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Poor Diagnostic Accuracy of Commercial Antibody-Based Assays for the Diagnosis of Acute Chikungunya Infection▽‖

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A Sri Lankan fever cohort ($n = 292$ patients; 17.8% prevalence) was used to assess two standard diagnostic Chikungunya IgM tests. The immunochromatographic test (ICT) acute sample sensitivity (SN) was 1.9 to 3.9%, and specificity (SP) was 92.5 to 95.0%. The enzyme-linked immunosorbent assay (ELISA) gave an acute sample SN of 3.9% and an SP of 92.5% and a convalescent sample SN of 84% and an SP of 91%. These assays are not suitable for the acute diagnosis of Chikungunya virus infection.

Chikungunya virus infection, a reemerging vector-borne disease, is an important cause of acute febrile illness in the tropics among both residents and travelers (7, 12). In this study, we retrospectively evaluated two commercial IgM antibody-based assays for utility in the diagnosis of acute Chikungunya infection on the basis of a fever patient cohort from Sri Lanka, where a Chikungunya virus epidemic occurred in 2006 and 2007 (4).

Patient samples were collected during the Ragama Fever Study conducted at the North Colombo Teaching Hospital, Sri Lanka, during June 2006 to June 2007 in an adult (≥16 years), febrile (≥38°C) patient cohort. Ethical clearance was granted by the University of Kelaniya in Sri Lanka, the Liverpool School of Tropical Medicine in the United Kingdom, and the Walter Reed Army Institute of Research in the United States. All patients gave informed written consent. Venous blood samples were collected on the day of admission (admission specimen) and where possible at discharge and follow-up 2 weeks later (convalescent specimens).

The two assays evaluated in this study were a Chikungunya IgM antibody rapid immunochromatographic test (ICT) device and a Chikungunya IgM antibody enzyme-linked immunosorbent assay (ELISA) manufactured by Standard Diagnostics (SD; Standard Diagnostics, South Korea). Assays were performed according to the manufacturers' instructions. The ICT was tested with acute specimens only, as would be the case in clinical practice, and the ELISA was tested with both acute and convalescent specimens. Three experienced operators read ICTs individually without conferring. Gold standard Chikungunya reference testing was performed at the Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand, and included testing for the presence of Chikungunya virus antibodies using the hemagglutination inhibition (HI) method (2) (≥1:10 dilution was considered positive) and the AFRIMS IgM antibody capture ELISAs (≥30 units was considered positive) and reverse transcription (RT)-PCR (8, 13). Samples that gave a positive result in one or more of the assays were considered positive for Chikungunya infection. All samples were labeled using a code that was devoid of personal identifiers. Non-Chikungunya reference testing was performed at AFRIMS (dengue) or Mahidol University—Oxford Tropical Medicine Research Unit (MORU) (rickettsial illnesses and leptospirosis) using previously described methods (1). Diagnostic accuracy was calculated for the Chikungunya rapid tests using the results of all three operators relative to the final patient diagnosis based on the results of reference testing. Equivocal ICT results were determined to be negative for the diagnostic accuracy evaluation. Diagnostic accuracy indices of sensitivity, specificity, negative predictive values (NPV), and positive predictive values (PPV) with exact 95% confidence intervals (CI) and interquartile ranges (IQR) of the number of days of fever and interrater Kappa values testing for significant differences between the readers ($P \leq 0.05$) were calculated using Stata/SE 10.0 (Stata Corp., College Station, TX).

Paired serum samples from 292 patients were examined in total. The median numbers of days of fever prior to collection was 5 (IQR, 3 to 7) days for the acute sample and 24 (IQR, 19 to 30) days for the convalescent sample. Based on the reference methods, 17.8% (52/292) of patients had a final diagnosis

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TABLE 1. Overall diagnostic accuracies and sensitivities of different diagnostic assays for the detection of IgM antibodies and RNA in patients with confirmed Chikungunya virus infections[a]

Diagnostic assay	Type of sample (n = 292)	% (no. of samples/total no. of samples; 95% CI)			
		Sensitivity	Specificity	PPV	NPV
SD IgM antibody ELISA	Acute	3.9 (2/52; 0–13)	92.5 (222/240; 88–96)	10.0 (2/10; 1–32)	81.6 (222/272; 77–86)
	Convalescent	84.1 (44/52; 72-93)	91.3 (219/240; 87–95)	67.7 (44/65; 55–79)	96.5 (219/227; 93–99)
SD IgM antibody ICT	Acute, operator 1	1.9 (1/52; 0–10)	92.5 (222/240; 88–96)	5.3 (1/19; 0–26)	81.3 (222/273; 76–86)
	Acute, operator 2	3.9 (2/52; 0–13)	94.2 (226/240; 90–97)	12.5 (2/16; 2–38)	81.9 (226/226; 77–86)
	Acute, operator 3	3.9 (2/52; 0–13)	95.0 (228/240; 91–97)	14.3 (2/14; 2–43)	82.0 (228/278; 77–86)
AFRIMS HI	Acute	21.2 (11/52; 11–35)	95.0 (228/240; 92–98)	47.8 (11/23; 27–69)	84.8 (228/269; 80-89)
AFRIMS IgM ELISA	Acute	21.2 (11/52; 11–35)	95.4 (229/240; 92–98)	50.0 (11/22; 28–72)	84.8 (229/270; 80–89)
AFRIMS RT-PCR	Acute	88.5 (46/52; 77–96)	100 (0/240; 99–100)	100 (46/46; 92–100)	97.5 (238/244; 95–99)

[a] Prevalence (reference methods), 17.8% (52/292 patients).

of acute Chikungunya infection. Of the non-Chikungunya cases (n = 240), dengue (28.8%; 69/240 cases) was the most common illness diagnosed in this cohort.

The ICT was used only to test acute samples. The sensitivity and specificity for the ICT ranged from 1.9 to 3.9% and 92.5 to 95.0% (Table 1), respectively, for the three operators. The overall kappa value for the three operators was 0.78 ($P \leq$ 0.0005), and for those patients with and without confirmed Chikungunya infections, the kappa values were 0.78 and 0.79, respectively.

The sensitivity and specificity of the SD IgM ELISA for acute samples were 4% (2/52 samples; 95% CI, 0.5 to 13%) and 92% (95% CI, 88 to 96%), and for follow-up samples they were 84% (44/52 samples; 95% CI, 72 to 93%) and 91% (95% CI, 87 to 95%) (Table 1). By comparison, the AFRIMS IgM ELISA gave a sensitivity and specificity of 21% (11/52 samples; 95% CI, 11 to 35%) and 95% (95% CI, 92 to 98%), respectively, for acute specimens.

Given the high proportion of viremic patients on admission with a positive RT-PCR result (46/292; 15.8%), the poor diagnostic performance of these assays could be attributable to low levels of IgM in patients presenting with acute Chikungunya infection. This is supported by the marked increase of sensitivity of the SD IgM ELISA for follow-up samples, to 84% (95% CI, 72 to 93%), while maintaining similar specificity of 91% (95% CI, 87 to 95%). The SD IgM ELISA demonstrated increasing positivity rates over time and approached 50% and 80% positivity at approximately days 10 and 18 after the onset of illness, respectively, although the AFRIMS IgM was more sensitive in the acute phase of infection (Table 2).

In order to make a reliable diagnosis of Chikungunya infection in the acute clinical setting, the assay should detect pathogen-specific analytes (RNA, viral antigen, specific antibodies) across the entire period of patient presentation. In this study, patient presentation was at a median of 5 days of fever/illness (range, 3 to 8 days), and both evaluated assays demonstrated poor (<4%) sensitivities. Comparatively, the AFRIMS RT-PCR approached 90% sensitivity for the same sampling period (Tables 1 and 2), which is strong evidence that the diagnostic accuracy of RT-PCR assays targeting pathogen genes is clinically acceptable for the diagnosis of acute Chikungunya infection up to 7 days of illness and is superior to antibody-based technologies (11). Unfortunately, however, the availability of the RT-PCR methodology is limited in low-resource settings where this disease is commonly found. There is an urgent need to design and evaluate simple Chikungunya RNA or antigen-based detection assays, such as loop-amplified (5, 6, 10) or antigen-capture ELISA (9) technologies in field settings.

While the antibody-based assays evaluated here are not suited for acute diagnosis, the ELISA may have sufficient sensitivity and specificity for use in seroepidemiological surveys. Also, convalescent specimens from patients with Chikungunya infections demonstrated a sensitivity approaching >85% and a specificity of >90%. The differential longevity of the anti-Chikungunya IgM and IgG antibody responses suggest that it may be useful to incorporate IgG antibody detection into the test for epidemiological purposes (3).

The results presented here demonstrate the importance of pathogen-immune response dynamics in the diagnostic accuracy of Chikungunya infections and of documenting the timing of sample collection (number of days of fever/illness). This study also highlights the importance of the development of new antigen/RNA-based commercial diagnostic assays for rapid and reliable clinical diagnosis on admission, as the majority of patients presenting at this stage are still in the viremic phase.

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TABLE 2. The effect of time (number of days of illness) on SD Chikungunya IgM antibody ELISA and ICT and AFRIMS reference assays

No. of days of fever	No. of patients	% positivity of Chikungunya infection-confirmed patients				
		SD IgM antibody ELISA	SD IgM antibody ICT	AFRIMS RT-PCR	AFRIMS IgM ELISA	AFRIMS HI
1	1	0	0	100	0	0
2	13	0	0	100	8	8
3	12	0	0	92	0	0
4	13	8	0	100	15	15
5	2	0	0	0	50	50
6	4	0	0	50	50	50
7	1	0	0	100	100	100
>7	6	17	17	83	67	67

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting true views of the United States Department of the Army or the Department of Defense. Standard Diagnostics provided the assays for evaluation purposes free of charge and did not play a role in the design of the study or have access to the manuscript prior to submission for publication. The authors have no personal conflicts of interest.

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