Clinical Performance of the FilmArray™ BioThreat-E test for the diagnosis of Ebola Virus Disease “in the field” in Guinea

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BACKGROUND

The recent West Africa Ebola outbreak has highlighted the importance of efficient diagnostic tools to contain such contagious and rapidly spreading epidemics. The non-specific clinical features of Ebola Virus Disease (EVD), its infectivity and occurrence in low-resource settings mean that a diagnostic test must have high sensitivity and specificity, be easy, safe and rapid to perform, and be able to be performed in the field without specialized expertise. The FilmArray (FA) system (BioFire Diagnostics, a bioMérieux company) is a qualitative in vitro diagnostic platform that combines nucleic acid purification and nested multiplex RT-PCR for the identification of infectious pathogens through a syndromic approach. The system is a highly accurate, fast, and easy-to-use PCR molecular diagnostic instrument which delivers test results in approximately one hour using a closed, sample-to-answer system. BioFire Defense has developed a commercially available FA test (BioThreat-E) to detect Ebola virus, Zaire species. Based on in vitro technical performance obtained on spiked-whole blood and urine specimens, an Emergency Use Authorization was obtained from the FDA on October 25, 2014 (1). On August 19, 2015, the BioThreat-E test was listed for WHO procurement (2). In the meantime, published data have demonstrated that the FilmArray panels are effective tests for evaluating patients with EVD (3-4), but no clinical performance study had yet been conducted so far. The aim of our study was to evaluate the clinical performance of the BioThreat-E test for the diagnosis of EVD in the field in Guinea.

DESIGN OF THE STUDY

The study was a prospective evaluation of FilmArray BioThreat-E test, conducted between March 7, 2015 and July 24, 2015 at the “Laboratoire des Fièvres Hémorragiques Virales” in Donka National Hospital (Conakry, Guinea), which is the Reference Laboratory for Hemorrhagic Fevers in Guinea. The reference based population included the regions of Conakry and Coyah. The study did not interfere with patient management according to routine practice in Conakry and Coyah Treatment Center. No specific invasive intervention was required for this research protocol: only one additional EDTA tube was drawn from a single patient during the sampling. Each participant was verbally informed before signing the consent form. The protocol has been approved by the Guinean Ethical Committee.

Inclusion criteria:

• All patients, male and female, older than 18, sent to Conakry or Coyah Ebola Treatment Centers, suspected of EVD according to WHO criteria, and considered eligible for Ebola routine diagnosis.
• Ability to deliver a written informed consent

The BioThreat-E test, performed on whole blood specimens, was compared with routine testing on serum using the following techniques: 1) QuantiTect Probe RT-PCR® (Qiagen) and 2) RealStar Filovirus Type RT-PCR Kit 1.0 (Altona). The status for EVD was defined for each patient based on the result of routine testing:

• Positive patients: patients enrolled in the study showing positive results with the two routine PCR tests performed in the lab: QuantiTect Probe RT-PCR and RealStar Filovirus Type RT-PCR Kit 1.0.
• Negative patients: patients enrolled in the study showing negative results with the two routine PCR tests performed in the lab: QuantiTect Probe RT-PCR and RealStar Filovirus Type RT-PCR Kit 1.0.
• Equivocal patients: all other results showing a discrepancy between the two routine tests.

RESULTS

From March 7 to July 24, 156 EVD-suspected patients were enrolled; 135 patients were considered eligible according to the following inclusion criteria:

• Patients meeting the inclusion criteria and with no exclusion criteria.
• Written informed consent and validated Clinical Research Form.
• Adequate biological samples for diagnosis: whole blood for FilmArray and serum for RT-PCR.

All three tests (QuantiTect Probe RT-PCR, RealStar Filovirus Type RT-PCR Kit 1.0 and BioThreat-E tests) performed within three days to guarantee comparable analytical conditions.

<table>
<thead>
<tr>
<th>RT-PCR status</th>
<th>NEG</th>
<th>POS</th>
<th>Equivocal</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>FA</td>
<td>87</td>
<td>2</td>
<td>1</td>
<td>90</td>
</tr>
<tr>
<td>Total</td>
<td>87</td>
<td>45</td>
<td>1</td>
<td>135</td>
</tr>
</tbody>
</table>

Considering only positive and negative patients in the analysis, the sensitivity of the BioThreat-E test is 95.7% [95% CI: 85.9-99.5] and the specificity is 100% [95% CI: 95.9-100]. If equivocal patients are included in the analysis (only one case), the sensitivity slightly decreases to 93.8% [95% CI: 82.8-98.7]. There were two discordant results between the BioThreat-E and the routine PCR assays; these may be true false negatives. However, the melting-curve data for the two BioThreat-E samples do not support the presence of a “missed” low concentration of Ebola Zaire nucleic acid; in fact, these two samples were retested and both showed positive results, suggesting that a user error is probably the underlying explanation for the initial discordant negative results. The equivocal sample could not be retested.

Conclusions

This field study confirmed that the BioFire FA BioThreat-E assay meets the requirements of an Ebola diagnostic test: high sensitivity and specificity, point-of-care use, safety, easy to perform, easy to read (“Yes/No result”). This last point was very much appreciated by the technicians. Thanks to the excellent characteristics of the BioThreat-E assay, we believe that this test can be very helpful for the detection of Ebola infections in various contexts, in complement with other tests:

• During an outbreak:
  - Rapid diagnosis or exclusion of disease in symptomatic healthcare workers
  - As an easy nucleic acid-based assay to verify the presence of Ebola virus in someone with a positive lateral-flow antigen assay
  - Rapid POC surveillance of new/arriving cases in neighboring countries
  - Rapid POC surveillance at pivotal transportation areas and borders
• Post-epidemic surveillance:
  - Early rapid detection of new suspected cases in neighboring geographic areas
  - Early rapid detection of re-emergence of the virus in the same outbreak area/country

References

3) LedéT.B et al. Use of FilmArray™ system for detection of Zaire ebolavirus in a small Hospital, Bo, Sierra Leone. J Clin Microbiol. 2015; Jul;53(7):2058-60