Preliminary evaluation of the EBOLA virus Antigen detection K-SeT rapid test in Sierra Leone

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Background

The 2013-2016 Ebola virus disease (EVD) outbreak highlighted that the efficient interruption of the EVD transmission chains critically depends on reliable and fast laboratory diagnosis. EVD diagnosis is currently performed mainly using reverse transcription-PCR (RT-PCR), highly sensitive and specific, but requiring skilled laboratory personnel and advanced equipment, as well as prolonged turnaround time to final results. New rapid diagnostic tests, performed as point-of-care or in resources-limited settings, are needed for a prompt epidemic control[1]. Coris BioConcept (Gembloux, Belgium) developed within the EbolaMoDRAD project a rapid antigen diagnostic test (RDT), EBOLA virus Antigen detection K-SeT, for Ebola virus (EBOV) detection. We evaluated RDT performance in EVD laboratory established at Princess Christian Maternity Hospital (PCMH) in Freetown (Sierra Leone) run by of EMERGENCY NGO and the Italian National Institute for Infectious Diseases (INMI).

Methods and Results

Aliquots of plasma (n=210) remaining from clinical specimens, collected from EVD positive and negative patients hospitalized at the EMERGENCY's Ebola Treatment Center in Goderich (Freetown, Sierra Leone) from 12 December 2014 to 21 June 2015 and stored at -20°C under controlled power supply until the study, were anonymized and retrospectively used for the evaluation of the RDT[2]. The RDTs were performed following the RDT instructions **(Fig 1)** and using high containment measures inside level III biosafety cabinet (BSCIII). The diagnostic efficacy of the RDT was evaluated comparing the clinical results previously obtained with the reference test RT-qPCR (RealStar® Filovirus Screen RT-PCR Kit 1.0, Altona Diagnostics GmbH), performed on SmartCycler® instrument.

All EBOLA Virus Antigen detection K-SeT RDTs performed within this study resulted valid, with a visible C line.
Overall, the RDT 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 with the reference test. The positive predictive value (PPV) of the RDT for the study population with EVD prevalence of 50%, was 97,9% (95% CI: 95,0–100,8). A negative predictive value (NPV) of 89,6% was achieved (95% CI: 84–95,2). **(Table 1)**
The sensitivity of the test strongly increased with samples having lower Ct values obtained in the EBOV RT-qPCR. Specifically, for 76 plasma samples (72,4%) with Ct values ≤25, the RDT had a sensitivity of 98,7% (95% CI: 96,1–101,2), with a PPV and NPV of 97,4% (95% CI: 93,8–101,0) and 99,4 % (95% CI: 97,21–100,9) respectively. **(Table 2a)** For the 29 samples (27,6%) with Ct values >25, the overall sensitivity resulted of 62,1% (95% CI: 44,4–79,7), the PPV was 90,0% (95% CI: 76,9–103,1) and the NPV was 90,3% (95% CI: 84,9–95,8). **(Table 2b)**

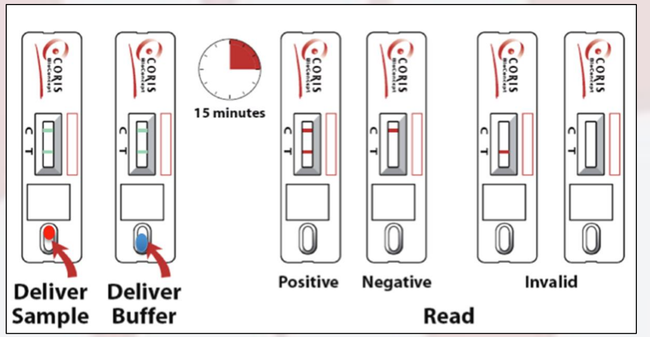

Fig 1. EBOLA virus Antigen detection K-SeT rapid test is a lateral flow immunoassay, which detects EBOV Zaire strain VP40 viral matrix protein, based on immunochromatographic technique using colloidal gold particles, nitrocellulose strips and two monoclonal antibodies specific for EBOV antigen. RDT is operated by introduction of 30 µL of plasma or serum and 4 drops of sample buffer onto the pad. Visual interpretation of results is performed after 15 minutes. The strip includes a control line to assess the correct sample migration.

	Proportion#	Percentage (95% CI)
Sensitivity	93 /105	88,6% (94,7 – 82,5)
Specificity	103/105	98,1% (100,7 – 95,5)
PPV	93/95	97,9% (100,8 – 95,0)
NPV	103/115	89,6% (95,2 – 84,0)

Table1. Performance of the EBOLA Virus Antigen detection K-SeT RDT versus RT-qPCR (Altona Diag.) # Data are n/N
Abbreviations: RT-qPCR, quantitative reverse transcription - PCR; CI, confidence interval; RDT, rapid diagnostic test; PPV, positive predictive value; NPV, negative predictive value.

a	Proportion#	Percentage (95% CI)
Sensitivity	75/76	98,7% (101,2 – 96,1)
Specificity	103/105	98,1% (100,7 – 95,5)
PPV	75/77	97,4% (101,0 – 93,8)
NPV	103/104	99,4% (100,9 – 97,2)

b	Proportion#	Percentage (95% CI)
Sensitivity	18/29	62,1% (79,7 – 44,4)
Specificity	103/105	98,1% (100,7 – 95,5)
PPV	18/20	90,0% (103,1 – 76,9)
NPV	103/114	90,3% (95,8 – 84,9)

Table 2. Performance of the EBOLA Virus Antigen detection K-SeT RDT based on RT-qPCR Ct values. a) Data obtained from RDT on samples with Ct value ≤ 25. b) Data obtained from RDT on samples with Ct value > 25

Conclusions

The results obtained in this preliminary study suggest that the EBOLA Virus Antigen detection K-SeT developed by Coris BioConcept could represent a new promising effective and rapid diagnostic tool for EVD diagnosis, meeting the need for minimal resources, point-of-care and rapid diagnosis for suspected EVD individuals.
The RDT is sensitive, specific and it performs well in resource-limited settings. It does not need any external instrumentation, it proved to be easy and rapid to use, can be performed by laboratory technicians with very little extra training and can be stored and transported at room temperature. It resulted to be a highly specific (98,1%) screening test , allowing high-risk suspected EVD cases to be rapidly and accurately identified for isolation and for confirmatory diagnostic testing and reducing non-EVD admissions and avoiding patients to be exposed to nosocomial EVD transmission. The sensitivity was very high (98,7%) for those samples in the RT-qPCR Ct range of 12 to 25, which identifies patients in acute phase of the disease and highly infectious. Although further investigation must be carried out in order to make a more complete evaluation of its performance, the EBOLA Virus Antigen detection K-SeT may be of great help in future EVD outbreaks to limit the burden on the health care systems, control transmission and improve patient outcomes in otherwise resource-constrained setting.

[1]*Singh B, Ganguly A, Sunwoo HH. Current and Future Diagnostic Tests for Ebola Virus Disease. J Pharm Pharm Sci. 2016;19(4):530-551.*
[2]*Colavita F, Venditti C, Vulcano et al. INMI/Emergency NGO Italian laboratory established in Sierra Leone during Ebola virus disease outbreak in West Africa. Clin Microbiol Infect Dis. 2016*