Inactivation of Zika Virus on a Hard Non-porous Surface

C. Wilde[1], Z. Chen[1], T. Kapes[1], R. W. Nims[2], S. Zhou[1]
[1]MicroBioTest, [2]RMC Pharmaceutical Solutions

Purpose

This study was conducted in order to provide information on the environmental stability of Zika virus (ZIKV) on a hard non-porous surface and to provide inactivation efficacy information for some commonly employed physical (dry heat) and chemical (disinfectant) inactivation approaches (70% isopropyl alcohol, a quaternary ammonium/alcohol product, chlorine at 500 to 10000 ppm, peracetic acid at 1000 ppm, and pH 4 or pH 10 solution).

Methods

Inactivation was examined in the presence of 5% fetal bovine serum (low organic load) or 90% sheep blood (high organic load). In addition, we have examined the similarities in inactivation susceptibility of ZIKV and other flaviviruses (BVDV, WNV) for which inactivation efficacy results have been reported previously in the literature.

Viruses. Zika virus (strain PRVABC59) was obtained from CDC. Its identity was confirmed by analysis of viral RNA using primers targeting the ZIKV virus capsid protein gene (GenBank accession number AMC13911). The virus was diluted in 1X MEM + 5% fetal bovine serum (FBS) to create a viral suspension. Vero E6 cells were washed with PBS and the viral suspension was added to T-75 flasks. The flasks were incubated at 36°C for 90 min. to allow for viral adsorption after which they were refed with MEM + 5% FBS. The flasks were incubated at 36°C until viral cytopathic effect involved ~90% of the cells within the T flasks. The flasks were frozen at -80°C and then thawed at room temperature. The conditioned medium was clarified at 2,000 rpm for 15 min., and the resulting supernatant was aliquoted and stored at -80°C until use. The certified titer of the stock ZIKV was determined to be 7.25 - 7.50 $\log_{10}$ tissue culture infectious $dose_{50}$ per mL ($TCID_{50}$/mL) in Vero E6 cells.

Surface Inactivation Studies. One physical inactivation approach (dry heat) and seven chemical disinfectants and were evaluated at two levels of organic load (5% serum, 90% blood), each at four contact times in duplicate (n = 2). For each organic load, 0.4 mL of virus was allowed to dry onto a 4-in^2 area of a glass Petrie dish at ambient temperature. After the drying period, 2.0 mL of the chemical disinfectant were added to the dried virus such that it completely covered the film for the duration of the contact time. For the dry heat treatment, the carriers were then placed in an incubator and held at 56 or 60°C for the duration of the contact time. For all disinfectants, contact times of 15 sec., 1 min., 2 min., and 5 min. were evaluated. For dry heat, contact times of 10, 20, 60, and 120 min. at 56 and 60°C were evaluated. At the completion of the contact time being evaluated, 2.0 mL (4.0 mL for the heat treatment) of an appropriate neutralizer were added to the coupon. The mixture was then scraped from the surface of the carriers using a cell scraper. This post-neutralized sample was considered to be a 10-fold dilution of the original virus stock.

Cell Infectivity Assays. The infectious virus titer of test and control samples was determined by generation of viral cytopathic effect in host cell cultures (Vero E6 for ZIKV, MDBK for BVDV, and Vero for WNV). Selected dilutions of test and control post-neutralized sample were inoculated onto host cell cultures (8 wells/dilution) and the culture plates were incubated at 36±2°C with 5±1% CO_2 for 7–14 days. The host cells were then examined microscopically for the presence of cytopathic effect or for evidence of cytotoxicity due to disinfectants. The cytopathic effect results were used to calculate the virus titer of the challenge virus stock of PNS in units of $TCID_{50}$/mL.

Results

ZIKV suspended in a matrix consisting of 90% blood and dried onto carriers displayed a minimal rate of inactivation (~0.06 $\log_{10}$ per hour) over the 8-hour period, while virus suspended in culture medium containing 5% FBS and dried onto carriers displayed inactivation at the rate of ~0.5 $\log_{10}$ per hour over the same 8-hour period. Results of surface inactivation studies performed with ZIKV alongside other flaviviruses such as BVDV and WNV indicate that in most respects, ZIKV displays susceptibility to commonly employed disinfectants similar to that of other flaviviruses. The only difference in susceptibility identified in these studies is in regard to the dry heat susceptibility of ZIKV at 56°C. In this respect, ZIKV appears to be less susceptible than BVDV or WNV.

Conclusion

These data suggest that disinfectant efficacy established on the basis of activity for BVDV or WNV should be applicable to ZIKV as well. Our results for ZIKV complement other recent reports describing efficacy of chemical and physical inactivation approaches for this newly emergent virus.