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Clinical evaluation of the BioFire FilmArray® BioThreat-E test for the diagnosis of Ebola Virus Disease in Guinea

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ABSTRACT

Background: The recent West Africa Ebola outbreak highlighted the need to provide access to rapid, safe and reliable Ebola Virus Disease diagnostics.
Objectives: The objective of this field study was to assess the clinical performance of the FilmArray® BioThreat-E test for the detection of Ebola Zaïre virus in whole blood in symptomatic patients suspected of Ebola Virus Disease in Conakry (Guinea) from March to July 2015.
Study design: The BioThreat-E test was compared to the two RT-PCRs, using serum, implemented at Donka Hospital in the emergency context: an in-house developed quantitative one-step RT-PCR adapted from the Weidmann technique, and the RealStar® Filovirus RT-PCR Kit 1.0 (Altona-Diagnostics). We also assessed the performance of this assay in noninvasive specimens (urine and saliva) to detect infected patients.
Results: Of 135 patients enrolled and eligible for performance assessment on whole blood, the sensitivity was 95.7% [95% CI: 85.5–99.5] and specificity 100% [95% CI: 95.9–100]. Of the 37 symptomatic infected patients able to provide saliva and/or urine samples, 34 of the 35 saliva samples and all 3 of the urine samples were positive with the BioThreat-E test.
Conclusions: This study showed that the FilmArray BioThreat-E test performs comparably to conventional molecular tests under field conditions, providing results and interpretation in approximately 1 h. Due to its operational characteristics, it can be easily deployed in the field during an epidemic and could also be a useful tool for post-outbreak surveillance.

1. Background

In March 2013, when Ebola Virus Disease (EVD) was detetected in West Africa [1], diagnosis was relying on complex techniques, based on the detection of Ebola virus, viral RNA viral antigens, or Ebola specific IgM antibodies. However, only viral RNA or antigens detection were recommended for early identification of EVD cases [1,2], but standard molecular assays for Ebola diagnosis were requiring sophisticated laboratories with skilled technicians. This is why, in November 2014, the WHO expressed the need to scale up development and implementation of rapid, sensitive, safe and simple diagnostic tests [3].

The FilmArray® (FA) system (BioFire Diagnostics, Salt Lake City, UT, a bioMérieux company) is a completely automated multiplex PCR system which enables simultaneous testing for bacteria, viruses, yeasts, parasites and/or antimicrobial resistant genes using several commercial comprehensive panels [4–7]. In addition to these panels, a FA test (BioThreat-E test) has been developed by BioFire Defense to detect Ebola virus, Zaïre species (EBOV). The Emergency Use Authorization (EUA), that permitted the use of the test for clinical diagnosis, was obtained from the United States Food and Drug Administration (US FDA) on October 25, 2014, based on results obtained on spiked-whole blood and urine specimens [8].

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2. Objectives

The aim of our study was to evaluate the clinical performance of the BioThreat-E test for the diagnosis of EVD under field conditions in Guinea. The BioThreat-E test was compared to the two conventional RT-PCRs implemented at Donka Hospital in the emergency context: an in-house developed quantitative one-step RT-PCR based on the Weidmann technique, specific to Zaire species, further adapted and then deployed on the field by the Institut Pasteur de Dakar [9–11], and the RealStar® Filovirus RT-PCR Kit 1.0 (CE-IVD) (Altona-Diagnostics, Hamburg, Germany), a pan-filovirus test. A second part of the study was designed to evaluate the use of the BioThreat-E test using saliva and urine specimens in the same context.

3. Study design

3.1. Patients

The study was conducted from March 7 through July 24, 2015 at the "Laboratoire des Fièvres Hémorragiques Virales" in Donka National Hospital (Conakry, Guinea). The reference based population came from the regions of Conakry and Coyah. The study did not interfere with the patient management according to ongoing practices and no separate blood drawing was required. Each participant was verbally informed before signing a consent form, which was then photographed and sent to the principal investigator. The protocol was approved by the Commission Recherche Ebola de la République de Guinée, and by the Comité National d'Ethique pour la Recherche en Santé (Conakry, Guinea).

Study inclusion criteria were: 1) patients older than 18, referred to Conakry or Coyah Ebola Treatment Centers for suspicion of EVD according to WHO criteria, and considered eligible for routine Ebola diagnostic testing and 2) ability to provide a written informed consent.

BioThreat-E test results were analyzed if they fulfilled the following criteria: 1) validated Clinical Report and Research Form available, 2) adequate biological samples for diagnosis obtained (whole blood for BioThreat-E test and serum for the two routine RT-PCRs), 3) all three tests [Weidmann in-house adapted PCR (W-PCR), Altona PCR and BioThreat-E test] performed within a total interval of three days to assure comparable analytical conditions.

Clinical data, routinely provided to the laboratory, were collected and recorded via a questionnaire. BioThreat-E test clinical sensitivity, specificity and clinical performance (positive and negative predictive values) in whole blood were evaluated in comparison with the routine RT-PCRs on serum (W-PCR and Altona PCR).

3.2. Procedures

When suspected cases were prescribed venipuncture for diagnostic testing, one additional EDTA tube was drawn for BioThreat-E test on whole blood, as well as additional non-invasive samples – urine and saliva – whenever possible, depending on the condition of the patient. Urine was collected in 60 mL polystyrene containers, and saliva collection was done using a transport device combining a foam swab, placed into 1 mL viral transport medium (Sigma Virocult®).

In Donka hospital, samples were sent to the lab on the same day. The distance between Coyah and Conakry made a same-day lab delivery impossible. Therefore, the Coyah samples were stored at 4 °C and transported to Conakry, once to twice a week according to local constraints, using a cooler during the transport.

All blood samples were analyzed by the two routine RT-PCRs on serum while the BioThreat-E test was performed on whole blood. Urine and saliva specimens were only analyzed by FA for positive cases as a preliminary exploration of the performance of the BioThreat-E test on non-invasive specimens. For all tests, specimens were processed according to WHO biosafety recommendation, using a Class III biosafety cabinet, by personnel wearing required Personal Protective Equipment (PPE). For FA testing, all pouches were inoculated in the biosafety cabinet, decontaminated using a 0.5% sodium chloride solution then loaded in the FilmArray® machine located in a conventional area of the laboratory.

3.3. Routine RT-PCRs

Viral RNAs were extracted from 100 µL of serum using a QIAamp Viral RNA mini kit (Qiagen, Germany) according to the manufacturer's protocol. RNA samples were processed immediately after extraction by the laboratory of hemorrhagic fevers at Donka hospital.

W-PCRs were carried out with QuantiTect RT-PCR kit (Qiagen) and a dried 10-fold primer and probe mix containing 100 pmol EBOZ FP and EBOZ RP and 50 pmol EBOZ P (TIB Molbiol, Hamburg, Germany) as a reference test. Each reaction was performed in a 25 µL total volume (QuantiTect Probe 10 µL, RT-PCR Master Mix 0.2 µL, H2O 9,8 µL, probe and primers FP 10 pmol, RP 10 pmol, P 5 pmol and 5 µL of RNA extracts) in a SmartCycler II system (Cepheid, Sunnyvale, CA). The following thermal cycle profile was used: 42 °C for 15 min, 95 °C for 15 min, followed by 40 cycles of 95 °C for 15 s and 55 °C for 45 s. Positive results above cycle threshold (Ct) 35 were regarded as equivocal and repeated for confirmation [11].

The Altona-PCR was performed using the RotorGene Q6 system, following manufacturer's recommendation using 10 µL of RNA extracted from 100 µL of serum [11]. The same RNA extract was used for both conventional RT-PCRs.

3.4. FilmArray® BioThreat-E assay

The BioThreat-E test was performed according to the manufacturer's instructions (Fig. 1), using 200 µL of whole blood, urine or transport

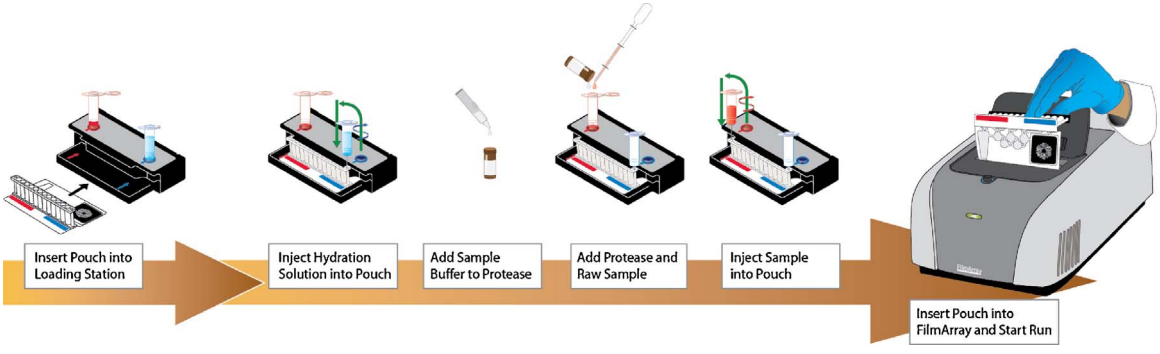

Fig. 1. This FilmArray BioThreat-E test quick guide details the five steps of the preparation of the pouch. The protease is used in processing whole blood samples. The protease step consists of rehydration of the lyophilized protease with the buffer and addition of the reconstituted protease to the sample vial, prior to adding the actual sample. It requires no incubation and adds only a few seconds to the whole process.