6694

Determination of Requirements to Ensure Effectivity Inactivation of Powassan virus as a Surrogate BSAT agent

Beth Flores[1], David Einfeld, Jennifer Gibbons[1], Rebecca Bradford[1], Helen Navin[1], Anna Ciera Albrecht[1], Michael Parker[1], Sujatha Rashid[1]
[1]ATCC Federal Solutions, Manassas, VA 20110 (Contact: bflores@atcc.org)

Abstract

To ensure Biological Select Agents and Toxins (BSAT) are effectively inactivate, it is imperative to employ best practices in the development, validation, production, authentication, inactivation, traceability, and disposition of the material. Inactivated BSAT should be subject to the highest level of oversight and confirmation testing due to the potential risk of incompletely inactivated pathogens in downstream use under resourced containment. Implementing inactivation provisions and methods for diverse agents has proven challenging since the effectiveness of the inactivation procedures can differ greatly between agent and sample matrix types. In this project, we determined inactivation protocols (inactivation by heat, chemical, or ŷ-irradiation) and pinpoint critical inactivation parameters for surrogate BSAT pathogen, Langat virus in pilot studies and Powassan virus for validation studies. Inactivation method parameters for Langat virus were then validated by treatment of multiple replicate samples. Parameter set points for the validation study were selected above the minimal effective pilot study parameters using increased dose exposure time, elevated temperature, or chemical concentration. The validated inactivation methods were then tested for effectiveness on Powassan virus (strain:LB). Powassan virus, while not a select agent, was used in place of Tick-borne encephalitis virus as a second closely related surrogate in the Flavivirus family. Using the surrogate method validation/verification test approach, we have identified parameters for the three different methods of inactivation of Powassan virus. The validated heat inactivation method parameters determined for the Langat strain were successfully transferred and verified on the BSAT surrogate Powassan virus. Formalin inactivation of Langat virus was accomplished using centrifugal filter units for buffer exchange of formaldehyde with PBS following treatment, and the inactivation method was successfully applied to Powassan virus. Finally, ŷ-irradiation doses were validated with Langat virus and effective parameters were successfully transferred and verified on Powassan virus.

Method

Based on published and internal data, set points for temperature, time and concentration was established for the initial pilot study (Table 1). Inactivation was determined by observation of CPE in permissible host cells, plaque formation in culture and qPCR. Langat virus does not form consistent plaques, so a $TCID_{50}$ assay was utilized for quantification.

Virus production/titration: For the pilot and validation study, surrogate Langat virus, strain: TP21 (BEI Resources, NR-51658) was grown on Vero E6 cells (ATCC©, CRL-1586), clarified after harvest and frozen in aliquots. Titration in the same cell line yielded a titer of $6.45x10^6$ $TCID_{50}$/mL.
For verification study, Powassan virus, strain LB (BEI Resources, NR-51181), was grown on Vero cells (ATCC© CCL-81), clarified after harvest and frozen in aliquots. Titration in Vero E6 cells (ATCC© CRL-1586) yielded a titer of 8.89×10^5 $TCID_{50}$/mL.

Infectivity detection by CPE: Inactivated material was passaged on host cells and observed for 14 days. CPE (syncytia, cell rounding) was graded on a 0 to 4+ ranking system and was used as a means of detecting viral infection. Pilot study material was passaged once, and validation/verification study material was passaged twice.

Infectivity detection by $TCID_{50}$: Inactivated material was serially diluted and plated on host cells. Plates were incubated and observed daily, scored for CPE.

Confirmation of inactivation by qPCR: Each replicate was sampled on day 0 and day 14, extracted (QIAGEN, QIAamp® Viral RNA Mini Kit). Increasing Ct values from day 0 to day 14, as evidence of non-replicative virus, were used to confirm viral inactivation (Invitrogen, SuperScript™ III Platinum™One-Step qRT-PCR, primers and probe developed in house).

Table 1: Inactivation Parameters.

Study	Heat Inactivation		Chemical Inactivation		y-irradiation
	Temperature (˚C)	Time (minutes)	Concentration (%)	Time (hour)	Dose (Mrad)
Pilot	50	10, 20, 30, 40	0	2	0.2
	55	10, 20, 30, 40	0.5	2	0.5
	60	10, 20, 30, 40	1.0	2	1
	26	40	1.5	2	2
			2.0	2	Room control
Validation/ Verification	**60**	**30**	**2**	**24**	**1**

Powassan virus Validation Study/Powassan virus Verification Study:

Validation of surrogate Powassan virus, LB inactivation was confirmed using 10 replicates each for heat inactivation, chemical inactivation and y-irradiation (Table 1). Two passages of each treated material were performed to allow for delayed amplification of virus and to increase detection sensitivity to ensure recovery of any viable virus. Detection was determined by CPE and PCR for both passage 1 and passage 2.

Ten independent replicates of Powassan virus were inactivated per method using the same parameters as the Langat virus validation. (Table 1).

Results

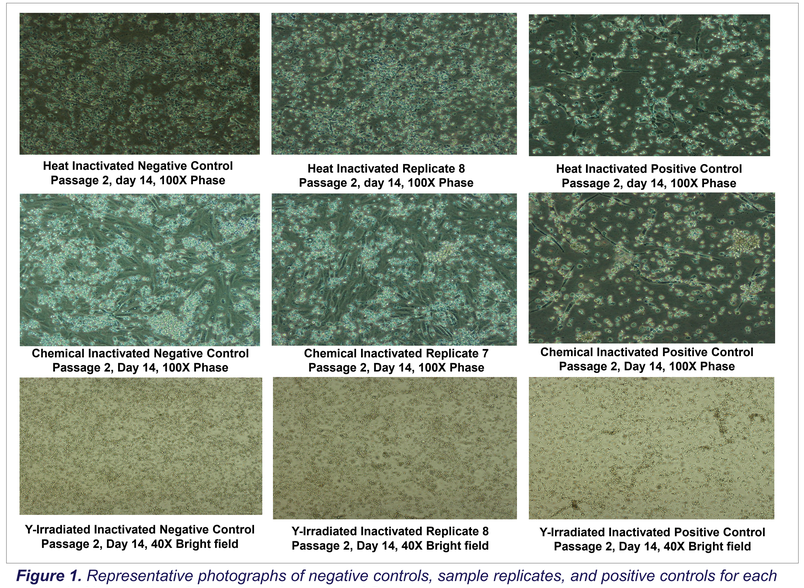
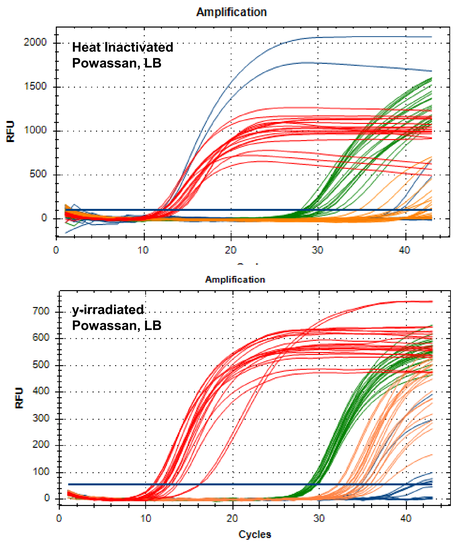


Figure 1. *Representative photographs of negative controls, sample replicates, and positive controls for each method of inactivation of Powassan virus, strain: LB.*



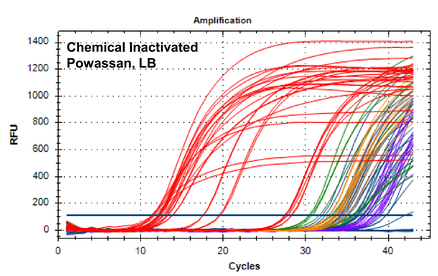


Figure 2. *qPCR of passage 1 and 2 of inactivated Powassan, LB following innocuity.*

Replicate Ct trace key:
Positive control-red
Passage 1 day 0-purple
Passage 1 day 14-green
Passage 2 day 14-orange
Negative control-navy blue.

Conclusions

Inactivation methods determined during surrogate validation studies were successfully used to inactivate select agents with no modifications to parameters and protocols.

The sensitivity of methods used to determine inactivation must be considered when developing protocols for validation purposes. Interpretation of CPE alone can be highly subjective due to degradation of cell monolayer over time, which may lead to false-positives. These studies require confirmatory methods, such as qPCR, to affirm the results.

Multiple passages of treated material with prolonged incubations should be used to identify low levels of virus that may survive inactivation methods.

Future studies would include assessment of inactivated material using applicable molecular, antigenic, or biochemical tests to determine retention of sufficient molecular integrity for research use.

Acknowledgements

This work was supported by the Centers for Disease Control and Prevention (CDC) Division of Select Agents and Toxins (DSAT), contract number 75D30118C03537, Public Health Emergency Preparedness and Response Applied Research (PHEPRAR), Topic 1: Determining Requirements to Ensure Entities Have Effectively Inactivated Biological Select Agents and Regulated Nucleic Acids.

Contributor of virus products to BEI Resources: BJ Russell, Centers for Disease Control and Prevention, Division of Vector-Borne Diseases, Arbovirus Reference Collection, Fort Collins, Colorado, USA.

BEI Resources is funded by the National Institute of Allergy and Infectious Diseases (NIAID) and managed by ATCC under contract HHSN272201600013C.

AFS 10801 University Boulevard, Manassas, VA 20110-2209 **Phone:** 800.638.6597 **Email:** atccfederalsolutions@atcc.org **Web:** www.atcc.org