Article DOI: https://doi.org/10.3201/eid3012.241470

Replication-Competent Oropouche Virus in Semen of Traveler Returning to Italy from Cuba, 2024

Appendix

Real-Time Reverse Transcription PCR (RT-PCR) Assays

We used the following specific real-time reverse transcription PCR (RT-PCR) assays from Altona Diagnostics GmbH, Germany:

• For dengue virus (DENV): RealStar Dengue RT-PCR Kit 2.0.

• For chikungunya virus (CHIKV): RealStar Chikungunya RT-PCR Kit 2.0.

• For Zika virus (ZIKV): RealStar Zika virus RT-PCR Kit 1.0.

We performed both an OROV-specific RT-PCR and an RT-PCR modified to target the S segment on whole blood, serum and urine as previously described by Lambert AJ (2009), and Naveca (2017) (*1,2*).

For the OROV RT–PCR analysis in semen, semen samples were collected into sterile cups. An aliquot 140 µL of semen was used for total RNA extraction using QIAamp® RNA viral kit.

Detection of Replication-Competent OROV in Semen

Infectious OROV was obtained from semen in cell culture under BSL-3 conditions. The seminal fluid sample (OROV Ct-value 25.4) was first diluted (1:1) with viral-inoculating-broth (VIB) 1× containing antibiotics. The mixture was kept at room temperature for 30 minutes and then diluted 1:5 in Minimum Essential Medium (MEM) and inoculated onto a Vero E6 cells semiconfluent monolayer. After adsorption (90 minutes), inoculum was removed and replaced with MEM containing 2% FBS and VIB 0.5x. Cytopathic effects were observed after 5 days by light microscopy. Viral replication was confirmed by an increased

OROV-RNA load (Ct-value 14.0) in spent cell growth medium. Subsequent passages were performed.

References

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