevention (CDC) to diagnose CHIKV infections is based on characteristics of CHIKV infection and the timing of specimen collection (Figure 1). CHIKV replicates rapidly to high titers in the host, and viral RNA generally can be detected by real-time RT-PCR in the first week after onset of clinical illness. Previously, in travelers to India who had been infected with CHIKV, viral RNA was detectable by real-time RT-PCR in samples collected out to 8 days after onset of illness [10]. Immunoglobulin M (IgM) antibodies elicited in the immune response are normally detectable in serum by days 5–7 after onset of illness. In the study of India travelers, CHIKV-specific IgM was detected in 1 patient on the day of illness onset but was not detectable in the rest until 9 days after onset of illness [10].

In the CHIKV testing algorithm developed by the CDC arbovirus diagnostic laboratory, samples collected <6 days after onset of illness are first tested by CHIKV real-time RT-PCR (Figure 2). Nucleotide sequences of 2 sets of primers and probes are listed in Table 1 [10–12]. The 3855 primer/probe set was designed to detect the East Central South African (ECSA) genotypes in travelers returning from India, and is broadly reactive, detecting both Asian and ECSA genotypes [10]. The 856 primer/ probe set is designed specifically for the Asian genotype CHIKV strain currently in the Caribbean and is slightly more sensitive for the Asian genotype than the 3855 set. Because of the higher sensitivity, the 856 set is useful for confirming positive results for specimens with low CHIKV RNA levels. Each laboratory should keep these specificity and sensitivity data in mind when designing an appropriate testing algorithm (ie, which sets to use and in what order). Specimens with positive results of tests that use both sets of primers and probes are considered to be confirmed CHIKV-positive specimens. If any of the negative controls test positive, the entire run is invalidated. Failure of the positive control to generate a positive result also invalidates the entire run.

At the CDC, samples collected on or after day 6 of illness and samples with negative real-time RT-PCR results are tested by the CHIKV IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA) [13, 14]. Positive MAC-ELISA results are confirmed by the 90% plaque reduction neutralization test [10, 15]. A sample with positive or equivocal MAC-ELISA results with a neutralizing titer is classified as a confirmed CHIKV-positive sample; those with negative results of plaque reduction neutralization testing are considered to have nonspecific reactivity (ie, to be negative for CHIKV).

There are numerous commercial molecular and serological CHIKV diagnostic assays available; those available as of June 2015 are listed in Table 2. Performance of the RealStar

Chikungunya RT-PCR Kit (Altona Diagnostics, Hamburg, Germany) was assessed by Panning et al in the premarket format and was found to have a sensitivity (approximately 5.3 RNA copies/ reaction) and specificity comparable to that of the in-house assay [16]. The product handbook of the genesig Chikungunya Nonstructural protein 2 standard (nsp2) RT-PCR kit (Primer-design, Southampton, United Kingdom) states that the test is sensitive to <100 RNA copies of target; however, no validity studies have been published in the literature. Inactivated CHIKV RNA–positive controls, to be used with published, validated primers and probes, are available through Vircell Microbiologists (Granada, Spain) and Zeptometrix (Buffalo, New York). Both positive-control RNAs, produced from CHIKV ECSA genotype strains, are detectable by the CDC primer/probe sets in Table 1 and the article by Lanciotti et al [10] (data not shown). RNA lysate positive controls prepared from an Indian (ECSA genotype) or Caribbean (Asian genotype) isolate are available from the CDC upon request (available at: http://www.cdc.gov/ncezid/dvbd/specimensub/index.html).

External evaluations of the CHIKV IgM detection assays listed in Table 2 have primarily focused on the rapid tests, as they are attractive options for testing at the point of care and/or elsewhere in resource-limited settings, where CHIKV outbreaks have primarily occurred [17–22] However, the performance of the rapid tests has been disappointing owing to low sensitivity. Therefore, to provide guidelines and recommendations to diagnostic laboratories on the use of these commercial assays, a comprehensive side-by-side comparison of the available (as of July 2015) commercial serological assays was performed at the CDC, the Public Health Agency of Canada National Microbiology Laboratory (NML), and the Caribbean Public Health Agency (CARPHA) in Trinidad-Tobago. With the exception of the CHIK IgM micro-capture ELISA kit manufactured by IBL International, all the CHIKV IgM detection assays listed in Table 2 were evaluated at the CDC, with a reference panel of 70–90 archived, well-characterized sera from CHIK outbreaks in Yap and the Caribbean and from travelers to India and the Philippines that had been submitted to the CDC arbovirus diagnostic laboratory. Positive control sera containing IgM to other alpha-viruses (Venezuelan equine encephalitis, eastern equine encephalitis, O′nyong-nyong, and Mayaro viruses) and other arboviruses (dengue virus) were included in the sample panel to calculate specificity. An in-house positive control was included in every run. At the NML, the performance of the Euroimmun ELISA was compared to in-house assays, using samples submitted to the NML for diagnostic testing from travelers to the Caribbean and other regions where CHIKV is circulating. CARPHA evaluated 4 kits (Abcam, Inbios, and Euroimmun MAC-ELISAs and the Euroimmun indirect immunofluorescence test [IIFT]), using samples submitted from the CARPHA member countries in the Caribbean that had been sent to the CDC for testing. The results of these evaluations have been published elsewhere and are not reported here [23]. However, conclusions of the CDC evaluations are as follows. The Euroimmun and Inbios CHIKV MAC-ELISA kits had the highest accuracy (99% and 100%, respectively) and reproducibility. The Euroimmun IIFT also had high performance (96% accuracy), but a technician skilled in immunofluorescence assay techniques is required, and a higher proportion of samples had equivocal results, necessitating retesting. There was considerable lot-to-lot variation with the Abcam ELISA kits. The initial Abcam kits evaluated had results highly concordant with CDC results; other lots had low sensitivity when used to test the same samples. All runs of all lots were valid

according to the criteria in the kit instructions (ie, the kit positive and cutoff control ODs were within the acceptable range). The CDC notified Abcam of the lot-to-lot variability in performance and the problem with the assay controls, after which the manufacturer replaced the controls with human serum containing CHIKV-specific IgM and improved quality control and quality assurance procedures. Because the kit components were modified, the reformatted Abcam ELISA kit needed to be re-evaluated at the CDC. The reformatted Abcam kits were evaluated with as many of the same samples as had been included in the previous Abcam kit evaluations; results were 99% concordant with CDC results. The 2 rapid tests and the remaining plate-based CHIKV MACELISA kits all had unacceptably low performance (sensitivity, <50%), including false-negative results with the CDC in-house positive control serum. Thus, the Inbios and Euroimmun MAC-ELISAs and the Euroimmun IIFT have performance comparable to the reference standard CHIKV MAC-ELISA [13, 14]. The rereleased Abcam MAC-ELISA containing CHIKV-specific IgM human serum controls also has high performance. However, any further changes to the composition of the Abcam kit will invalidate these results and necessitate reevaluation.

In summary, there are numerous validated, commercially manufactured molecular and serological CHIKV diagnostic assays available from which laboratories can choose. The CDC also provides CHIKV RNA–positive controls and primer/probe nucleotide sequences for real-time RT-PCR testing. Nine of 10 commercial CHIKV IgM detection assays were evaluated at the CDC, but only the Inbios and Euroimmun MAC-ELISAs, the Euroimmun IIFT, and the reformatted Abcam MAC-ELISA had acceptable performance. The 2 rapid tests and the 3 remaining plate-based CHIKV MAC-ELISA kits lacked sensitivity and are not recommended for use in their current format. Information on kit prices, the availability of the kits in the United States, and whether the kits have received Food and Drug Administration approval is not provided here, as this information is continually changing and would quickly become outdated and inaccurate.

The CDC CHIKV diagnostic testing algorithm shown in Figure 2 can be used to guide CHIKV testing at public health laboratories. Laboratories with only real-time RT-PCR or MAC-ELISA capacity should test all received samples at all days after onset of illness with the test that they have. Negative results should be interpreted as inconclusive, and the laboratory should consider sending the sample to a reference laboratory where comprehensive testing can be done.

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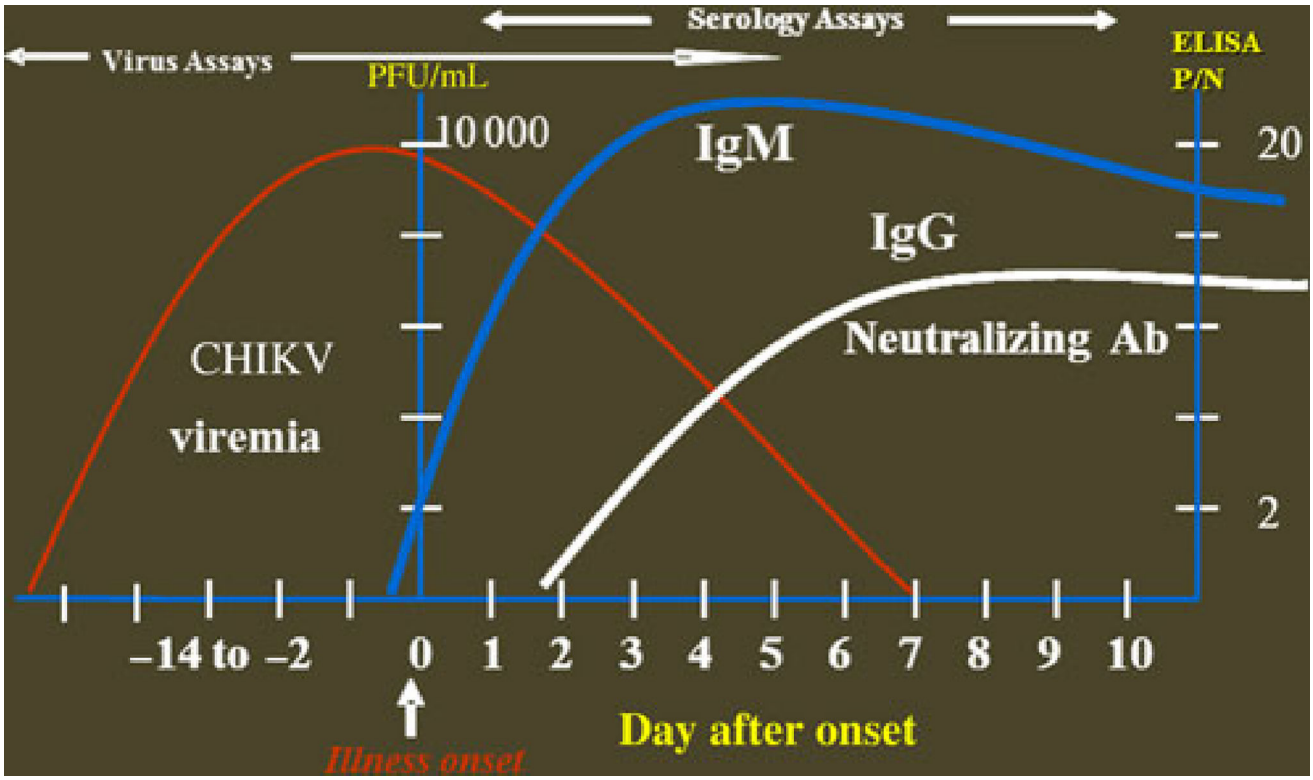

Figure 1.

Time course of chikungunya virus (CHIKV) viremia and immune response. Limit of detection (LOD) of real-time reverse transcription–polymerase chain reaction (RT-PCR) assay is approximately 100 plaque-forming units (PFU)/mL (approximately 1 RNA transcript/reaction); the LOD of the immunoglobulin M (IgM) antibody capture enzyme-linked immunosorbent assay (ELISA) positive-to-negative ratio (P/N) is >2. Abbreviations: Ab, antibody; IgG, immunoglobulin G.

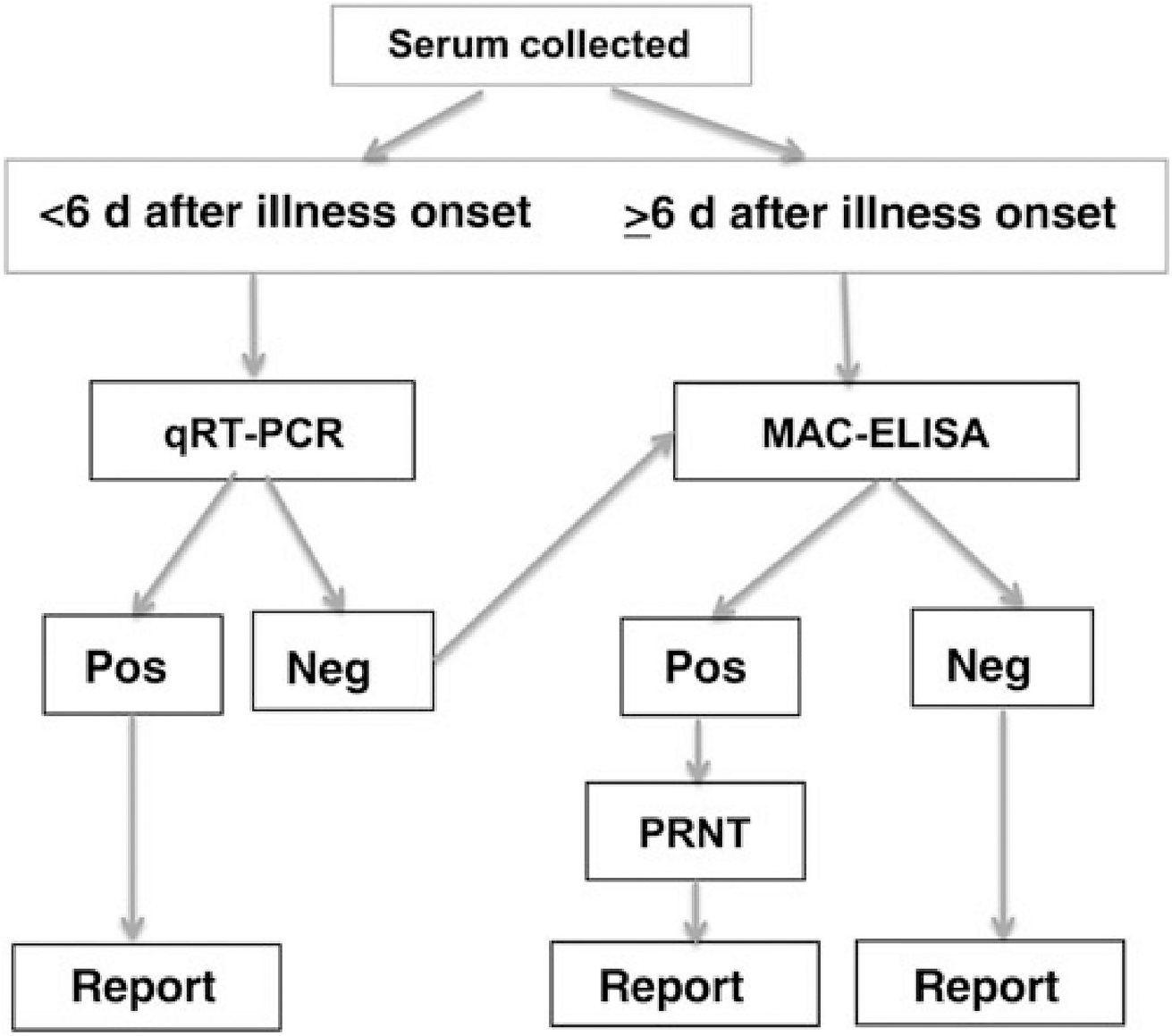

Figure 2.

The Centers for Disease Control and Prevention diagnostic testing algorithm for detection of chikungunya virus (CHIKV) infection. Serum is collected from patients meeting the clinical case definition of fever and arthralgia who have returned from a region where CHIKV is endemic or CHIKV infection is epidemic. All samples with positive and equivocal real-time quantitative reverse transcription–polymerase chain reaction (qRT-PCR) results are repeated with a second set of primer/ probes for confirmation. Specimens with positive results of tests using both sets of primers and probes are considered to be confirmed CHIKV-positive specimens. Samples with positive or equivocal immunoglobulin M capture enzyme-linked immunosorbent assay (MAC-ELISA) results are confirmed to be positive if plaque reduction neutralizing testing (PRNT) yields positive results. Abbreviations: Neg, negative result; Pos, positive result.

Table 1

Chikungunya Virus (CHIKV) Oligonucleotide Primers and Probes Used in Centers for Disease Control and Prevention Real-time Reverse Transcription–Polymerase Chain Reaction Assays Designed to Detect CHIKV Asian Genotype Strains

Primer/Genome 5′ Position	Nucleotide Sequence
CHIKV3855F	GAGCATACGGTTACGCAGATAG
CHIKV3957C[a]	TACTGGTGATACATGGTGGTTTC + TGCTGGTGACACATGGTGGTTTC
CHIKV3886FAM (probe)[a]	ACGAGTAATCTGCGTACTGGGACGTA + ACGAGTCATCTGCGTATTGGGACGCA
CHIK856F	ACCATCGGTGTTCCATCTAAAG
CHIK962C	GCCTGGGCTCATCGTTATT
CHIK908FAM (probe)	ACAGTGGTTTCGTGTGAGGGCTAC

The 3855 primer/probe set is broadly reactive, detecting both Asian and the East/Central/South African genotypes. The 856 set is designed specifically for the Asian genotype CHIKV strain currently in the Caribbean and is slightly more sensitive for the Asian genotype than the 3855 set. The threshold of detection of both sets of primer/probes is approximately 1 RNA transcript.

[a]Primer CHIKV3957C is produced by mixing equal volumes of the 100 μM stock solutions of the 2 individual primers; the CHIKV3886FAM probe is an equal mixture of the 25 μM individual probe stocks.

Table 2

Sources of Commercially Manufactured Chikungunya Virus (CHIKV) Diagnostic Assays as of June 2015

Manufacturer	Location	Assay Name and Format	Reference	Samples/Tests per Kit, No.
CHIKV real-time RT-PCR kit				
Altona Diagnostics	Germany	RealStar Chikungunya RT-PCR Kit	011013	96 reactions
Primerdesign	United Kingdom	genesig Chikungunya Non structural protein 2 standard (nsp2) RT-PCR kit	CHIKV-EASY	150 reactions
CHIK RNA positive control (inactivated)				
Vircell	Spain	Amplirun CHIKV RNA control (strain S27)	MBC099	Approximately 1000 runs
Zeptometrix	USA	NATtrol inactivated virus (strain India 2006)	NATCHIKV-ST	Approximately 200 runs
Plate MAC-ELISA				
IBL International	Germany	CHIK IgM micro-capture ELISA	RE58841	91
CTK Biotech	USA/China	RecombiLISA CHIK IgM Test	E0315	91
Genway	Germany	CHIKV IgM μ-capture ELISA	40-521-475066	91
Abcam	Germany	Anti-CHIKV IgM human ELISA kit	ab177848	91
SD Diagnostics	Korea	CHIKa IgM ELISA	16EK10	91
Euroimmun	Germany	Anti-CHIKV ELISA (IgM)	EI293a-9601M	93
Inbios	USA	CHIKjj Detect MAC-ELISA	CHKM-C	92
CHIKV IgM detection rapid test				
CTK Biotech	USA	On-site CHIK IgM Combo Rapid test	R0066C	30
SD Diagnostics	Korea	SD BIOLINE Chikungunya IgM	46FK10	25
CHIKV IgM immunofluorescence assay				
Euroimmun	Germany	Anti-CHIKV IIFT (IgM)	FI293a-1010 G/M	50

Abbreviations: IgM, immunoglobulin M; IIFT, indirect immunofluorescence test; MAC-ELISA, IgM antibody capture enzyme-linked immunosorbent assay; RT-PCR, reverse transcription–polymerase chain reaction.