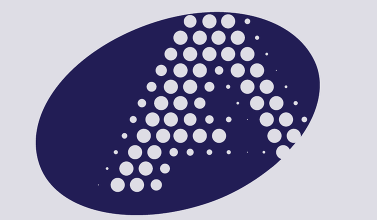


ATCC®

Credible leads to Incredible™

AFS ATCC FEDERAL SOLUTIONS

Determined Requirements to Ensure the effectivity of Inactivated SARS coronavirus

Beth Flores, Jennifer Gibbons, Sujatha Rashid, Rebecca Bradford, Timothy Stedman, Helen Navin, Anna Ciera Albrecht, Adam Wood

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) had 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 (for inactivation by heat, chemical, or y-irradiation) and pinpoint critical inactivation parameters for SARS-CoV-2 using pilot studies. Inactivation method parameters for SARS-CoV-2 were then validated by treatment of multiple replicate samples. Parameter setpoints 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 the select agent virus (SARS-CoV, Urbani).

Using the surrogate method validation/verification test approach, we have identified parameters for three different methods of inactivation of SARS coronaviruses. The validated heat inactivation method parameters determined for the surrogate SARS-CoV-2 strain were successfully transferred and verified on the select agent SARS-CoV. Formalin inactivation of SARS-CoV-2 was accomplished using centrifugal filter units for buffer exchange of formaldehyde with PBS following treatment, and the inactivation method was successfully applied to SARS-CoV. Finally, y-irradiation doses were validated with SARS-CoV-2 and effective parameters were successfully transferred and verified on the select agent SARS-CoV, Urbani.

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.

Virus production/titration: For the pilot and validation study, SARS-CoV-2/USA-WA1/2020 (BEI Resources, NR-52281) was grown on Vero E6 cells (ATCC©, CRL-1586), clarified after harvest, for a final titer of 6.45×10^6 $TCID_{50}$/mL.
For verification study, SARS-CoV, Urbani (BEI Resources, NR-15418), was grown on Vero E6 cells (ATCC© CRL-1586), clarified after harvest, for a final titer of 2.81×10^7 $TCID_{50}$/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 plaque assay: Inactivated material was serially diluted and plated on host cells. Following adsorption, an overlay was added, and plates were incubated for 3 days prior to staining. Plaques were counted and recorded to determine the limit of detection.

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 were used as confirmation of viral inactivation (Invitrogen, SuperScript™ III Platinum™One-Step qRT-PCR, primers and probe developed in house).

Table 1: Inactivation parameters.

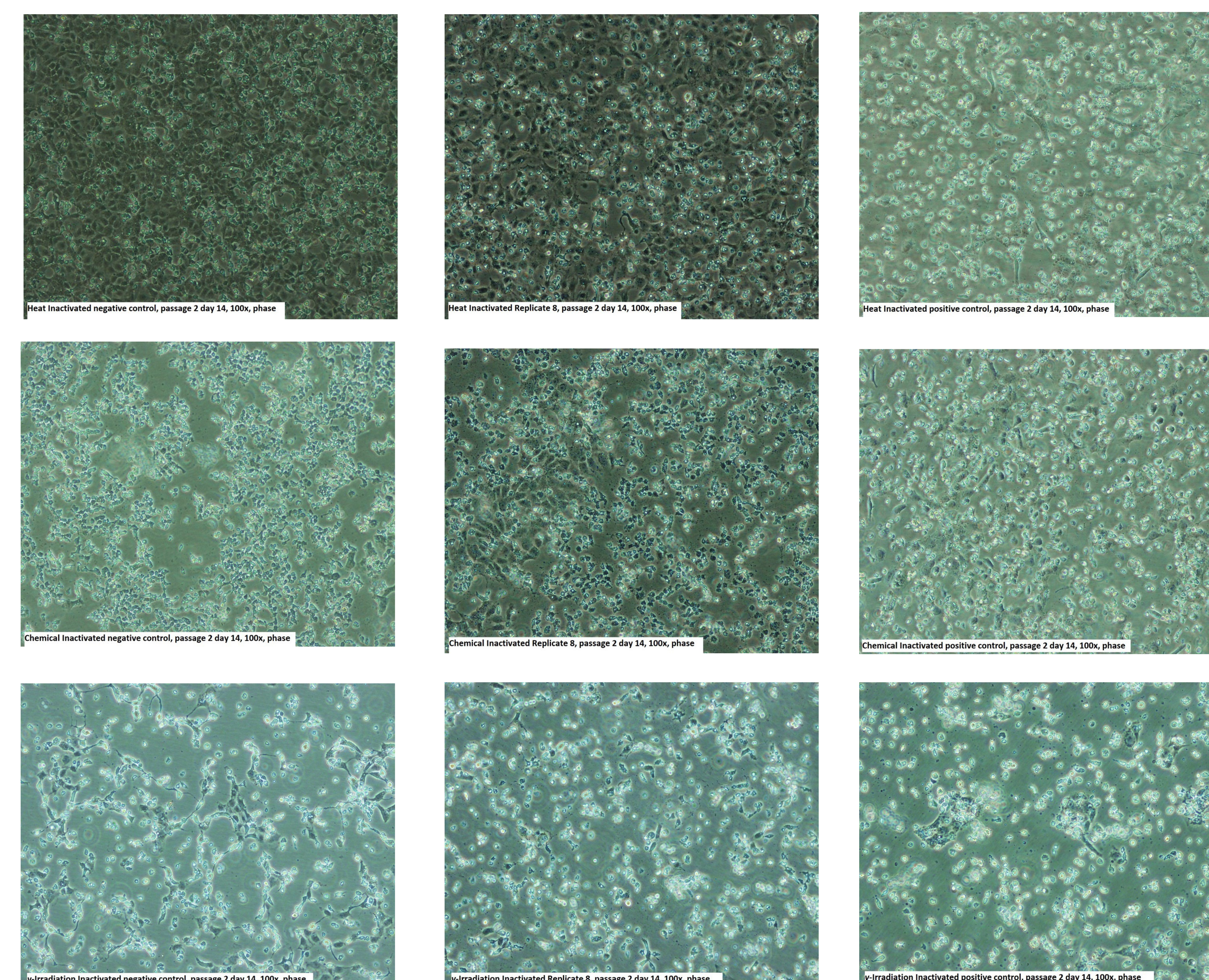
Study	Heat Inactivation		Chemical Inactivation		y-irradiation
	Temperature (˚C)	Time (minutes)	Concentration (%)	Time (hour)	Concentration (Mrad)
Pilot	50	30	0	2	0.2
	55	5, 10, 15, 20, 25	0.1	2	0.5
	60	5, 10, 15	0.25	2	1
	26	30	0.50	2	2
			1	2	Room control
Validation/ Verification	**65**	**30**	**0.5**	**4**	**2**

SARS-CoV-2 Validation Study/SARS-CoV Verification Study:

Validation of SARS-CoV-2 inactivation was confirmed using 6 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 SARS-CoV were inactivated per method using the same parameters as the SARS-CoV-2 validation (Table 1).

Results



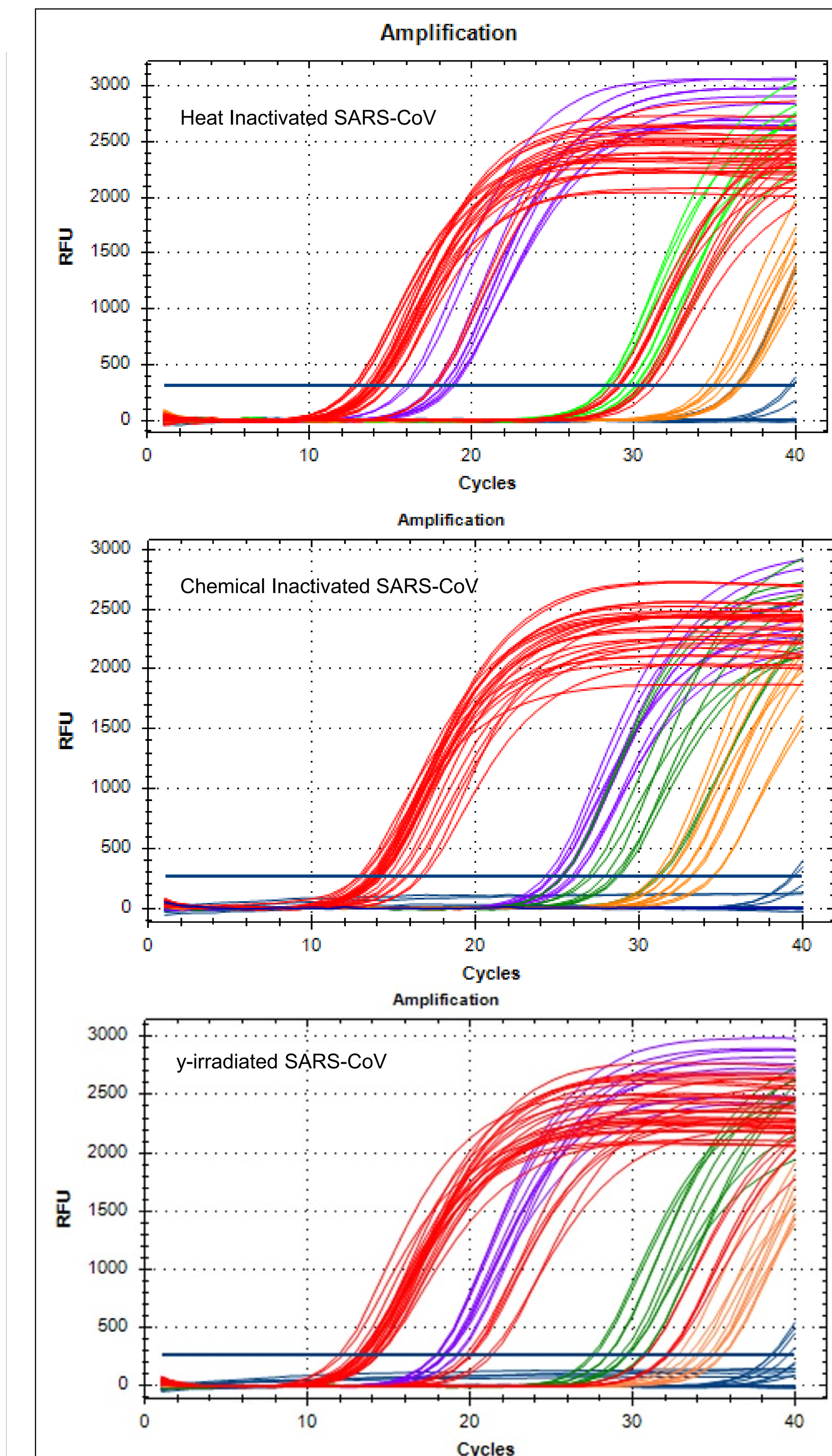


Figure 1. qPCR of passage 1 and 2 of inactivated SARS-CoV, Urbani following innocuity. Positive control-red, passage 1 day 0-purple, passage 1 day 14-green, passage 2 day 14-orange, negative control-navy blue.

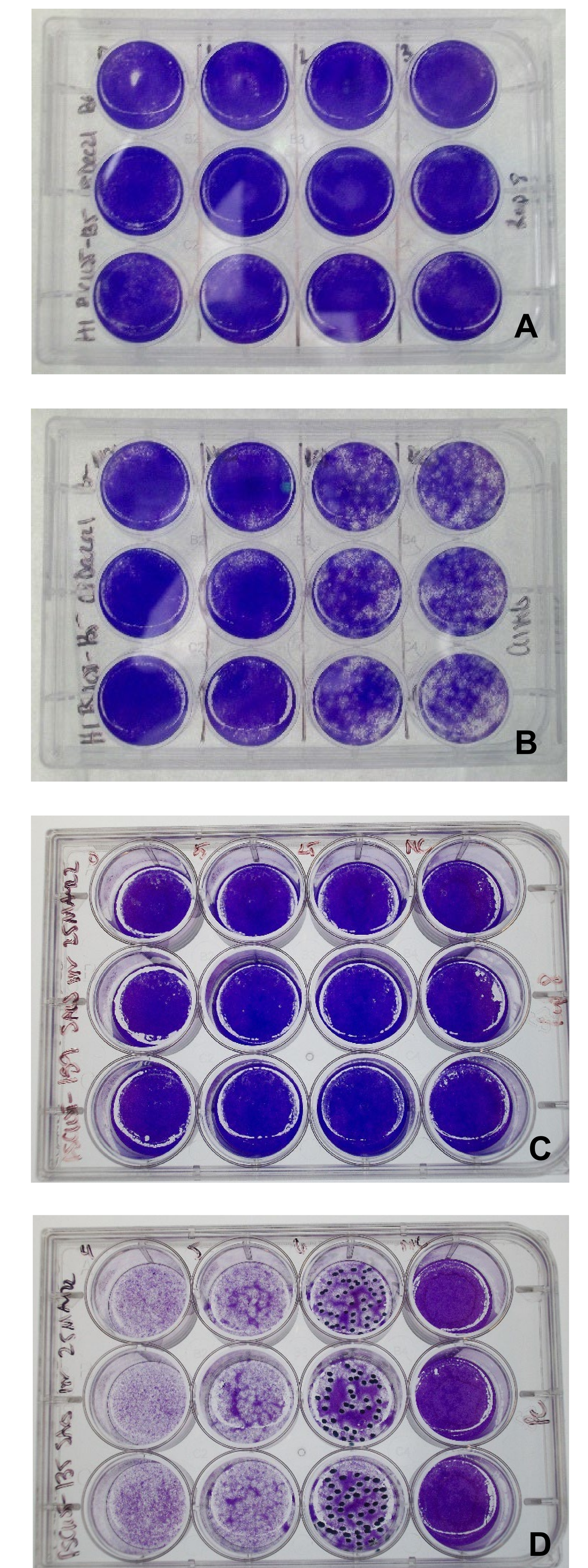


Figure 2. SARS-CoV plaque assays (A) following heat inactivation, (B) controls for heat inactivation, (C) following y-irradiation, and (D) controls for y-irradiation. Treated material dilutions: neat (undiluted), 1:10, 1:100, 1:1000. Control dilutions: 1:10,000, 1:100,000, 1:1,000,000.

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.
Potential 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.

ATCC 10801 University Boulevard, Manassas, Virginia 20110-2209 **Phone:** 800.638.6597 **Email:** sales@atcc.org **Web:** www.atcc.org

© 2018 American Type Culture Collection. The ATCC trademark and trade name, and any other trademarks listed in this publication are trademarks owned by the American Type Culture Collection unless indicated otherwise.