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Performance of the RealStar Chikungunya Virus Real-Time Reverse Transcription-PCR Kit▽

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A novel commercial Chikungunya virus real-time reverse transcription-PCR (RT-PCR) kit was evaluated on a comprehensive panel of original patient samples. The assay was 100% sensitive and specific in comparison to a published real-time RT-PCR. Viral loads from both assays were highly correlated. The kit proved to be suitable for routine use in patient care.

Since 2005, an epidemic of Chikungunya fever has been ongoing in the Indian Ocean region (2). The disease is caused by Chikungunya virus (CHIKV), an arthropod-borne RNA virus endemic in parts of sub-Saharan Africa and Southeast Asia. Along with the current Indian Ocean epidemic, hundreds of imported cases have been reported worldwide (5, 7). In humans, CHIKV infection is characterized by acute febrile illness, rash, and severe polyarthralgia (3, 11). The clinical picture can mimic that of dengue fever or malaria, which are often coendemic (9). Definite laboratory diagnosis of CHIKV is therefore crucial for case management and prognostic appreciation. The viremic phase in infected patients is short-lived, and sensitive methods are required for accurate diagnosis. Pilot studies have shown that reverse transcription-PCR (RT-PCR) assays are reliable in diagnosing acute infections (7). Concomitantly from a public health perspective, active surveillance is of importance to contain epidemics (10). However, laboratory methods for CHIKV were not widely available before the current epidemic. We have recently demonstrated that the provision of a preformulated RT-PCR assay could significantly improve the performance of laboratories in diagnosing CHIKV infection (6). This preformulated real-time RT-PCR provided desirable features similar to other assays commercially available for more prevalent viruses, including quality-controlled oligonucleotides and RNA standards.

Despite the apparent lack of diagnostic tools, the industry did not prioritize for a long time on the development of CHIKV test kits. In this study, we did the first premarket evaluation of a commercial CHIKV real-time RT-PCR kit and compared it with a published CHIKV real-time RT-PCR assay on a comprehensive panel of CHIKV patient plasma samples.

The RealStar CHIKV RT-PCR kit (astra Diagnostics, Hamburg, Germany) used a target region in the nonstructural protein (nsP-1) gene similar to that of our previously evaluated in-house assay (7). The published oligonucleotides were updated upon the latest gene bank entries as of 2008. The RealStar kit claimed to provide detection of all three CHIKV genotypes (West African, East/Central African, Asian) upon addition of auxiliary oligonucleotides. Two versions of the assay were available for evaluation. One was specified for LightCycler 1.1, 1.2, 1.5, and 2.0 (Roche) and the other for ABI Prism 7000/7500 (Applied Biosystems), LightCycler 480 (Roche), and RotorGene 600 (Corbett-Research) instruments, respectively. Both versions included noncompetitive internal controls along with four external RNA standards for quantification purposes (the underlying in-house assay had a competitive internal control) (7).

In the first step, the analytical sensitivity of the commercial RT-PCR kit was determined by probit analysis, using in vitro-transcribed RNA copies of a fragment of the CHIKV nsP-1 gene of CHIKV Indian Ocean strain 899, isolated in 2006 (GenBank accession number FJ959103) (7). The 95% limit of detection (LOD) was 5.3 RNA copies per RT-PCR (95% confidence interval [CI], 4.0 to 8.0 RNA copies/RT-PCR) with the LightCycler 2.0 and 3.8 RNA copies/RT-PCR (95% CI, 2.8 to 6.1 RNA copies/RT-PCR) with the LightCycler 480, respectively. This corresponded to a projected 190 and 137 copies of viral RNA per milliliter of plasma, respectively. The LOD was further assessed using plaque-purified and plaque-quantified CHIKV (strain 899) spiked at different concentrations into human plasma negative for CHIKV. Viral RNA was extracted by means of a viral RNA mini kit (Qiagen, Hilden, Germany) as recommended by the manufacturer. The 95% LOD was determined to be 0.51 PFU/ml (95% CI, 0.31 to 2.11 PFU/ml) with the LightCycler 2.0 and 0.34 PFU/ml (95% CI, 0.22 to 0.76 PFU/ml) with the LightCycler 480, respectively. Specificity was assessed by testing a panel of undiluted tissue culture supernatants of reference strains of different alphaviruses. Neither of the commercial test kits yielded a positive result with any of the following non-CHIKV alphaviruses: Ross River virus,

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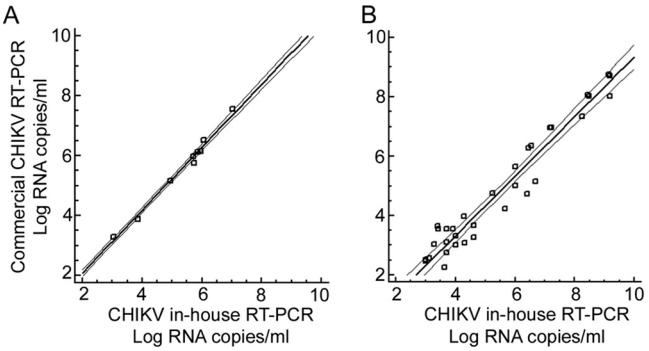

FIG. 1. Correlation curves of CHIKV RNA copies/ml obtained after comparison between commercial RT-PCR and expected results of EQA study and in-house real-time RT-PCR assay. (A) Correlation of CHIKV RNA copies/ml determined by commercial CHIKV RT-PCR (*x* axis) and expected CHIKV RNA copies/ml of EQA study as determined by in-house RT-PCR ($n = 9$) (*y* axis). (B) Correlation of CHIKV RNA copies/ml determined by commercial CHIKV RT-PCR (*x* axis) and RNA concentrations by in-house RT-PCR ($n = 35$) (*y* axis).

Semliki Forest virus, Sindbis virus, Eastern equine encephalitis virus, Western equine encephalitis virus, Venezuelan equine encephalitis virus, Mayaro virus, O'nyong-nyong virus, and Barmah Forest virus. In addition, both versions of the commercial kit did not react with RNA extracted from tissue culture supernatants of lymphocytic choriomeningitis virus, hepatitis C virus, highly pathogenic avian influenza virus (H5N1), Japanese encephalitis virus, dengue virus types 2 and 4, yellow fever virus, and West Nile virus. Specificity was further assessed on a panel of clinical samples ($n = 25$) of patients with fevers, obtained from a large outpatient department at the University of Bonn Medical Centre. None of these patients reported a recent travel history. As expected, all samples tested negative. Intra-assay variability was calculated on eight parallel tests of a sample containing 14 CHIKV nsP-1 RNA transcript copies/microliter. For the LightCycler 2.0, an intraassay coefficient of variation of 0.72% (based on an average crossing point value of 33.04) was determined. Interassay variability was determined on 15 independent runs on a sample containing a calculated 140 copies of CHIKV nsP-1 RNA transcript per microliter. An interassay coefficient of variation of 0.78% was calculated at an average crossing point value of 30.13 for LightCycler 2.0. Finally, LightCycler 2.0 was used to analyze 12 coded samples from a recent CHIKV external quality assurance (EQA) study (6). All samples were correctly detected. The correlation coefficient between CHIKV RNA concentrations determined by the commercial assay and the expected EQA panel results was 0.99 ($P < 0.01$; Pearson's goodness of fit) (Fig. 1).

To evaluate the clinical performance of the assay, a total of 57 samples from 53 individual patients were tested. All 53 patients had been seen with laboratory-confirmed acute CHIKV infection in 2006 (7). Thirty-five of 57 (61%) samples tested positive by in-house RT-PCR upon receipt. All specimens were retested with LightCycler 2.0 and quantified using the RNA standards provided with the assay. Results were expressed in RNA copies per milliliter. All samples that tested positive by using the in-house RT-PCR were also determined to be positive by the commercial RT-PCR. Next, viral loads determined by both assays were compared. Median viral loads were 4.6 log RNA copies/ml (range, 3 to 10.1 log RNA copies/ml) by the in-house assay and 4 log RNA copies/ml by the commercial assay (range, 1.6 to 9.6 log RNA copies/ml) (Fig. 1). Viral loads were highly correlated when analyzed by Pearson's goodness of fit test (correlation coefficient, 0.97; $P < 0.01$). All 22 samples that tested negative by using the in-house RT-PCR also tested negative by the commercial kit, and all yielded a valid internal control signal. Compared with the in-house method, the clinical sensitivity and specificity of the commercial test kit were 100%.

This is the first evaluation of a commercial real-time RT-PCR kit for CHIKV. Experiences during the severe acute respiratory syndrome (SARS) outbreak have proven that the rapid provision of preformulated diagnostic tools can assist in outbreak management (1). Commercial test kits, which were rapidly provided by the industry during the SARS epidemic, have yielded superior performance compared to in-house assays (4). Analogous to the SARS outbreak, laboratories were not prepared for CHIKV diagnostics when the Indian Ocean epidemic began. In a similar way, this commercial CHIKV kit may assist laboratories in affected regions and serve the needs of outpatient travel medicine clinics worldwide. The capability of quantifying virus RNA concentrations may facilitate the monitoring of disease progression and the assessment of risks of transmission in the nosocomial situation (8). In addition, this kit may help in regions where CHIKV vectors *Aedes aegypti* and *Aedes albopictus* are subject to virus surveillance.

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