Use of Existing Diagnostic Reverse-Transcription Polymerase Chain Reaction Assays for Detection of Ebola Virus RNA in Semen

James Pettitt, Elizabeth S. Higgs, Rick D. Adams, Peter B. Jahrling, and Lisa E. Hensley

Integrated Research Facility, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Frederick, Maryland

Sexual transmission of Ebola virus in Liberia has now been documented and associated with new clusters in regions previously declared Ebola free. Assays that have Emergency Use Authorization (EUA) and are routinely used to detect Ebola virus RNA in whole blood and plasma specimens at the Liberian Institute for Biomedical Research were tested for their suitability in detecting the presence of Ebola virus RNA in semen. Qiagen AVL extraction protocols, as well as the Ebola Zaire Target 1 and major groove binder quantitative reverse-transcription polymerase chain reaction assays, were demonstrably suitable for this purpose and should facilitate epidemiologic investigations, including those involving long-term survivors of Ebola.

Keywords. Ebola; PCR; semen; diagnosis.

In March 2015, an Ebola case was identified in Liberia. The single reported risk factor was unprotected vaginal intercourse with a survivor who had been discharged from an Ebola treatment unit >150 days prior [1]. It was previously determined that Ebola virus (EBOV) is present and can be shed in a wide range of bodily fluids, including semen, during acute disease and that it can persist for months after recovery [2]. However, sexual transmission of Ebola had never been previously documented. As a consequence, the potential for sexual transmission of Ebola in West Africa has become a critical concern [3, 4], and the urgent need to screen survivors for the presence of EBOV RNA in semen specimens has been realized. Unfortunately, many of the assays that have been developed and made available under section 564 of the Federal Food, Drug, and Cosmetic Act, Emergency Use Authorization (EUA) were developed and approved for detection of viral RNA in whole-blood or plasma

specimens. The lack of data and experience using these assays for detection of EBOV RNA in semen specimens raised significant concerns regarding their use in the screening of survivors. To address these concerns, seminal fluid specimens were spiked with live EBOV and analyzed with the Ebola Zaire Target 1 (EZ-1) quantitative reverse-transcription polymerase chain reaction (qRT-PCR) assay [5], and findings were compared with those of EZ-1 qRT-PCR of EBOV-spiked whole-blood specimens.

METHODS

Seminal fluid samples were obtained over a two week period from 6 donors as were blood samples from another 6 donors. Blood samples were collected in 9-mL tubes containing K₃ ethylenediaminetetraacetic acid and stored at 2°C–8°C until use; seminal fluid samples were collected using urine collection cups and stored at room temperature (approximately 25°C) until use. Samples were from healthy donors who were deemed immunocompetent and had not ejaculated in the last 48–120 hours.

EBOV/H.sapiens-tc/GIN/14/WPG-C05 was kindly provided by Dr Gary P. Kobinger, Public Health Agency of Canada. Vero E6 cells (catalog no. NR-596; BEI Resources, Manassas, Virginia) were infected at a multiplicity of infection of 0.001. On day 7 after infection, when the cytopathic effect was visible, virus-containing supernatants were collected and clarified by centrifugation. The resulting EBOV/Mak virus stock (Vero E6 p3) was titrated on Vero E6 cells by plaque assay in 6-well plates before use in these studies.

Semen samples were spiked with logarithmic dilutions of EBOV/H.sapiens-tc/GIN/14/WPG-C05 (EBOV/Mak, GenBank accession no. KP096420) ranging from 10 000 plaque-forming units (PFU)/mL to 10 PFU/mL, along with a negative matrix control for each donor. Spiked Roswell Park Memorial Institute (RPMI) 1640 medium and negative RPMI 1640 medium were also used as cell-free controls. After the dilution schemes were generated, samples were extracted using the Qiagen QIAamp viral RNA mini kit (catalog no. 52906), using procedures outlined in the EUA instruction booklet [5]. RNA samples generated from the dilution samples were tested using the EUA Critical Reagent Program (CRP) EZ1 RT-PCR reagents and assay parameters [5], as well as CRP ribonuclease P (RNAseP) and major groove binder (MGB) reagents and assay parameters [6, 7]. Data were plotted and analyzed using Prism GraphPad. Multiple grouped *t* tests were used, with significance determined using the Holm-Sidak method.

RESULTS

No significant variability was observed in the extractions using the control assay targeting the housekeeping gene, RNaseP

Received 12 August 2015; accepted 3 September 2015; published online 15 September 2015.

Correspondence: P. B. Jahrling, Integrated Research Facility Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, B-8200 Research Plaza, Fort Detrick, Frederick, MD 21702 (jahrlingp@niaid.nih.gov).

The Journal of Infectious Diseases® 2016;213:1237–9

Published by Oxford University Press for the Infectious Diseases Society of America 2015. This work is written by (a) US Government employee(s) and is in the public domain in the US. DOI: 10.1093/infdis/jiv454

Figure 1. Ribonuclease P reverse-transcription polymerase chain reaction assay performed on samples extracted using the Qiagen viral RNA matrices tested were whole blood (wb), semen (s), and Roswell Park Memorial Institute (RPMI) 1640 medium. RPMI 1640 samples did not produce a threshold cycle (CT) value and, consequently, are not represented. Abbreviation: PFU, plaque-forming units.

(Figure 1). Donor threshold cycle (CT) values were constant across the different spiked concentrations, allowing for accurate comparisons between sample matrices. As expected, RPMI 1640 medium controls did not produce CT values in the RNA-seP assay (data not shown).

Comparison of spiked semen and whole-blood specimens from donors demonstrated no significant differences between these sample types (Figure 2). The RPMI 1640 medium control samples had significantly higher dilutions than those for both whole-blood and semen samples. Also of note was the higher assay sensitivity of the RT-PCR using MGB master mix in this study.

DISCUSSION

In this study, we evaluated the suitability of diagnostic assays currently in use at the National Reference Laboratory in Liberia, as well as in other locations in West Africa, for testing semen specimens for the presence of EBOV RNA. The results from these studies demonstrate that these assays were able to detect EBOV RNA in spiked semen and whole-blood specimens with no observed loss in sensitivity. The assumption that performance of these assays is the same for spiked versus authentic samples is reasonable but should be monitored in the field. This study now opens the door to test semen specimens from EBOV survivors for the presence of residual viral RNA and to facilitate epidemiological investigations to rapidly identify sources of new cases and potential at-risk contacts. In addition, these studies suggest that the MGB-1 assay may be more sensitive than the EZ-1 assay for detection of the Makona EBOV variant. Additional studies, which were outside of the scope of this project, will be required to better determine whether the

Figure 2. Comparison of different matrices, using quantitative reverse-transcription polymerase chain reaction analysis. The Ebola Zaire Target 1 (EZ1; A) and major groove binder (MGB; B) assays were compared after extraction using the Qiagen viral RNA kit. Semen ($n = 6$) and whole blood ($n = 6$), along with Roswell Park Memorial Institute (RPMI) 1640 medium ($n = 4$), were compared using t tests to determine the statistical significance of differences at each log dilution; samples were evaluated in duplicate. Data represent findings from 2 separate experiments performed over a 2-week period. Abbreviations: NA, not applicable; PFU, plaque-forming units.

difference in EBOV RNA recovery and detection between the MGB-1 assay and the EZ1 EUA assay is significant. In the future, it would also be desirable to determine the performance of these selected assays with other bodily fluids, especially urine and saliva specimens. In the interim, public health officials and laboratorians can be confident that the existing EZ1 EUA assay performs as well on semen specimens as they do on whole-blood specimens.

Notes

Financial support. This work was supported by the intramural program of the Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health.

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Christie A, Davies-Wayne GJ, Cordier-Lasalle T, et al. Possible sexual transmission of Ebola virus—Liberia, 2015. *MMWR Morb Mortal Wkly Rep* **2015**; 64:479–81.
2. Bausch DG, Towner JS, Dowell SF, et al. Assessment of the risk of Ebola virus transmission from bodily fluids and fomites. *J Infect Dis* **2007**; 196:S142–7.
3. Rogstad KE, Tunbridge A. Ebola virus as a sexually transmitted infection. *Curr Opin Infect Dis* **2015**; 28:83–5.
4. World Health Organization. Interim advice on the sexual transmission of the Ebola virus disease. 8 May **2015**. <http://www.who.int/reproductivehealth/topics/rtis/ebola-virus-semen/en/>. Accessed 12 September 2015.
5. JVAP-MCS. Ebola Zaire (EZ1) rRT-PCR (TaqMan) assay on ABI 7500 Fast Dx, Lightcycler, and JBAIDS: instruction booklet. <http://www.fda.gov/downloads/MedicalDevices/Safety/EmergencySituations/UCM408334.pdf>. Accessed 23 July 2015.
6. Trombley AR, Wachter L, Garrison J, et al. Comprehensive panel of real-time TaqMan polymerase chain reaction assays for detection and absolute quantification of filoviruses, arenaviruses, and New World hantaviruses. *Am J Trop Med Hyg* **2010**; 82:954–60.
7. Sozhamannan S, Holland MY, Hall AT, et al. Evaluation of signature erosion in Ebola virus due to genomic drift and its impact on the performance of diagnostic assays. *Viruses* **2015**; 7:3130–54.