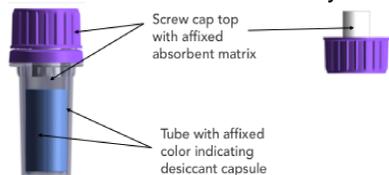


Recovery of Live Virus from ViveST™ After Storage at Ambient Temperature

Introduction

Major barriers to performing virological field studies in developing nations are the lack of ultralow freezers and the difficulty of shipping frozen samples. Traditionally, biological samples could be shipped dried on filter paper or FTA cards; however, viruses often lost viability. A recently developed product, ViveST™, provides the ability to store up to 1mL of biological specimen on a synthetic matrix at ambient temperature for use in specimen storage and transportation. The matrix is housed on the screw-cap of a tube such that the sample is self-contained during storage and shipping. This product has been approved for the recovery of viral nucleic acids from plasma and cell culture supernatants, but its applications as a viral recovery device have not been evaluated. Here we report on the capacity of the ViveST™ to preserve live virus at ambient temperature.

The ViveST™ Nucleic Acid Recovery Device



Methods

Viruses

- Enteric Cytopathic Human Orphan virus 3 (ECHOV)
- Human Rhinovirus 15 (HRV)
- Human Coxsackievirus B5 (CBV)
- Herpes simplex virus 1 (HSV)
- Dengue virus serotype 2 (DENV)
- Human Adenovirus 14 (HAdV)

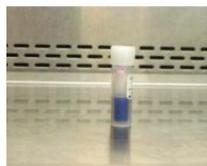
Cells

- Human lung cells, A549
- Rhesus macaque kidney cells, LLC-MK2
- Human cervical cells, HeLa
- African green monkey kidney cells, Vero
- Canine kidney cells, MDCK

1) Infect column with 500ul sample



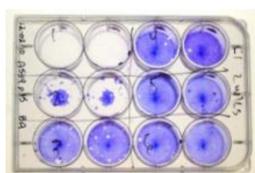
2) Store sample



3) Rehydrate sample with 500ul water



4) Titer sample



Results

ViveST™ Preserves HAdV for 4 Weeks

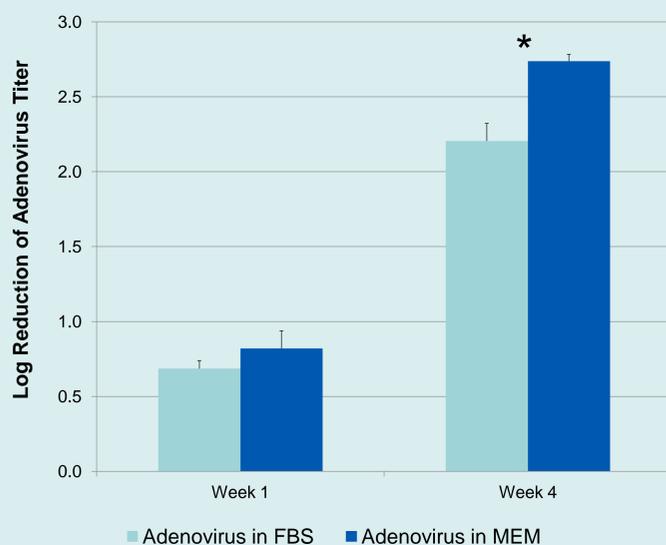


Figure 1. ViveST™ preserves human adenovirus for 4 weeks at ambient temperature. Mean log reduction of HAdV titer after 1 week and 4 weeks stored at room temperature on the ViveST™ column with two media. At a 95% confidence limit, there was a significant difference (*) between fetal bovine serum (FBS) and minimum essential medium (MEM) at week 4, but not at week 1. Error bars represent 1 SEM of log reduction for 6 replicates.

ViveST™ Preserves Virus at Ambient Temperature

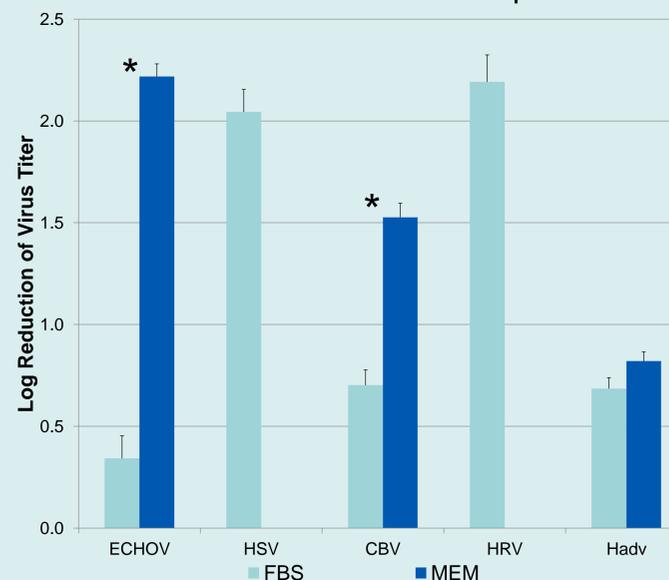


Figure 2. ViveST™ preserves virus for 1 week at ambient temperature. Mean log reduction of titer for 5 viruses stored for 7 days at room temperature on the ViveST™ column. At a 95% confidence limit, there was a significant difference (*) between fetal bovine serum (FBS) and minimum essential medium (MEM) for 2 viruses. Error bars represent 1 SEM of log reduction for 3 replicates.

Table 1: Mean log reduction (±SEM) of viral titer for six viruses after 7 days at ambient temperature in the ViveST™ viral recovery device. Each virus was evaluated in triplicate in both fetal bovine serum (FBS) and minimum essential medium (MEM).

Virus	Mean log reduction of virus titer in FBS	Mean log reduction of virus titer in MEM
ECHOV*	0.34 (±0.1)	2.19 (±0.06)
HSV	2.04 (±0.1)	No virus recovered
CBV*	0.70 (±0.08)	1.53 (±0.07)
HRV	2.19 (±0.1)	No virus recovered
HAdV	0.69 (±0.05)	0.82 (±0.05)
DENV	No virus recovered	No virus recovered

*significant difference (95% CL) between FBS & MEM for this virus.

Discussion

The application of ViveST™ as a viral recovery device should not be based on a single pathogen as viral morphology and physiology are as diverse as the syndromes they cause.

Significant quantities of most of the viruses tested were recovered from ViveST™ after 7 days storage at ambient temperature. With a log or less in reduction of titer from the original stock, the ViveST™ may be an economical alternative for obtaining specimens in remote areas or in the absence of cold chains. Non-enveloped viruses had a higher rate of recovery than enveloped viruses. DENV was not preserved. This may be due to the fact that the envelope glycoproteins of DENV mediate fusion with target cells via molecular and structural changes and are required to remain intact for infection to occur.

Virus stored in FBS exhibited a higher rate of recovery than virus stored in MEM which may be due to the physical properties of FBS or the action of one or more of its constituents. Similar performance of FBS has been observed in cryopreservation. Perhaps the data presented here reflect a similar phenomenon.

Conclusion

- Preliminary viral titer studies indicate that ViveST™ preserved several types of live virus for periods ranging from 1 to 4 weeks at ambient temperature.
- These data suggest that ViveST™ might be useful in field specimen collection scenarios where ultra-cold storage is not available.

Acknowledgements

Dengue virus (DENV) serotype 2 was kindly provided by Dr. Barry Alto of the Florida Medical Entomology Laboratory. Human adenovirus type 14p (HAdV), a prototype strain, was kindly provided by Dr. Dean Erdman of the Centers for Disease Control and Prevention (CDC). This work was supported by grants from the US Department of Defense Armed Forces Health Surveillance Center's Global Emerging Infections Surveillance and Response Program (I0179_10_UN Dr. Gray) and from the National Institute of Allergy and Infectious Diseases (R01 AI068803-Dr. Gray).

