Clinical Virology Symposium
Presentation: 5
September 11, 2023

DEVELOPMENT OF INACTIVATED SARS-COV-2 CONTROL FOR ANTIGEN-BASED ASSAYS

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ABSTRACT

BACKGROUND: Rapid antigen tests for SARS-CoV-2 were an important tool during the COVID-19 pandemic because of their significant cost advantage and ease of use. Effective and efficient development and validation of these tests require high-quality control materials that have proven performance on this test method. The objective of this study was to develop an inactivated SARS-CoV-2 control for use on antigen-based assays.
METHODOLOGY: We developed an irradiation-based inactivation protocol that sought to provide complete inactivation of the virus and improved retention of viral protein over heat inactivation. Infectivity assay was used to confirm the absence of infectious virus, while ELISA was used to quantify nucleocapsid protein (N protein) in inactivated SARS-CoV-2 samples. PROtrol SARS-COV-2 samples were further evaluated for reactivity on four commercially available lateral flow immunoassay tests (LFIA).
RESULTS: The infectivity assay showed that inactivated samples did not exhibit any cytopathic effects (CPE), thus indicating the absence of infectious virus. The N protein was measured in infectious (CF), irradiated (PROtrol), and heat-inactivated (CFHI) culture fluids. (Figure 1) The results demonstrate that when compared to infectious CFs, PROtrol products show superior retention of SARS-CoV-2 N protein immunoreactivity than CFHIs. Retention of the N protein reactivity was further demonstrated through consistent and predictable reactivity on 4 commercially available SARS-COV-2 LFIAs.
CONCLUSION: This study demonstrates that we have successfully developed PROtrol™ SARS-CoV-2, an inactivated control for antigen-based assays. Irradiation effectively inactivated the virus while preserving protein structure better than heat inactivation.
The infectivity assay confirmed the absence of infectious virus, and ELISA detected nucleocapsid protein in the inactivated samples.

INTRODUCTION

The COVID-19 pandemic prompted the rapid development of diagnostic assays for the detection of SARS-COV-2. This included both molecular tests designed to detect SARS-COV-2 RNA and lateral flow immunoassays designed to detect SARS-COV-2 antigens. Rapid antigen tests, specifically, were an important tool during the COVID-19 pandemic because of their significant cost advantage and ease of use. Effective and efficient development and validation of these tests required high-quality control materials that have proven performance on this test method. To support the growing use of these tests it was important to develop inactivated non-infectious virus derivatives that could be used by laboratories and assay manufacturers. The objective of this study was to develop an inactivated SARS-CoV-2 control for use in the development and validation of antigen-based assays.

METHODOLOGY

We developed an irradiation-based inactivation protocol that sought to provide complete virus inactivation and improved retention and integrity of viral proteins than heat inactivation. We confirmed the absence of infectious virus by infectivity assay and quantified nucleocapsid protein (N protein) in inactivated samples using ELISA. The study was performed at ZeptoMetrix LLC, Buffalo NY.

Objectives:

- To evaluate the efficacy of irradiation-based inactivation protocol for SARS-CoV-2
- To demonstrate the absence of infectious virus after irradiation using infectivity assay
- To detect the N protein in inactivated SARS-CoV-2 samples using Enzyme-Linked Immunosorbent Assay (ELISA)
- To demonstrate performance of PROtrol SARS-COV-2 on commercially available lateral-flow immunoassays (LFIA)

Methods:

Two independent lots of SARS-COV-2 infectious Culture Fluid (Isolate: USA-WA1/2020) and 1 lot of each variant (Delta, Omicron & Omicron BA.5) were inactivated using irradiation (PROtrol) and heat-based inactivation protocols.

All samples were evaluated for viral infectivity using the Tissue Culture Infectious Dose 50 (TCID50) Assay which monitored cytopathic effect (CPE) in VeroE6 cells for 7 days.
The SARS-COV-2 N protein was evaluated using an ELISA developed at ZeptoMetrix. SARS-COV-2 live culture fluid (CF), irradiated (PROtrol), and heat-inactivated (CFHI) culture fluids, were evaluated for N protein concentration.

PROtrol SARS-COV-2 samples were further evaluated for reactivity on four commercially available lateral flow immunoassay tests from iHealth, On/Go, Abbot (BinaxNOW) and Siemens (Clinitest). PROtrol samples were serially diluted (1:100 to 1:256,000) into PBS (phosphate-buffered saline). 50uL of diluted PROtrol was dispensed into tubes and collected using the swabs from each test. The test was performed according to the manufacturer's instructions. Dilutions were tested until a negative test was observed. The last dilution that tested positive was reported as the end-point dilution. Endpoint titration in pre-inactivation TCID50/mL and ng/mL were estimated using the values reported on the Certificate of Analysis. (Table 1)

RESULTS

Inactivated samples did not exhibit any cytopathic effects (CPE) in the infectivity assay, thus indicating the absence of infectious virus and complete inactivation.

The ELISA showed a notable reduction in N protein concentration after heat inactivation (CFHI). Conversely, the N protein levels remained largely unaffected in irradiated (PROtrol) samples when compared to the levels observed in the infectious CF. The PROtrol samples exhibited superior retention of SARS-CoV-2 N protein immunoreactivity compared to CFHIs (Fig-1).

Retention of the N protein reactivity was further demonstrated through consistent and predictable reactivity on 4 commercially available SARS-COV-2 lateral flow immunoassay tests. We observed that the mean endpoint titer of the 5 PROtrol samples met or exceeded the TCID50 limit of detection of each of the commercial kits evaluated. Furthermore, PROtrol samples exhibited more consistent performance when endpoint titration was evaluated using the N protein concentration (Table 2).

Further investigation is needed to understand the relationship between TCID50 and N protein concentration of SARS-COV-2 as titer increases.

FIGURES AND TABLES

Sample Part #	Virus/Variant	Lot	N Protein (ng/mL)	Pre-inactivation TCID50/mL
PROSARS(COV2)-587	SARS-CoV-2 USA-WA1/2020	Lot 1	2774	8.17E+05
PROSARS(COV2)-587	SARS-CoV-2 USA-WA1/2020	Lot 2	956	2.97E+04
PROSARS(COV2)-624	B.1.617.2; Delta Variant	Lot 3	1110	9.87E+04
PROSARS(COV2)-642	B.1.1.529; Omicron Variant	Lot 4	1085	2.72E+04
PROSARS(COV2)-658	BA.5; Omicron Variant	Lot 5	1260	6.08E+05

TABLE 1 Reported testing data for PROtrol Samples used in the study

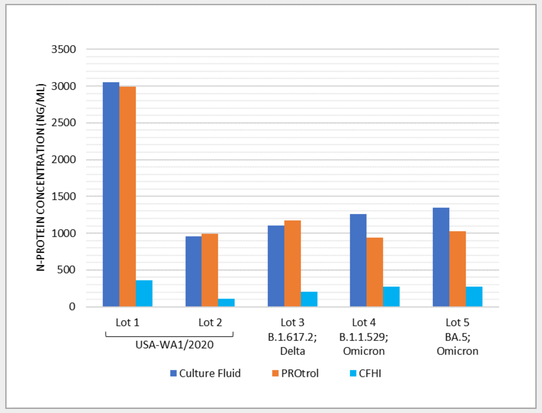

FIGURE 1 Two lots of SARS-COV-2 (USA-WA1/2020) and 3 SARS-COV-2 variants were evaluated for nucleocapsid protein concentration by ELISA under different conditions including culture fluid (CF), irradiated (PROtrol), and heat-inactivated (CFHI) culture fluids.

	Titer (TCID50)			Protein Concentration (ng/mL)		
Test	Average	SD	%CV	Average	SD	%CV
A	51.0	78.3	153.4%	0.26	0.12	45.1%
B	150.2	159.9	106.4%	0.84	0.21	24.9%
C	148.5	161.7	108.8%	0.78	0.31	39.8%
D	21.0	18.0	85.6%	0.19	0.12	65.6%

TABLE 2 Endpoint titration data for commercially available lateral flow immunoassays

CONCLUSIONS

Heat-inactivated cultures can exhibit high variability in antigen tests, likely due to protein aggregation or denaturation. To overcome this, we developed a novel irradiation-based method to inactivate the virus while preserving protein integrity and epitopes used for antigen tests.

This study demonstrates the successful development of PROtrol SARS-CoV-2, an inactivated control for antigen-based assays. Irradiation was found to be more effective in inactivating the virus while preserving protein structure compared to heat inactivation. The infectivity assay confirmed the absence of infectious virus, and ELISA successfully detected the presence of nucleocapsid protein in the inactivated samples. The study successfully achieved its objective of providing laboratories and assay manufacturers with safe and reliable control materials to ensure accurate testing.

PROtrol™ (Protein Control) is a non-infectious, ready-to-use, control material with demonstrated performance on commercially available SARS-COV-2 lateral flow immunoassay tests. This material serves as an excellent control for analytical and quality control testing in antigen-based assays. It can be utilized in assay validation, verification, and personnel training. PROtrol ensures consistent, reliable, and accurate quality control solutions, evaluating the consistency of test kits and assay reagents across different batches. Additionally, it provides an unbiased and independent assessment of testing proficiency.

ACKNOWLEDGEMENTS

Lindsey Ruszczyk, BS, Amber Deering, BS, Nicole Williamson, BS, Karuna Sharma, PhD

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