Abstract

To ensure Biological Select Agents and Toxins (BSAT) are effectively inactivated, it is imperative to employ best practices in the development, validation, production, authentication, inactivation, traceability, and ultimate disposition of the material. Inactivated BSAT should be subject to the highest level of oversight and confirmation testing possible due to the potential risk of incompletely inactivated pathogens in downstream use under reduced containment. Implementing inactivation provisions for diverse agents and inactivation methods (e.g., heat, chemical, irradiation) has proven challenging since the effectiveness of the inactivation procedure and the viability testing approach used differs greatly between agents and test samples.

In this project, we determined inactivation protocols for inactivation by heat, chemical or y-irradiation and pinpoint critical inactivation parameters for Venezuelan equine encephalitis virus (VEEV). TC-83 was used for pilot studies. Inactivation method parameters for VEEV were then validated by treatment of multiple replicate samples. To incorporate a safety margin, individual parameter set points were selected above effective pilot study parameters such as increased dose exposure time, elevated temperature or chemical concentration. The validated inactivation methods were then tested for effectiveness on a select agent strain (VEEV, Beck/Wycoff).

Using the surrogate method validation/verification test approach, we have identified parameters for three different methods of inactivation of VEEV. The validated heat inactivation method parameters determined for the surrogate VEEV, TC-83, were successfully transferred and verified on the select agent strain VEEV, Beck/Wycoff. Formalin inactivation of VEEV, TC-83 was accomplished using centrifugal filter units for buffer exchange of formaldehyde with PBS following treatment, and the inactivation method was successfully applied to VEEV, Beck/Wycoff. Finally, y-irradiated doses were validated with VEEV, TC-83 and effective parameters were successfully transferred and verified on the select agent VEEV, Beck/Wycoff.

Method

Set points for temperature, time, and concentration were established using published literature and internal data (Table 1). Inactivation was determined through CPE observation in the same cell line and was used as a means of detecting viral infection. Pilot study material was passaged once, and validation/verification material was passaged twice.

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 with CPE of 4+ correlating with 90% or greater of infected cells.

Infectivity detection by plaque assay: Inactivated material was serially diluted and plated on host cells. Following adsorption, an overlay was added, and plates were incubated for 2 days prior to staining. Plaques were counted and recorded to determine the limit of detection.

Conclusion of inactivation by qPCR: Each replicate was sampled on day 0 and day 14, and 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 (multiplicon, SuperScript™ III Platinum One-Step qRT-PCR, primers and probe developed internally).

Table 1: Inactivation Parameters.

<table>
<thead>
<tr>
<th>Study</th>
<th>Heat Inactivation</th>
<th>Chemical Inactivation</th>
<th>y-irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temperature (°C)</td>
<td>Time (minutes)</td>
<td>Concentration (%)</td>
</tr>
<tr>
<td>Pilot</td>
<td>55</td>
<td>0, 10, 20, 30, 60</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0, 10, 20, 30, 60</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>0, 10, 20, 30, 60</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>0, 10, 20, 30, 60</td>
<td>1.5</td>
</tr>
<tr>
<td>Validation/ Verification</td>
<td>65</td>
<td>30</td>
<td>2.0</td>
</tr>
</tbody>
</table>

VEEV, TC-83 Validation Study/VEEV, Beck/Wycoff Verification Study:

Validation of surrogate VEEV, TC-83 inactivation was confirmed using 8 replicates for heat inactivation and 10 replicates each for chemical 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 passages and plaque infection for passage 1.

Ten independent replicates of VEEV, Beck/Wycoff were inactivated per method using the same parameters as the VEEV, TC-83 validation (Table 1).

Results

Infectivity detection by qPCR:

A. VEEV, Beck/Wycoff, passage 1, day 14, 100X Phase
B. VEEV, Beck/Wycoff, passage 2, day 14, 100X Phase
C. y-irradiated VEEV, Beck/Wycoff plaque assay (A) following heat inactivation, (B) controls for heat inactivation, (C) following y-irradiation, (D) controls for y-irradiation. Treated material diluted: neat (undiluted), 1:5, 1:25, NC. Control dilutions: 1:100,000, 1:1,000,000, 1:10,000,000.

Conclusions

Inactivation methods determined during surrogate (VEEV, TC-83) validation studies were successfully used to inactivate select agent (VEEV, Beck/Wycoff) with no modifications to the parameters and protocols.

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.

The following reagents were obtained through BEI Resources, NIAID, NIH: NR-63, NR-21720.

© ATCC 2019. The ATCC trademark, trade name, any and all ATCC catalog numbers listed in this publication are trademarks of the American Type Culture Collection unless indicated otherwise.