Clinical Microbiology and Infection xxx (2017) 1–5

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Original article

EBOLA Ag *K*-SeT rapid test: field evaluation in Sierra Leone

F. Colavita [1, †], M. Biava [1, †], P. Mertens [2], Q. Gilleman [2], C. Borlon [2], M. Delli Guanti [3], A. Petrocelli [3], G. Cataldi [3], A.T. Kamara [3, 4], S.A. Kamara [3, 4], K. Konneh [3, 4], D. Vincenti [1], C. Castilletti [1], S. Abdurahman [5], A. Mirazimi [5, 6, 7], M.R. Capobianchi [1], G. Ippolito [1], R. Miccio [3], A. Di Caro [1, *]

[1] *National Institute for Infectious Diseases 'L. Spallanzani' IRCCS, Rome, Italy*
[2] *Coris BioConcept, Gembloux, Belgium*
[3] *EMERGENCY Onlus NGO, Milan, Italy*
[4] *Diagnostic Ebola Virus Diseases Laboratory, 'Princess Christian Maternity Hospital', Freetown, Sierra Leone*
[5] *Public Health Agency of Sweden, Stockholm, Sweden*
[6] *National Veterinary Institute, Uppsala, Sweden*
[7] *Department of Medicine, Karolinska Institute, Stockholm, Sweden*

ARTICLE INFO

Article history:
Received 7 July 2017
Received in revised form
19 October 2017
Accepted 20 October 2017
Available online xxx

Editor: F. Allerberger

Keywords:
Antigen detection
Ebola virus disease
Point of care
Rapid diagnostic test
Western Africa

ABSTRACT

Objectives: Efficient interruption of Ebola virus disease (EVD) transmission chains critically depends on reliable and fast laboratory diagnosis. We evaluated the performance of the EBOLA Virus Antigen Detection *K*-SeT (EBOLA Ag *K*-SeT), a new rapid diagnostic antigen test in field settings.
Methods: The study was conducted in a field laboratory located in Freetown (Sierra Leone) by the Italian National Institute for Infectious Diseases 'L. Spallanzani' and the EMERGENCY Onlus NGO. The EBOLA Ag *K*-SeT was tested on 210 residual plasma samples (EVD prevalence 50%) from patients hospitalized at the EMERGENCY Ebola treatment center in Goderich (Freetown), comparing the results with quantitative real-time PCR.
Results: Overall, the sensitivity of EBOLA Ag *K*-SeT was 88.6% (95% confidence interval (CI), 82.5–94.7), and the corresponding specificity was 98.1% (95% CI, 95.5–100.7). The positive and negative predictive values were 97.9% (95% CI, 95.0–100.8) and 89.6% (95% CI, 84–95.2), respectively. The sensitivity strongly increased up to 98.7% (95% CI, 96.1–101.2) for those samples with high virus load (≥6.2 log RNA copies/mL).
Conclusions: Our results suggest that EBOLA Ag *K*-SeT could represent a new effective diagnostic tool for EVD, meeting a need for resource-poor settings and rapid diagnosis for individuals with suspected EVD.
F. Colavita, Clin Microbiol Infect 2017;▪:1

Introduction

The 2013–2016 Ebola virus disease (EVD) outbreak in Western Africa was the largest and most complex outbreak of filoviruses ever reported since the discovery of the virus. Its magnitude reached a total number of 28 646 cases and 11 323 deaths, mostly reported in three heavily affected countries: Guinea, Liberia and Sierra Leone [1]. The recent resurgence of EVD in the north of the Democratic Republic of Congo (DRC) shows that the Ebola virus (EBOV) still poses a relevant threat and that the international community must remain on high alert and ready to respond. Especially in the early stage of infection, EVD patients normally show nonspecific clinical signs and symptoms (i.e. fever, headache, diarrhoea and vomiting), which overlap with those of other diseases endemic in Western Africa (i.e. malaria, typhoid fever, measles, dengue and Lassa), thus complicating the clinical diagnosis and management of the patients. In addition, there is still no proven specific and effective treatment available; therefore, it is crucial to

* Corresponding author. A. Di Caro, The National Institute for Infectious Diseases 'L. Spallanzani', Via Portuense 292, 00149 Rome, Italy.
E-mail address: antonino.dicaro@inmi.it (A. Di Caro).
† The first two authors contributed equally to this article, and both should be considered first author.

https://doi.org/10.1016/j.cmi.2017.10.019
1198-743X/

limit the transmission of the infection [2–5]. Laboratory testing plays an essential role in confirming EVD cases, thus permitting necessary isolation measures to be executed, early treatment to be provided and monitoring of high-risk individuals put into place. In an outbreak scenario, especially in resource-limited settings, the entire EVD diagnosis process, from sample collection to confirmatory diagnostic results, takes several hours, or even days if the reference laboratory is located far from where the suspected EVD cases are triaged [5].

Current methods to diagnose EBOV infection include reverse transcription–PCR, antigen-capture ELISA, and IgM and IgG ELISA. EVD diagnosis implemented in the field and in outbreak settings mostly relies on quantitative real-time PCR (qPCR) technology [6–8]. This test is highly sensitive and specific; however, it is costly and requires laboratory personnel with expertise in molecular techniques, a laboratory infrastructure, and operation and maintenance of complex equipment, as well as the provision of a continuous power supply, refrigeration and appropriate environmental temperatures for reagents, and a longer time to results [9–11]. Handheld lateral flow assays that detect virus antigens in blood or other body fluids are ideal for rapid EVD diagnosis, especially in resource-limited settings: they require no electricity and no skilled laboratory personnel. Further, they can often be stored at room temperature and can provide results within approximately 10 to 20 minutes.

Accordingly, World Health Organization (WHO) guidelines recommend initial testing with a rapid diagnostic antigen test (RDT), if available, to assist in triage and case management when clinical and laboratory resources are overwhelmed and when reverse transcription–PCR (RT-PCR) testing is not immediately available (http://apps.who.int/iris/bitstream/10665/134009/1/WHO_EVD_GUIDANCE_LAB_14.1_eng.pdf) [12]. Since the Western Africa outbreak, several RDTs have been developed. A few have obtained emergency use authorisation from the WHO and the US Food and Drug Association [7,13–15]. Some of them have been recently trialled and tested in DRC. To effectively respond to future epidemics, and for continuous refinement of surveillance, research towards developing novel RDTs for EVD diagnosis is still critical.

In this study, we present the results obtained from a preliminary assessment of the EBOLA Virus Antigen Detection *K*-SeT (EBOLA Ag *K*-SeT), a RDT developed by Coris BioConcept (Gembloux, Belgium) within the framework of the EbolaMoDRAD project (http://www.ebolamodrad.eu; see also http://www.corisbio.com/). The evaluation of its performance was carried out in the EVD laboratory established at the Princess Christian Maternity Hospital in Freetown (Sierra Leone), run by EMERGENCY Onlus NGO (EMERGENCY; http://www.emergency.it/) and the Italian National Institute for Infectious Diseases 'L. Spallanzani' (INMI) (http://www.inmi.it).

Methods

The EBOLA Ag *K*-SeT (Coris BioConcept) is a lateral flow immunoassay which detects Ebola virus VP40 viral matrix protein. Because of its abundance in the infected host cells, VP40 results are a good candidate for developing antigen detection assays [16]. EBOLA Ag *K*-SeT is designed to test blood, plasma and serum samples. It is based on an immunochromatographic technique using colloidal gold particles and two monoclonal antibodies specific for EBOV VP40 antigen on nitrocellulose strips. The strip includes a control (C) line to assess the correct migration of the sample, and visual interpretation of results is performed after 15 minutes' incubation. RDTs showing a failed C line are considered invalid (Fig. 1).

Aliquots of plasma samples ($n = 210$) remaining from clinical specimens collected from EVD-positive and -negative patients hospitalized at the EMERGENCY Ebola treatment center in Goderich (Freetown, Sierra Leone) from 12 December 2014 to 21 June 2015 were anonymized and retrospectively used for the evaluation of the EBOLA Ag *K*-SeT [17]. All residual samples had been stored at −20°C under controlled power supply until the study (April–May 2016); no substantial power interruptions were reported during sample storage. The RDTs were performed following the manufacturer's instructions using high containment measures (Biological Safety Cabinet Class III). The diagnostic efficacy of the EBOLA Ag *K*-SeT was evaluated comparing the results with those previously obtained on fresh venipuncture plasma samples tested for EVD with the reference qPCR test (RealStar Filovirus Screen RT-PCR Kit 1.0; Altona Diagnostics, Hamburg, Germany; limit of detection, 3.11 log RNA copies/mL (cp/mL)), performed on a SmartCycler instrument (Cepheid, Sunnyvale, CA, USA) at the Italian laboratory in Goderich during the outbreak [17,18]. Virus genome quantification, expressed as cp/mL, was based on a standard reference curve provided by the kit producers, spanning up to 9 log cp/mL. As retesting all samples with qPCR was not feasible due to logistic issues (i.e. sample volume constraints and lack of reagent availability), qPCR was performed at the time of the study only on a set of samples to verify the integrity. No significant differences were observed compared to the original results.

Experiments to assess the reactivity of EBOLA Ag *K*-SeT to other pathogens were conducted in two different European BSL4 laboratories (INMI and Public Health Agency of Sweden) depending on specimen or virus stock availability. Clinical residual anonymized samples from patients with non-EBOV infections or plasma from healthy donors spiked with virus preparations were tested. The concentrations of the pathogens fluctuated owing to the available stock, and the clinical specimens with the highest virus load were selected. The study was approved by the INMI ethical board (16-2015) and by the ethics and scientific review committee and pharmacy board of Sierra Leone (SLESRC 09/06/2015, PBSL 17/08/2015).

Results

In this study, 105 of 210 plasma samples resulted in EBOV-positive findings for the reference test qPCR, with an EVD prevalence of 50%. Of the 210 patients tested, 120 (58.0%) were male, and the median age was 32 years (interquartile range, 36–17 years). Data on the onset of symptoms were available for 154 (73.3%) of 210 cases. In these patients, the median time of symptom duration before the date of EVD diagnostic testing was 6 days (interquartile range, 10–4 days). Of the patients who tested positive for EVD, 47 (45.8%) recovered from the disease, and 58 (55.2%) died. All 105 EVD-negative patients considered in this study presented with fever as well as diarrhoea or vomiting. Two consecutive negative qPCR results on samples collected after an interval of at least 24 hours were required for patient discharge. The average virus load detected among the positive samples tested in this study was 7.19 log cp/mL in a range from 10 (threshold cycle value, $C_t = 12.01$) to <3.11 log cp/mL ($C_t = 33.56$), with a median of 7.43 log cp/mL. In addition, 76 samples tested (72.4%) had a virus load of ≥6.2 log cp/mL ($C_t = 25$) and 29 (27.6%) <6.2 log cp/mL.

All the RDTs performed in this study were valid. Ninety-five patients had positive RDT results with both the C and the T lines. Among all 95 patients who had positive EBOLA Ag *K*-SeT results, 93 (97.9%) were positive by qPCR. Twelve of 105 patients who were qPCR positive were negative by the RDT (Table 1).

Overall, EBOLA Ag *K*-SeT had a sensitivity of 88.6% (95% confidence interval (CI), 82.5–94.7) and a specificity of 98.1% (95% CI, 95.5–100.7) compared to the reference test. For a study population with an EVD prevalence of 50%, the positive predictive value (PPV) of EBOLA Ag *K*-SeT was 97.9% (95% CI, 95.0–100.8) and the negative predictive value (NPV) was 89.6% (95% CI, 84–95.2) (Table 2).

Please cite this article in press as: Colavita F, et al., EBOLA Ag *K*-SeT rapid test: field evaluation in Sierra Leone, Clinical Microbiology and Infection (2017), https://doi.org/10.1016/j.cmi.2017.10.019