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Evaluation of first rapid diagnostic kit for Anti-Crimean-Congo Hemorrhagic Fever virus IgM antibody using clinical samples from Iran

Vahid Baniasadi[a], Mohammad Hassan Pouriayevali[a], Tahmineh Jalali[a,b], Mehdi Fazlalipour[a], Kayhan Azadmanesh[c,d], Mostafa Salehi-Vaziri[a,c,*]

[a] Department of Arboviruses and Viral Hemorrhagic Fevers (National Reference Laboratory), Pasteur Institute of Iran, Tehran, Iran
[b] Department of Biology, Shahed University, Tehran, Iran
[c] Research Center for Emerging and Reemerging Infectious Diseases, Pasteur Institute of Iran, Tehran, Iran
[d] Department of Virology, Pasteur Institute of Iran, Tehran, Iran

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ABSTRACT

Crimean-Congo Hemorrhagic Fever (CCHF) is a potentially fatal tick-borne viral disease, which is in the WHO list for emerging infections likely to cause major epidemics in the near future. Early diagnosis of CCHF is very important for both patient treatment and infection control. An efficient CCHF rapid test therefore is of great significance. This study was conducted to evaluate the sensitivity and specificity of the first CCHF rapid diagnostic kit (CCHF Sero K-SeT, CORIS BioConcept, Belgium) for detection of IgM specific antibody in patients' sera or plasma, using 87 clinical serum samples from Iranian patients. Although the assay showed an acceptable specificity of 92.9% (13/14), a low sensitivity rate of 39.7% (29/73) was observed. There was no association between the results of CCHF rapid diagnostic kit and the genotype of CCHF virus. This evaluation revealed that the CCHF Sero K-SeT is not suitable for screening of CCHF suspected cases due to its poor sensitivity.

Crimean-Congo Hemorrhagic Fever (CCHF) is the most widespread tick-borne viral disease in the world, which has been listed by the world health organization (WHO) as one of the top eight emerging diseases likely to cause major epidemics in the near future (http://www.who.int/blueprint/priority-diseases/en/). CCHF is caused by an RNA virus belonging to genus *Orthonairovirus* from family *Nairoviridae* which is transmitted to human by infected tick bite or through direct exposures to infected blood and animal tissues. It is important to note that transmission of the CCHF virus infection among humans and nosocomial cases have been reported from affected countries (Bente et al., 2013). Despite the fact that human being is the accidental host of CCHF virus and the incidence rate of the disease is relatively low, the virus is highly virulent and infected individuals can experience a lethal hemorrhagic fever with a significant case fatality rate of up to 80% (Al-Abri et al., 2017). Timely diagnosis is of critical importance not only for proper treatment of the patient, but also for infection control practices (Tezer and Polat, 2015). Nevertheless, due to the similarity of pathological characteristics of CCHF to other infections and also some non-communicable medical conditions, laboratory analysis is necessary to obtain unambiguous diagnosis (Fazlalipour et al., 2016b; Kara et al., 2016).

Routine CCHF laboratory diagnosis is based on detection of viral RNA and anti-CCHF virus IgM/IgG antibodies by reverse transcription polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) or Immunofluorescence Assay (IFA) tests, respectively. Although these assays have high sensitivity and specificity, they need sample preparation, specific equipment and skilled personnel. Consequently, they are not suitable for point of care testing, field deployment and in remote areas where the majority of CCHF cases occur and there is no proper infrastructure for routine laboratory diagnosis. Therefore, there is an urgent need for development of a sensitive, specific and simple rapid test to be employed in the aforementioned settings. To the best of our knowledge, the only commercially available rapid diagnostic test for CCHF is the CCHF Sero K-SeT (CORIS BioConcept, Belgium), which is a lateral flow strip for detection of anti-CCHF virus IgM antibody. Since there was no information about the sensitivity and specificity of the kit, this study was designed to evaluate these parameters.

In accordance with the Iranian national expert committee on viral hemorrhagic fevers guideline, all CCHF probable samples are referred to the department of Arboviruses and Viral Hemorrhagic Fevers, Pasteur Institute of Iran (National Ref. Lab). In this study, serum

* Corresponding author at: Department of Arboviruses and Viral Hemorrhagic Fevers (National Reference Laboratory), Pasteur Institute of Iran, 69 Pasteur Ave., 1316943551 Tehran, Iran.
E-mail address: m.salehi@pasteur.ac.ir (M. Salehi-Vaziri).

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Fig. 1. CCHF Sero K-SeT IgM rapid test showing the positive and negative results. (I) Positive result: appearance of control line (C region) and strong positive test line (T region), (II) Weakly positive result: appearance of control line (C region) and weak positive test line (T region), (III) Negative result: appearance of control line (C region) and absent test line.

samples collected during 2017 were used to analyze the sensitivity and specificity of the CCHF Sero K-SeT kit. For each patient, two serum samples were obtained. The first serum sample taken during the first week after onset of symptom was tested by RT-PCR for specific viral RNA. The second serum sample taken at least 1 week following the first sample was tested by ELISA (VectoCrimea-CHF-IgM ELISA kit, Vector-Best, Novosibirsk, Russia) for anti-CCHF virus IgM antibody.

RT-PCR was done according to a homemade protocol using Iran F2 and Iran R3 primers targeting the small (S) segment of the viral genome (Fazlalipour et al., 2016a). RNA extraction was performed by the QIAamp Viral RNA Kit according to the manufacturer's instructions (Qiagen GmbH, Hilden, Germany). RT-PCR was carried out using the One-Step RT-PCR Kit (Qiagen GmbH, Hilden, Germany). The reaction mix consisted of 5x QIAGEN OneStep RT-PCR buffer (10 µl), dNTP mix (10 mM), primers (0.6 µM each), QIAGEN OneStep RT-PCR Enzyme mix (2 µl) and 500 ng of extracted viral RNA in a total volume of 50 µl. The RT-PCR condition was programed as follows: 30 min at 50 °C, 15 min at 95 °C, followed by 40 cycles including 30 s at 95 °C, 30 s at 50 °C, 45 s at 72 °C, before a final extension step at 72 °C for 10 min. Genotyping of CCHF virus had been done by sequencing the RT-PCR products. Sequencing was carried out using an Applied Biosystems 3730/3730xl DNA Analyzer and phylogenetic analysis was done by MEGA 6 software (Salehi-Vaziri et al., 2016).

VectoCrimea-CHF-IgM ELISA kit (Vector-Best, Novosibirsk, Russia) was used for IgM detection. The kit sensitivity has been reported previously to be 87.8% (95% CI 78.6%–96.9%) with specificity of 98.9% (95% CI 96.7%–100.0%), compared to in-house methods from different laboratories. (Vanhomwegen et al., 2012). The ELISA test was performed according to manufacturer's instruction. In brief, for detection of anti-CCHF virus IgM antibody, serum samples were diluted 1:100 in serum dilution buffer. Per well, 100 µl of diluted serum was applied and the plates were incubated for 60 min at 37 °C. After washing the plates three times with wash buffer, 100 µl conjugate was added and the plates were incubated for 90 min at 37 °C. Plates were washed again 5 times with wash buffer and 100 µl TMB solution was added. Plates were incubated for 25 min at room temperature (18–25 °C) and the reaction was then stopped by the addition of 100 µl stop solution. The HRP reaction product was quantified by measuring optical density (OD) at 450 nm and 620 nm (reference wavelength) on an ELISA reader. Samples were interpreted positive if the OD sample ≥ OD cutoff and results were considered negative if $OD_{sample} < 0.8 \times OD_{cutoff}$. Samples with $0.8 \times OD_{cutoff} \leq OD_{sample} < OD_{cutoff}$ were considered equivocal.

In the current study, a total of 87 human serum samples were included for evaluating the CCHF Sero K-SeT rapid test. These consisted of 73 CCHF IgM positive and 14 CCHF IgM negative samples. The positive group included IgM positive secondary serum samples (confirmed

by VectoCrimea-CCHF-IgM ELISA kit) obtained from patients whose first acute phase serum sample were tested positive for viral RNA by RT-PCR. The IgM-negative group consisted of IgM negative secondary serum samples (confirmed by VectoCrimea-CHF-IgM ELISA kit) obtained from CCHF probable patients whose first acute phase serum samples were tested negative for viral RNA. The IgM positive serum samples were chosen from patients infected with different CCHF virus genotypes to cover all of the available genotypes in Iran. The most common clinical symptoms among all 87 included cases were fever (100%) (87/87), headache (41.4%) (36/87), myalgia (31%) (27/87), hemorrhagic manifestations (29.9%) (26/87) and nausea/vomiting (23%) (20/87). Thrombocytopenia was the most important laboratory finding observed in 72.4% (63/87) of patients.

CCHF Sero K-SeT is an immunochromatographic rapid test for the qualitative detection of anti-CCHF virus IgM antibody from human serum or plasma sample. Specimens were tested under code and interpreted without knowledge of the results of ELISA test. The rapid test was performed according to manufacturer's instructions. In brief, 5 µl of serum sample was added to the sample well followed by adding 3 drops of ST-A Buffer and kept in plastic bag for 15 min. Two individuals read the strips independently and in case of disagreement, a third read was done by another person. The results were interpreted as positive if both control and test lines appear and negative if only the control line appears. Fig. 1, represents a CCHF Sero K-SeT strip before use; and positive, negative and weakly positive tests. To exclude the effect of freeze thawing, IgM ELISA test was repeated at the time of testing samples with the CCHF Sero K-SeT kit.

Sensitivity and specificity of CCHF Sero K-SeT Rapid diagnostic test were calculated as follows: Sensitivity: Number of true positive samples / (Number of true positive samples + Number of false negative samples) × 100 and Specificity: Number of true negative samples /(Number of true negative samples + Number of false positive samples) × 100. The Chi square test was employed to test categorical data between groups. A P value less than 0.05 was considered as statistically significant. Statistical analysis was performed using SPSS software version 16.0. (SPSS Inc., Chicago, IL, USA).

Table 1 shows the results of RT-PCR, IgM ELISA and CCHF Sero K-SeT IgM rapid test for each sample. CCHF positive samples from four different genotypes including Asia-1, Asia-2, Europe-1 and Europe-2 were used in this study. There were also two samples infected with an out-group related to the Kerman22 strain detected in 2012 in Iran (Chinikar et al., 2016).

A total of 73 IgM ELISA positive serum samples were characterized using CCHF Sero K-SeT IgM rapid test; of those, 29 were confirmed to be IgM positive for CCHF (10 strongly positive and 19 weakly positive) demonstrating a sensitivity rate of 39.7% (29/73). Among the 14 IgM ELISA negative serum samples, 13 were negative by the CCHF Sero K-SeT IgM rapid test indicating specificity of 92.9% (13/14). Positive and negative predictive values were 96.7% (29/30) and 22.8% (13/57), respectively.

We considered IgM ELISA OD of 2 as a threshold for strongly and weakly positive samples and evaluated the association between ELISA OD and CCHF Sero K-SeT kit results. There was a statistically significant correlation between OD in IgM ELISA test and the positivity of CCHF Sero K-SeT kit. As represented in Table 2, most of CCHF Sero K-SeT positive samples (25/29) had OD of ≥2 in ELISA test (p value = 0.001). No association was observed between the genotype of the virus and the positivity of CCHF Sero K-SeT kit (Table 3).

The World Health Organization has listed CCHF virus among the priority pathogens for which research and product development should be accelerated (http://www.who.int/blueprint/priority-diseases/en/). A sensitive, reliable and simple rapid diagnostic test is crucial for screening CCHF positive cases at the early stage of infection as a point-of-care test (POCT) especially in rural areas where the incidence rate is higher than urban regions and the laboratory infrastructure is limited (http://www.who.int/blueprint/priority-diseases/key-action/crimean-congo-haemorrhagic-fever/en/).

Rapid diagnostic tests are mainly based on lateral flow immunoassay format, which require minimal specimen processing, provide results in 5–30 min and are easily field deployable. However, in agreement with our results these assays suffer from lower detection sensitivity in comparison with their ELISA equivalents (Peeling et al., 2017). Our study demonstrates the CCHF Sero K-SeT rapid test used herein has lower sensitivity (39.7%) compared to the commercial ELISA kit routinely used for determining the presence of IgM against CCHF. This may be due to impurity or misfolding of recombinant CCHFV

Table 1
Results of anti-CCHF virus IgM antibody by ELISA and CCHF Sero K-SeT rapid diagnostic test in sera from 73 patients who were infected with different genotypes of CCHF virus.

Genotype*	CCHF Sero K-SeT results		
	IgM ELISA Result (OD)	CCHF Sero K-SeT results**	Number
Asia 1 (n = 42)	≥2	++	6
	< 2	++	1
	≥2	+	10
	≥2	–	8
	< 2	–	17
Asia 2 (n = 3)	≥2	–	3
Europe 1 (n = 20)	≥2	++	3
	≥2	+	5
	< 2	+	2
	≥2	–	7
	< 2	–	3
Europe 2 (n = 2)	≥2	–	1
	< 2	–	1
Kerman 22 (n = 2)	< 2	+	1
	≥2	–	1
ND*** (n = 4)	≥2	+	1
	≥2	–	2
	< 2	–	1

* RT-PCR and Genotyping of the virus was performed on the first sample of patients and IgM detection by ELISA and CCHF Sero K-SeT (rapid test) was carried out in the second sample.
** ++: Strongly positive, +: Weakly positive, -: Negative.
*** ND: Not determined due to low RNA concentration or quality of sample.

Table 2
Relation between OD of IgM ELISA and CCHF Sero K-SeT results in patients who were infected with five different genotypes of CCHF virus.

		IgM ELISA OD, n (%)		Total	P value
		< 2	≥2		
CCHF Sero K-SeT	Negative	23 (52.3)	21 (47.7)	44 (100)	0.001
	Positive	4 (13.8)	25 (86.2)	29 (100)	

Table 3
Correlation between genotype of CCHF virus and CCHF Sero K-SeT results.

		CCHF Sero K-SeT, n (%)		Total	P value
		Negative	Positive		
Genotype*	Asia 1	25 (59.5)	17 (40.5)	42 (100)	0. 220
	Europe 1	10 (50.0)	10 (50.0)	20 (100)	
	Others(Asia 2, Europe 2, Kerman 22 and ND**)	9 (81.8)	2 (18.2)	11 (100)	
Total		44 (60.3)	29 (39.7)	73 (100)	

* RT-PCR and Genotyping of the virus was performed on the first sample of patients and IgM detection by CCHF Sero K-SeT (rapid test) was carried out in the second sample.
** ND: Not determined.

nucleocapsid protein used in the kit or the low sample volume (5 µl) (Baneyx and Mujacic, 2004; Koczula and Gallotta, 2016). Moreover, inactivated CCHF virus is used as antigen in the IgM ELISA kit while as previously mentioned CCHF Sero K-SeT rapid test is based on recombinant nucleocapsid protein. This may also explain the low sensitivity of the rapid test.

Nevertheless, the number of test and control samples in this study was limited (73 and 14 samples, respectively). Therefore, the specificity and sensitivity of the CCHF Sero K-SeT rapid test should be further investigated in studies with larger sample size within different affected countries to have better evaluation of these parameters. It is noteworthy that low sensitivity was also observed in initial evaluation of CCHF Sero K-SeT using 42 serum samples at the National Public Health laboratory in Ankara (https://cordis.europa.eu/result/rcn/173337_en.html). However, the exact rate of sensitivity has not been mentioned in that preliminary evaluation.

Although no correlation between genotypes of the virus and positivity of Sero K-SeT RDT was observed, due to high genetic variation of CCHF virus strains (20% in nucleocapsid gene, 22% in polymerase gene and 31% in glycoprotein gene), we recommend that different genotypes of CCHF virus should be considered for the development of the CCHF diagnostic kits.

Recent approaches for developing rapid tests are highlighting the necessity of nucleic acid detection. Combination of the specificity, sensitivity and flexibility of nucleic acid diagnostics with the rapidity and simplicity of Lateral flow immunoassays has been proposed as an ideal strategy (Myhrvold et al., 2018). Myhrvold et al. (2018) developed a Cas13-based field deployable test that is able to detect Zika and Dengue arboviruses at very low concentration of 1 copy per microliter. In addition, detection of viral components e.g. nucleic acid and antigen would provide a faster diagnosis than IgM antibody, because viral elements are detectable from first day of clinical symptoms onset while IgM usually can be detectable after 5 days.

In conclusion, this evaluation demonstrated that the CCHF Sero K-SeT kit is not sensitive enough to be employed as a rapid diagnostic test for screening of CCHF virus specific IgM in suspected patients. However, the kit showed an acceptable rate of specificity and thus could be useful for laboratory confirmation of CCHF virus infection.

Conflict of interest

None to declare.

Ethical approval

All procedures involving human participants were in accordance with the National Expert Committee on Viral Hemorrhagic Fevers and Iranian Center for Disease Control and Prevention guidelines.

Informed consent

Informed consent was not obtained from participants included in this study due to lack of access.

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