ASM Microbe
CPHM10 Diagnostic Virology
Poster Number: 4670

DEVELOPMENT OF INACTIVATED INFLUENZA CONTROLS OPTIMIZED FOR USE WITH ANTIGEN-BASED ASSAYS

Andrew Zenger, Chen Katz, Doug Mason, Tanya Cruz-Morris, Lindsey Ruszczyk, Amber Deering, Rahul Chandel, Karuna Sharma
ZeptoMetrix, Buffalo, NY

ABSTRACT

BACKGROUND: High-quality control materials are vital for the efficient and effective development of diagnostic tests. QC material that is specifically designed for use with a particular testing method can help provide a more accurate picture of test performance by helping to minimize variability in analytical studies. The cost and ease of use advantages of rapid antigen tests were instrumental in their widespread use in combating the COVID-19 pandemic. A sharp rise in cases of SARS-COV-2, RSV, and Influenza Virus between September 2022 and March 2023 illustrated a need for updated antigen tests that can accurately detect and differentiate between these pathogens. Furthermore, an increase in treatment options for respiratory infections and improved sensitivity of rapid antigen tests have provided an opportunity to improve patient outcomes. To aid in this effort we have developed PROtrol Influenza, inactivated quality controls optimized for use with antigen tests.

METHODS: We employed a novel irradiation method to inactivate Influenza virus. The absence of infectious virus was confirmed through a standard tissue culture-based infectivity assay, while ELISA was used to quantify Influenza nucleoprotein (NP) in the inactivated samples. The performance of PROtrol was further confirmed through testing with widely available Influenza rapid antigen tests (RATs).

RESULTS: The inactivated samples did not exhibit any cytopathic effects (CPE) in the infectivity assay, confirming the absence of infectious virus. ELISA results measuring NP concentration, revealed that PROtrol displayed comparable Influenza NP immunoreactivity to infectious culture fluid (CF), whereas heat-inactivated culture fluid (CFHI) exhibited a significant decrease in immunoreactivity

CONCLUSION: This study demonstrates the successful development of PROtrol Influenza A Virus and Influenza B Virus, inactivated controls optimized for antigen-based assays. Irradiation effectively inactivated the virus while preserving protein structure compared to heat inactivation. The infectivity assay confirmed the absence of infectious organisms, while the ELISA and RATs demonstrated the expected performance of PROtrol.

INTRODUCTION

Rapid antigen tests were a useful public health tool during the COVID-19 pandemic. Between September 2022 and March 2023, independent spikes in cases of influenza, SARS-COV-2, and RSV highlighted the need for improved antigen tests that could differentiate these infections (1). Furthermore, an increase in treatment options for respiratory infections and improved sensitivity of rapid antigen tests have provided an opportunity to improve patient outcomes (2). To aid in this effort we have developed PROtrol Influenza, inactivated quality controls optimized for use with antigen tests.

In December 2023 the FDA published document titled Premarket Validation Recommendations for Development of In Vitro Diagnostics Tests for SARs-COV-2 Antigen. Appendix 5 details recommendations for SARS-COV-2 multianalyte antigen tests with Influenza and/or RSV. This states “For all analytical study with influenza A/B live virus strains should be used. Use of inactivated virus for influenza and RSV is not acceptable.” We sought to develop inactivated influenza controls that would perform comparably to live virus on antigen assay, and provide manufacturers a safer solution for ongoing quality control testing (3).

RESULTS

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

The ELISA showed comparable nucleoprotein reactivity between the irradiated (PROtrol) samples and the infectious CF. Conversely, the heat-inactivated culture fluids (CFHIs) exhibited a significant decrease in nucleoprotein reactivity. This effect was most pronounced in the Influenza B samples which showed an over 80% decrease in nucleoprotein reactivity relative to the infectious CF (Fig-1).

The retention of the nucleoprotein reactivity was further demonstrated through consistent and high performance on 4 commercially available multianalyte lateral flow immunoassay tests, designed to detect Influenza A, and Influenza B (Table-2). PROtrol demonstrated consistent, high performance in all tests. A further comparison of PROtrol and live culture fluid reactivity was done using one LFA commercial test (Quidel, QuickVue) demonstrating comparable performance for both products (Fig-2).

Influenza A&B immunoreactivity evaluation using ELISA

Virus	Strain	LOT	Lot #	PROtrol Conc. (ng/mL)	Pre-inactivation TCID50/mL
Flu A H1N1	Guangdong-Maonan/SWL 1536/19	Lot 1	556870	1838.3	3.98E+05
		Lot 2	556871	5788.3	3.16E+06
Flu A H3N2	Brisbane/10/07	Lot 3	553806	5776.4	1.41E+05
		Lot 4	555468	3820.51	1.41E+05
Flu B	Washington/02/19	Lot 5	557748	6633.6	1.02E+08
		Lot 6	557749	10161.1	3.16E+06

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

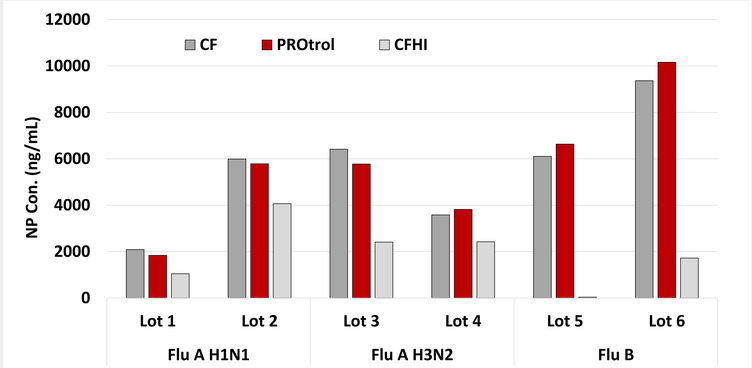

Figure 1 Two lots of Influenza A H1N1, Influenza A H3N2 and Influenza B were evaluated for nucleoprotein concentration by ELISA under different conditions including culture fluid (CF), irradiated (PROtrol), and heat-inactivated (CFHI) culture fluids.

Evaluation Using Lateral Flow Assay (LFA)

Virus	strain	Lot #	Consult	Status	Osom	Quidel
Flu A H1N1	Guangdong-Maonan/SWL 1536/19	556870	✓	✓	✓	✓
Flu A H3N2	Brisbane/10/07	555468	✓	✓	✓	✓
Flu B	Washington/02/19	557749	✓	✓	✓	✓

Table 2 PROtrol reactivity was tested on four commercial lateral flow immunoassays. All PROtrol samples showed reactivity, with results ranging from 23.0-331.7 ng/ml and 1.77e3-5.12e6 TCID50/ml.

Comparable Reactivity Of PROtrol and Infectious Culture Fluid

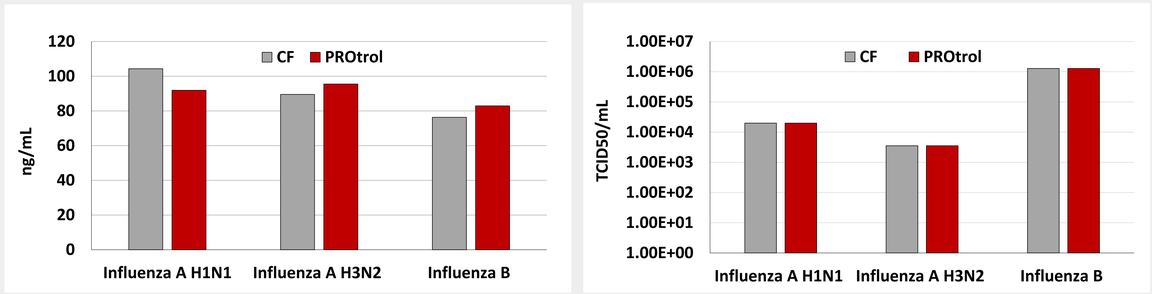

Figure 2 Comparison of the lower limit of detection (LLOD) for a select lateral flow immunoassay (Quidel, QuickVue) between PROtrol and live culture fluids. (A) LLOD of nucleoprotein concentration(ng/ml). (B) LLOD of Titer (TCID50/mL).

METHODS

Influenza A H1N1, A H3N2, and Influenza B strains were propagated in MDCK cells. Culture fluids were inactivated using heat and irradiation protocols. Viral infectivity was assessed using the TCID50 assay, and the Influenza N protein was evaluated using ELISA for live, irradiated (PROtrol), and heat-inactivated culture fluids (Table-1). PROtrol Influenza samples were also tested for reactivity on four commercial lateral flow immunoassays (LFA) from McKesson, Lifesign, Sekisui, and Quidel. Following the positive results a further comparison of PROtrol versus live culture fluid reactivity was done using the Quidel LFA kit

CONCLUSIONS

- Irradiation was more effective than heat inactivation for preserving protein structure while inactivating the virus.
- Infectivity assay confirmed no infectious virus.
- Heat-inactivated cultures show high variability in antigen tests due to protein aggregation or denaturation. for example, Influenza B virus was almost undetectable using standard nucleoprotein ELISA assay.
- Due to this variability FDA recommends using live Influenza virus for multianalyte lateral flow assays.
- Here developed a novel irradiation-based method to inactivate the virus while preserving protein integrity and epitopes for antigen-based assays such as ELISA and LFA
- PROtrol products provide safe, reliable control materials for accurate testing in laboratories and assay manufacturers.

PROtrol™ is a non-infectious, ready-to-use control material that has shown performance on four commercial multianalyte lateral flow immunoassays designed to detect SARS-CoV-2, Influenza A, and Influenza B. This material is ideal for quality control testing in antigen-based assays and can be used for assay validation, verification, and personnel training. It ensures consistent, reliable, and accurate quality control across different batches, providing an unbiased and independent assessment of testing proficiency

REFERENCES

1. RESP-Net Interactive Dashboard | CDC. Center for Disease Control. (n.d.). https://www.cdc.gov/resp-net/dashboard/
2. Stamm, Et. Al. , The Influence of Rapid Influenza Diagnostic Testing on Clinician Decision-Making for Patients With Acute Respiratory Infection in Urgent Care, Clinical Infectious Diseases, Volume 76, Issue 11, 1 June 2023, Pages 1942–1948, https://doi.org/10.1093/cid/ciad038
3. FDA (2023) Premarket Validation Recommendations for Development of In Vitro Diagnostics Tests for SARs-COV-2 Antigen

CONTACT

Tech. Assistance + Customer Service
T (716) 882-0920 | (800) 274-5487
E zepto.customerservice@antylia.com

WWW.ZEPTOMETRIX.COM