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Timothy R. Southern, University of Nebraska Medical Center
Lori D. Racsa, Emory University
César G. Albariño, Centers for Disease Control and Prevention
Paul D. Fey, University of Nebraska Medical Center
Steven H. Hinrichs, University of Nebraska Medical Center
Caitlin N. Murphy, University of Nebraska Medical Center
Vicki L. Herrera, Nebraska Public Health Laboratory
Anthony R. Sambol, Nebraska Public Health Laboratory
Charles Hill, Emory University
Emily L. Ryan, Emory University

Only first 10 authors above; see publication for full author list.

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Comparison of FilmArray and Quantitative Real-Time Reverse Transcriptase PCR for Detection of Zaire Ebolavirus from Contrived and Clinical Specimens

Timothy R. Southern,a,b Lori D. Racsa,c César G. Albariño,d Paul D. Fey,a Steven H. Hinrichs,a Caitlin N. Murphy,a,b Vicki L. Herrera,b Anthony R. Sambol,b Charles E. Hill,c Emily L. Ryan,d Colleen S. Kraft,c,e Shelley Campbell,d Tara K. Sealy,d Amy Schuh,d James C. Ritchie,5 G. Marshall Lyon III,c Aneshk K. Mehta,c Jay B. Varkey,b Bruce S. Ribner,d Kent P. Brantly,b Ute Ströher,4 Peter C. Iwen,a,b Eileen M. Burd).e
Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska, USA;Department of Pathology and Laboratory Medicine, Emory University, Atlanta, Georgia, USA;Centers for Disease Control and Prevention, Atlanta, Georgia, USA;Department of Medicine, Division of Infectious Diseases, Emory University School of Medicine, Atlanta, Georgia, USA;Samaritan’s Purse, Boone, North Carolina, USA

Rapid, reliable, and easy-to-use diagnostic assays for detection of Zaire ebolavirus (ZEBOV) are urgently needed. The goal of this study was to examine the agreement among emergency use authorization (EUA) tests for the detection of ZEBOV nucleic acids, including the BioFire FilmArray BioThreat (BT) panel, the FilmArray BT-E panel, and the NP2 and VP40 quantitative real-time reverse transcriptase (qRT) PCR assays from the Centers for Disease Control and Prevention (CDC). Specimens used in this study included whole blood spiked with inactivated ZEBOV at known titers and whole-blood, plasma, and urine clinical specimens collected from persons diagnosed with Ebola virus disease (EVD). The agreement for FilmArray and qRT-PCR results using contrived whole-blood specimens was 100% (6/6 specimens) for each ZEBOV dilution from 4 × 10⁻⁶ to 4 × 10⁻⁵ 50% tissue culture infective dose (TCID50)/ml, as well as the no-virus negative-control sample. The limit of detection for FilmArray and qRT-PCR assays with inactivated ZEBOV, based on duplicate positive results, was determined to be 4 × 10⁻⁶ TCID50/ml. Rates of agreement between FilmArray and qRT-PCR results for clinical specimens from patients with EVD were 85% (23/27 specimens) for whole-blood specimens, 90% (18/20 specimens) for whole-blood specimens tested by FilmArray testing and matched plasma specimens tested by qRT-PCR testing, and 85% (11/13 specimens) for urine specimens. Among 60 specimens, eight discordant results were noted, with ZEBOV nucleic acids being detected only by FilmArray testing in four specimens and only by qRT-PCR testing in the remaining four specimens. These findings demonstrate that the rapid and easy-to-use FilmArray panels are effective tests for evaluating patients with EVD.

Ebolavirus is an enveloped, single-stranded RNA virus that is the cause of Ebola virus disease (EVD). EVD is characterized by fever, enesis, diarrhea, and a hemorrhagic disorder that can include maculopapular rash, petechiae, ecchymoses, and mucosal hemorrhage (1). Zaire ebolavirus (ZEBOV) was the cause of the 2014–2015 outbreak of EVD in West Africa, which to date has resulted in 27,352 total cases, 15,052 laboratory-confirmed cases, and 11,178 deaths (2,3).

Early detection of ZEBOV is critical for the management of cases of EVD and for outbreak control. A significant challenge in areas without Ebola, such as the United States, is the rapid assessment of individuals with a history of travel to West Africa who present with symptoms of EVD. Currently, the standard protocol for EVD testing involves collection of whole blood or plasma, followed by testing using quantitative real-time reverse transcriptase (qRT) PCR assays. Although they are highly sensitive and specific, qRT-PCR assays for ZEBOV are complex, which limits their use to state public health laboratories and the Centers for Disease Control and Prevention (CDC) (4,5). Depending on the location of the patient being tested and the laboratory performing the testing, the turnaround time for qRT-PCR results could be measured in days, whereas initial testing performed at or near the point of care using a rapid test could be completed within hours. Rapid, reliable, and easy-to-use tests for the detection of ZEBOV are needed not only for testing in areas in which the disease is endemic but also for screening of health care workers, international travelers, and other potentially exposed individuals.

The first U.S. nationals infected in Africa were returned to our facilities for treatment in the Serious Communicable Diseases Unit (SCDU) at Emory University (EU) and the Nebraska Biocontainment Unit at Nebraska Medicine, the academic medical hospital affiliated with the University of Nebraska Medical Center (UNMC), between 2 August and 28 October 2014 (6–8). At that

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Address correspondence to Eileen M. Burd, eburd@emory.edu.
T.R.S. and L.D.R. contributed equally to this article.
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time, the assays available for the detection of ZEBOV were the research-use-only FilmArray BioThreat (BT) panel (BioFire Defense, Salt Lake City, UT) and the ZEBOV nucleoprotein 2 (NP2) and matrix protein (VP40) gene assays from the Centers for Disease Control and Prevention (Atlanta, GA). The NP2 and VP40 qRT-PCR assays and the FilmArray BT-E assay (a modified version of the BT panel) were granted emergency use authorization (EUA) in October 2014, during the course of the treatment of our patients. The FilmArray BT assay detects a panel of biothreat agents, but the ZEBOV primers detecting the L-gene are identical in the FilmArray BT test and the FilmArray BT-E (EUA) test. The FilmArray BT-E assay also includes a freeze-dried protease to add with the blood and loading buffer and has an additional primer in the second-stage PCR that perfectly matches the current circulating strain. This study provides a comparative analysis of the FilmArray BT-E panel and the NP2 and VP40 qRT-PCR assays for the detection of ZEBOV using contrived whole-blood specimens. This study also provides a prospective analysis of the FilmArray BT panel and qRT-PCR assays using whole-blood, plasma, and urine specimens from 6 persons with EVD who were treated in our facilities.

MATERIALS AND METHODS

FilmArray assay. The BioFire FilmArray system (BioFire Diagnostics LLC, Salt Lake City, UT) is an automated nested PCR system that allows the extraction and detection of nucleic acid targets in a closed system. The BT and BT-E panels (BioFire Defense, Salt Lake City, UT) are used with the FilmArray system to detect ZEBOV in whole-blood or urine specimens (9). Briefly, each panel was rehydrated with the provided rehydration solution, and whole blood (100 μl) or urine (200 μl) was mixed with the sample buffer provided. The resulting solution was injected into the panel pouch, and the panel pouch was loaded into the FilmArray instrument. One sample was tested at a time, with a result of detected or not detected being provided in approximately 1 h. FilmArray testing was performed in a satellite laboratory within the SCDU at EU, at the Nebraska Public Health Laboratory (NPHL) biosafety level 3 (BSL3) facility located on the UNMC campus, and at the CDC in the Viral Special Pathogens Branch (VSPB) laboratory. Specimens were processed for FilmArray testing in a class II biological safety cabinet, by individuals wearing enhanced personal protective equipment, as defined by the biosafety committees at each institution.

qRT-PCR assays. Testing was also performed by the VSPB at the CDC using the qRT-PCR assays for the detection of NP2 and VP40 genes (10, 11). While only whole blood was evaluated with the FilmArray system, plasma and whole-blood samples were tested with qRT-PCR assays at various times. Results were generated in about 3 h and up to 96 specimens could be analyzed simultaneously. Total RNA was extracted from whole-blood, plasma, or urine specimens by using the bead Retrieval system (Invitrogen, Grand Island, NY) and the MagMax Pathogen RNA/DNA isolation kit (Applied Biosystems, Grand Island, NY). The NP2 qRT-PCR assay was then performed with the extracted nucleic acids, and a cycle threshold ($C_T$) value was reported back to each institution for use in patient care. Cycle threshold values of ≤40 were interpreted as positive. Specimens with no specific amplification or with amplification curves that did not cross the baseline threshold were interpreted as negative. Specimens that yielded positive results with $C_T$ values of >38 in the NP2 assay were confirmed using the VP40 assay and/or an additional serological assay, at the CDC VSPB laboratory (the EUA provides for equivocal interpretation for specimens with $C_T$ values of 38 to 40 and suggests that additional analysis may be required).

Specimens. Contrived specimens were prepared at the VSPB using inactivated ZEBOV at known titers, to examine concordance between the FilmArray BT-E panel, the NP2 qRT-PCR assay, and the VP40 qRT-PCR assay for detection of ZEBOV nucleic acids. ZEBOV (specimen number 812592) at a titer of $4 \times 10^5$ 50% tissue culture infective dose (TCID$_{50}$/ml) was inactivated by gamma irradiation. Ten-fold serial dilutions of the inactivated virus, from $4 \times 10^7$ to $4 \times 10^3$ TCID$_{50}$/ml, and a no-virus negative-control sample were prepared in whole blood. Contrived specimens at each dilution of virus, and the negative-control sample, were tested in duplicate using the FilmArray BT-E panel and the NP2 and VP40 qRT-PCR assays.

Clinical specimens from individuals with EVD who were treated at EU or UNMC were evaluated using the FilmArray BT panel and qRT-PCR assays. Whole-blood ($n = 27$), plasma ($n = 20$), and urine ($n = 13$) specimens were collected and tested at various times throughout the course of patient management. Twenty-seven matched whole-blood specimens and 13 matched urine specimens were tested using the FilmArray BT panel and qRT-PCR testing. An additional 20 whole-blood specimens were tested using the FilmArray BT panel with qRT-PCR testing on matched plasma specimens, for a total of 60 pairwise tests on clinical specimens from patients being treated for EVD. FilmArray BT panel testing was performed at the clinical sites, using whole-blood and urine specimens. Matched clinical specimens were tested by qRT-PCR at the VSPB, once per sample. Only the qRT-PCR results were used for patient management at that time. The interval between on-site FilmArray testing and testing at the VSPB ranged from 24 h before to 5 days after qRT-PCR results were received.

Calculations. Agreement was calculated for FilmArray and qRT-PCR assay results.

Ethics statement. Approval was obtained from the institutional review boards at Emory University and the University of Nebraska.

RESULTS

Ten-fold serial dilutions of inactivated ZEBOV in whole blood, from $4 \times 10^7$ to $4 \times 10^1$ TCID$_{50}$/ml, were tested in duplicate using the FilmArray BT-E panel, the NP2 qRT-PCR assay, and the VP40 qRT-PCR assay, at the VSPB (Table 1). ZEBOV nucleic acids were detected in contrived whole-blood specimens at virus concentrations of $4 \times 10^7$ to $4 \times 10^2$ TCID$_{50}$/ml, resulting in 100% agreement among the 3 ZEBOV tests (6/6 specimens for each titer and 36/36 specimens overall). The VP40 qRT-PCR assay detected ZEBOV nucleic acids in 1 of 2 tests at the $4 \times 10^3$ virus dilution, while ZEBOV nucleic acids were not detected by either the FilmArray panel or the NP2 qRT-PCR assay, which resulted in 83.3% agreement (5/6 specimens) for that dilution. ZEBOV nucleic acids were not detected in whole-blood samples that did not contain inactivated virus, resulting in 100% agreement (6/6 specimens) among the three assays. The limit of detection (LOD), i.e., the lowest virus dilution at which each assay detected ZEBOV nucleic acids in duplicate tests, was determined to be $4 \times 10^1$ TCID$_{50}$/ml for the FilmArray panel and both qRT-PCR assays.

Sixty specimens (47 whole-blood specimens and 13 urine specimens) from individuals with EVD were tested using the FilmArray system (see Table S1 in the supplemental material). Testing of matched whole-blood, plasma, and urine specimens was performed by qRT-PCR testing at the CDC. Twenty-seven whole-blood specimens were tested using both the FilmArray and qRT-PCR assays. An additional 20 whole-blood specimens were tested using the FilmArray system and the corresponding plasma specimens were tested using the qRT-PCR assay. Thirteen urine specimens were also tested using both assays.

Of the 60 specimens tested, ZEBOV nucleic acids were detected in 40 specimens using both FilmArray and qRT-PCR assays, while 12 specimens were negative by both assays (Table 2). Eight discrepant results were noted, with ZEBOV nucleic acid results being
positive by the FilmArray test but negative by the qRT-PCR assay in four cases and positive by the qRT-PCR assay but negative by the FilmArray test in four cases. The specimens that yielded discrepant results included four whole-blood specimens, two plasma specimens (paired with whole-blood specimens), and two urine specimens (see Table S1 in the supplemental material). The overall agreement between FilmArray and qRT-PCR results was 87% (52/60 specimens). The agreements were 85% (23/27 specimens) for whole-blood specimens tested by both the FilmArray and qRT-PCR assays, 90% (18/20 specimens) for whole-blood specimens tested by the FilmArray assay and matched plasma specimens tested by the qRT-PCR assay, and 85% (11/13 specimens) for urine specimens (Table 3).

Two plasma specimens tested ZEBOV negative by qRT-PCR testing but the corresponding whole-blood specimens tested positive by FilmArray testing. To explore this further, a series of six specimens from one of the patients at EU were collected every 24 h for 6 days and tested on the day of collection, using whole blood and plasma obtained from the same collection tube. ZEBOV was detected in whole blood by both the FilmArray and qRT-PCR assays for all six specimens; however, detectable virus was found in only two of the corresponding plasma specimens by FilmArray testing, suggesting a trend toward the virus being cleared from plasma before being cleared from whole blood (Table 4).

DISCUSSION

Rapid, reliable, and easy-to-use assays for the detection of ZEBOV in clinical specimens are needed in response to the unprecedented outbreak in West Africa and the emergence of infected individuals beyond outbreak zones. This study evaluated the FilmArray assay and two qRT-PCR assays for detection of ZEBOV in whole-blood specimens spiked with inactivated virus at known titers and whole-blood, plasma, and urine clinical specimens from 6 individuals with EVD who were treated in the United States.

Parallel testing of contrived whole-blood specimens at virus dilutions of $4 \times 10^2$ to $4 \times 10^6$ TCID$_{50}$/ml revealed 100% agreement between the three diagnostic assays evaluated in this study. The only disagreement noted was a single positive result in VP40 qRT-PCR testing for the dilution of $4 \times 10^5$ TCID$_{50}$/ml, which resulted in 83.3% agreement (5/6 specimens). Detection of the VP40 qRT-PCR target in one sample at a 10-fold lower dilution, compared to FilmArray and NP2 qRT-PCR results, may suggest somewhat greater sensitivity for the VP40 qRT-PCR assay.

Parallel testing of contrived whole-blood specimens with inactivated virus also provided insight into the limits of detection, which, given the concentrations tested, were determined to be $4 \times 10^2$ TCID$_{50}$/ml for all three assays. Inactivation is known to affect nucleic acid integrity and to shift the detection limit, usually about 10- to 100-fold higher, compared to that obtained with viable virus (10, 11). Studies performed by BioFire Defense with the BT-E panel indicated an LOD of $6 \times 10^5$ PFU/ml of whole blood with inactivated ZEBOV (9). BioFire Defense also reported successful BT-E panel detection of a synthetic ZEBOV L-gene RNA template in Tris-EDTA buffer at $1 \times 10^8$ genome equivalents (8). Studies at the CDC documented limits of detection for both the NP and VP40 qRT-PCR assays of $30$ TCID$_{50}$/reaction ($\sim 5,400$ TCID$_{50}$/ml), using inactivated ZEBOV in both whole blood and urine (10, 11). The apparent differences likely have more to do with variations in the harshness of the protocols used to inactivate the virus in the analyte preparations than the ability of the test systems to detect ZEBOV. Our studies did not evaluate the specificity of the assays, but the EUA documentation includes results of extensive studies that were performed with the help of the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) and the U.S. Department of Defense, which confirmed the high analytical specificities of all three tests (9–11). Further, clinical specificity was documented in a study in Sierra Leone in which the FilmArray assay was used for research purposes to test patients and health care workers who had been referred for diagnostic testing (12). In that study, 83 individuals were tested. Six of the individuals tested positive, with 5 of the 6 being confirmed as positive by CDC mobile laboratories using the NP2 and VP 40 tests in Sierra Leone. The remaining patient had a throat swab that tested positive in FilmArray testing, but the patient died with typical EVD symptoms before a specimen for con-

### TABLE 1 Results of FilmArray BioThreat E panel and qRT-PCR testing using serial dilutions of inactivated Zaire ebolavirus at known titers in whole blood

<table>
<thead>
<tr>
<th>Virus titer (TCID$_{50}$/ml)</th>
<th>NP2 results</th>
<th>VP40 results</th>
<th>FilmArray results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test 1</td>
<td>Test 2</td>
<td>Test 1</td>
</tr>
<tr>
<td>40,000,000</td>
<td>Positive ($C_T = 20$)</td>
<td>Positive ($C_T = 20$)</td>
<td>Positive ($C_T = 18$)</td>
</tr>
<tr>
<td>4,000,000</td>
<td>Positive ($C_T = 23$)</td>
<td>Positive ($C_T = 23$)</td>
<td>Positive ($C_T = 22$)</td>
</tr>
<tr>
<td>400,000</td>
<td>Positive ($C_T = 26$)</td>
<td>Positive ($C_T = 26$)</td>
<td>Positive ($C_T = 25$)</td>
</tr>
<tr>
<td>40,000</td>
<td>Positive ($C_T = 30$)</td>
<td>Positive ($C_T = 30$)</td>
<td>Positive ($C_T = 28$)</td>
</tr>
<tr>
<td>4,000</td>
<td>Positive ($C_T = 33$)</td>
<td>Positive ($C_T = 33$)</td>
<td>Positive ($C_T = 32$)</td>
</tr>
<tr>
<td>400</td>
<td>Positive ($C_T = 37$)</td>
<td>Positive ($C_T = 35$)</td>
<td>Positive ($C_T = 34$)</td>
</tr>
<tr>
<td>40</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>0</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

a Zaire ebolavirus (strain 812592) stock at a known titer of $4 \times 10^6$ TCID$_{50}$/ml was inactivated by gamma irradiation prior to testing. EUA-approved NP2 and VP40 qRT-PCR assays and FilmArray BT-E panel testing were performed in duplicate, using 10-fold serial dilutions of inactivated virus in whole blood, at the CDC.

### TABLE 2 Results of FilmArray BioThreat panel and qRT-PCR testing of clinical specimens throughout the course of disease for patients diagnosed with EVD

<table>
<thead>
<tr>
<th>FilmArray result</th>
<th>No. with NP2 qRT-PCR result of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive ($C_T \leq 40$)</td>
</tr>
<tr>
<td>Detected</td>
<td>40</td>
</tr>
<tr>
<td>Not detected</td>
<td>4b</td>
</tr>
</tbody>
</table>

a Testing was performed using both the FilmArray BioThreat panel and the CDC qRT-PCR assay, with whole-blood, plasma, and urine specimens.

b Includes two samples that involved whole blood paired with plasma.

c The $C_T$ values for these four samples were 38, 39, 39, and 39.
firmatory testing could be obtained. Notably, one asymptomatic health care worker tested positive, became symptomatic the following day, and also tested positive by the CDC assay with a blood specimen collected 4 days later. There were 19 asymptomatic individuals who had been exposed to patients with confirmed EVD but tested negative in FilmArray testing, did not meet the suspected EVD case definition, and so were not tested further. Whole-blood specimens from 57 symptomatic patients tested negative in FilmArray testing and were confirmed as negative by the CDC mobile laboratory. During follow-up monitoring, none of those patients developed EVD. One symptomatic patient with a urine specimen that tested negative in FilmArray testing was diagnosed as having EVD on the basis of a blood specimen that was tested 3 days later by the CDC mobile laboratory. This study showed high specificity and perfect correlation between the FilmArray and CDC assays when they were used as diagnostic tests with whole blood as the specimen type.

Testing of clinical specimens in the current study using FilmArray and qRT-PCR assays resulted in overall agreement of 87% (52/60 specimens), with agreements of 85% (23/27 specimens) for whole-blood specimens and 85% (11/13 specimens) for urine specimens. Eight discrepancies between FilmArray and qRT-PCR results were noted, with four from whole-blood specimens, two from plasma specimens, and two from urine specimens, which occurred when EVD was resolving and viral loads were waning, as indicated by patient improvement and qRT-PCR results with high or negative Ct values. Agreement between the FilmArray and NP2 qRT-PCR results, regardless of specimen type, was 100% (30/30 specimens) for testing of clinical specimens obtained initially, during the early stages of disease, and through early recovery in our patients. These specimens all had Ct values of 37 or lower. In the later stages of recovery, there was some variability between the assays. Of the 14 specimens with qRT-PCR Ct values of 38 or higher, 4 were not detected by FilmArray testing. Conversely, of the 16 specimens that tested negative by qRT-PCR assay, 4 had detectable virus by FilmArray testing.

Specimen type, sample volume, handling, and storage are important to consider when interpreting the results of FilmArray and qRT-PCR testing for ZEBOV. Plasma and whole blood were tested with qRT-PCR testing, while only whole blood was evaluated with FilmArray testing. In this study, two discrepancies were noted when plasma specimens tested by qRT-PCR testing were negative for ZEBOV while corresponding whole-blood specimens were positive using FilmArray testing. Evaluation of six additional specimens corroborated the results and showed that this discordance occurred when viral loads were waning, as evidenced by Ct values of 36 or higher. This observation gives some insight into viral kinetics and might suggest that whole blood is a more-appropriate specimen type for ZEBOV detection, since corresponding plasma specimens may not contain virus at detectable levels. This is perhaps not unexpected, since monocytes are infected early in EVD and the plasma compartment clearing first has been shown for other RNA viruses (13, 14). Sample volume is also an important consideration for molecular detection of ZEBOV. We initially performed FilmArray testing with 100 μl of whole blood, but the EUA FilmArray BT-E instructions for use indicate that 200 μl of whole blood should be used (9). We tested two whole-blood specimens using the FilmArray assay to compare input volumes of 100 μl and 200 μl. Positive FilmArray results were obtained with both input volumes. The corresponding Ct values from the NP assay were 33 and 37. Specimens with higher Ct values were not tested. Four whole-blood specimens that tested negative using FilmArray testing were also negative in qRT-PCR testing. Finally, specimen handling and storage are important considerations for molecular detection of ZEBOV. Whole-blood specimens should be collected in plastic collection tubes, with EDTA, sodium polyanethol sulfonate, or citrate as preservative, and tested promptly following collection. If necessary, specimens may be stored for a short time at 4°C or frozen to prevent degradation of the specimens and viral nucleic acids. If shipping is required, then the specimens should be packaged and shipped at 2 to 8°C with cold packs, according to the Department of Transportation recommendations for a category A infectious substance, using an approved courier (15).

The unprecedented outbreak of ZEBOV in West Africa highlights the need for rapid, reliable, and easy-to-use tests for the detection of ZEBOV in clinical specimens. Data presented here suggest that the FilmArray BT and BT-E panels perform comparably to the CDC qRT-PCR assays for rapid detection of ZEBOV in blood and urine specimens from individuals suspected of having EVD. Reduced manipulation of clinical specimens, ease of use, and rapid turnaround time make this an appropriate screening test for health care institutions and public health laboratories that

### TABLE 3 Comparison of FilmArray BioThreat panel and CDC NP2 qRT-PCR assay results for detection of Zaire ebolavirus in whole-blood, plasma, and urine specimens

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>No. of specimens evaluated</th>
<th>No. with result of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RT+/FA+</td>
</tr>
<tr>
<td>Whole blood</td>
<td>27</td>
<td>19</td>
</tr>
<tr>
<td>Plasma</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td>Urine</td>
<td>13</td>
<td>8</td>
</tr>
</tbody>
</table>

* Testing was performed using both the FilmArray (FA) BioThreat panel and the CDC NP2 qRT-PCR (RT) assay.

### TABLE 4 Detection of low-level ZEBOV viremia using whole blood versus plasma, in serial samples from a single patient

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>FilmArray results</th>
<th>Plasma</th>
<th>NP2 qRT-PCR result for whole blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Detected</td>
<td>Detected</td>
<td>Positive (Ct = 36)</td>
</tr>
<tr>
<td>2</td>
<td>Detected</td>
<td>Not detected</td>
<td>Positive (Ct = 36)</td>
</tr>
<tr>
<td>3</td>
<td>Detected</td>
<td>Detected</td>
<td>Positive (Ct = 37)</td>
</tr>
<tr>
<td>4</td>
<td>Detected</td>
<td>Detected</td>
<td>Positive (Ct = 37)</td>
</tr>
<tr>
<td>5</td>
<td>Detected</td>
<td>Not detected</td>
<td>Positive (Ct = 37)</td>
</tr>
<tr>
<td>6</td>
<td>Detected</td>
<td>Not detected</td>
<td>Positive (Ct = 40)</td>
</tr>
</tbody>
</table>

* Testing was performed using both the FilmArray BioThreat panel and the CDC NP2 qRT-PCR assay.
lack qRT-PCR capabilities but need the ability to provide presumptive identification of ZEBOV for individuals suspected of having EVD. According to the EUA, all FilmArray BT-E results should be confirmed by state public health laboratories or the CDC using EUA methods.

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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