Evaluation of Hydrogen Peroxide to Generate Non-infectious Influenza Virus for Serology

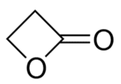
David Einfeld, Yasmeen Zoubi, Yasmin Rahmani, Lenny Godinho, Heather Couch

ABSTRACT

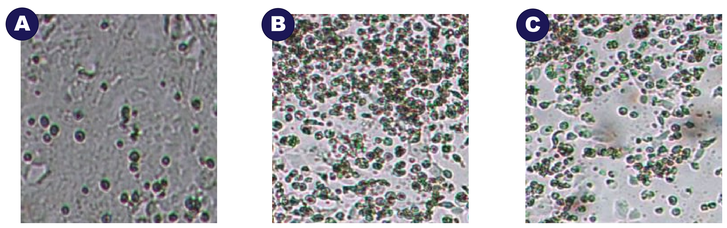
The International Reagent Resource (IRR) provides registered users with reagents that support the study and detection of bacterial and viral pathogens. Beta-propiolactone (BPL) inactivated influenza viruses are currently available for use in serological assays. BPL is a known carcinogen and requires additional personal protective equipment (PPE) including a full-facepiece respirator during use. BPL waste created during the inactivation process requires special handling and disposal as well. We have explored the utility of hydrogen peroxide (H_2O_2) as an inactivating reagent in comparison to the conventional use of BPL for this purpose since the PPE is the same as a regular BSL2 laboratory and the waste is non-toxic. Representatives of different influenza subtypes from the IRR catalog were treated with varied concentrations of H_2O_2 and analyzed for loss of infectivity. Effective inactivation conditions were confirmed in validation studies. Inactivated viruses were found to retain the hemagglutination titer of untreated controls using turkey red blood cells. Antigenicity was evaluated using monoclonal antibodies to hemagglutinin (HA) and subtype specific polyclonal antisera from the IRR catalog. Antibody binding was examined using ELISA assays on untreated and inactivated viruses. While a high concentration of H_2O_2 could disrupt antibody binding, conditions effective at inactivating virus allowed retention of antibody binding. Antigenicity of inactivated virus was also evaluated in hemagglutination inhibition assays. Influenza viruses inactivated with BPL were examined in parallel to compare the impact of the two methods on the utility of treated virus for serological assays. In addition to the antigenicity analysis, the integrity of nucleic acids of treated virus was examined using reverse transcription and PCR.

VIRUS INACTIVATION

- Inactivation of viruses can be an important technology, enabling their handling under less restrictive conditions which is particularly of interest for pathogens that require the use of BSL-3 or higher facilities.
- Inactivation needs to preserve key aspects of the virus that are of interest to the investigator.
- With a focus on antigenicity, can hydrogen peroxide provide a safer alternative to BPL?

 Beta-Propiolactone	• Used historically for virus inactivation, including vaccine generation • An OSHA-regulated carcinogen requiring engineering controls for handling • Reacts with nucleotides and proteins • Evidence that it can block membrane fusion by flu virus
H_2O_2 Hydrogen peroxide	• Widely employed in disinfectants • Published reports that under appropriate conditions peroxide-treated viruses can be effective as vaccines • Toxicity can be removed by addition of catalase • Mechanism of action unclear

INNOCUITY ASSAYED BY CPE



A. MDCK cells at day 4 of 3rd passage of B/Texas/02/2013 (B/TX) exposed to 1% H_2O_2.
B. MDCK cells at day 3 of inoculation with 10(-6) dilution of B/TX (no peroxide).
C. MDCK cells at day 3 of inoculation with B/TX diluted 10(-6) in media pre-treated with H_2O_2.
Peroxide treatment was 3 hr at room temperature followed by two additions of catalase to degrade residual peroxide before addition to cells.

Innocuity Monitored By HA Activity			
H_2O_2	Time	Passage 2 d4	Passage 3 d4
0.3%	2 h	4	<2
	4h	4	<2
	8h	4	<2
1.0%	2 h	4	<2
	4h	4	<2
	8h	4	<2
3.0%	2 h	2	<2
	4h	<2	<2
	8h	<2	<2

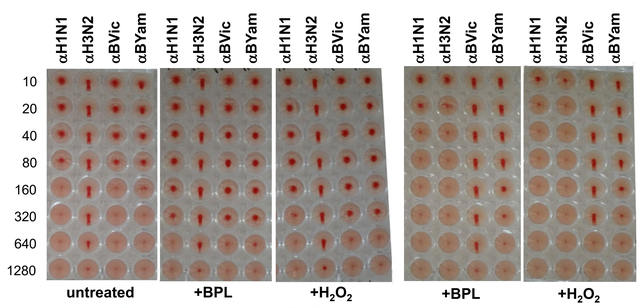
EFFECT OF H_2O_2 ON HA TITER

Virus	0% 3h	0% 24h	1% 3h	1% 24h	3% 3h	3% 24h
BYam1	256	128	256	256	256	< 4
BYam2	512	512	512	1024	1024	< 4
BVic1	128	128	128	128	64	<4
BVic2	128	128	128	128	128	64
H3N2-1	128	256	256	256	512	256
H3N2-2	256	256	512	512	512	256
H3N2-3	512	512	512	256	512	512

Viruses: BYam1 – B/Guangdong-Liwan/1133/2014, BYam2 – B/Massachusetts/2/2012, BVic1 - B/Bangladesh/5278/2006, BVic2 – B/Nevada/03/2011, H3N2-1 A/Brisbane/10/2007 IVR-147, H3N2-2 A/New York/55/2004, H3N2-3 A/Switzerland/9715293/2013

HA titers were assayed on turkey RBCs after peroxide exposure followed by catalase addition

EVALUATION OF H_2O_2 TREATED VIRUS IN HEMAGGLUTINATION INHIBITION ASSAYS

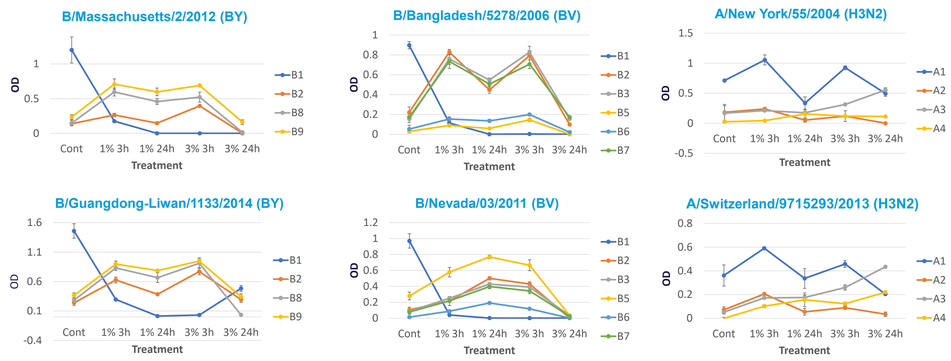


A/Delaware/01/2021 (H3N2) B/Michigan/01/2021 (BV)

Virus	Treatment	Antisera specificity			
		a H1N1	a H3N2	a BVic	a BYam
A/Delaware/01/2012 (H3N2)	None	< 10	640	< 10	< 10
	BPL	< 10	640	< 10	< 10
	H_2O_2	< 10	640	< 10	< 10
B/Michigan/01/2021 (BVic)	None	< 10	< 10	320	80
	BPL	< 10	< 10	640	80
	H_2O_2	< 10	< 10	320	80
B/Phuket/3073/2013 (Byam)	None	20	< 10	20	1280
	BPL	80	10	20	640
	H_2O_2	20	10	80	1280

Viruses treated with 1% peroxide for 3 hr give comparable HAI titers to the BPL-inactivated versions of the same virus.

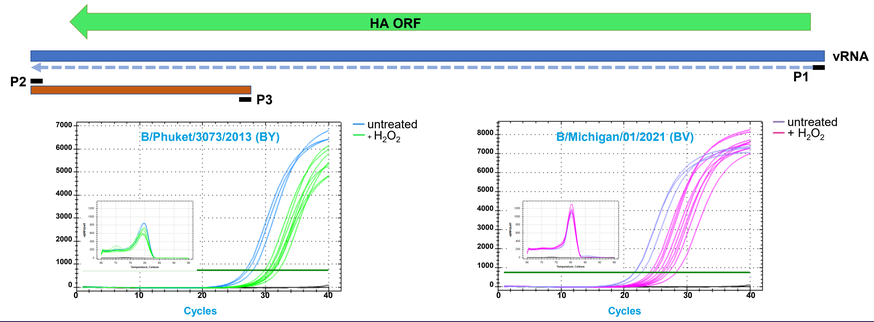
EFFECT OF H_2O_2 ON ANTIGENICITY



To analyze the impact of peroxide exposure on antigenicity, influenza viruses were exposed to H_2O_2 at concentrations of 1 and 3% and incubated for 3 and 24 h. Samples were treated with catalase to degrade remaining peroxide and used to coat ELISA plates. Binding of monoclonal antibodies reactive with HA, with the exception of the NP-binding antibody B1, were compared on treated and untreated virus. Antibodies B3 – B9 have shown subtype specificity with variable binding among virus strains. The majority of these antibodies were generated to baculovirus-expressed HA proteins. While they show strong binding to infected cells, binding to plated virus samples appeared relatively weak. Exposure to peroxide at 1% or for 3 h resulted in elevated binding for some antibodies. While these antibodies may not be optimal for assessing the retention of antigenicity, the results suggest peroxide can impact antigen recognition. In most cases, exposure to 3% peroxide for 24 h had a negative impact on antibody recognition.

EFFECT OF H_2O_2 ON VIRAL RNA

To examine the integrity of genomic RNA after exposure of virus to 1% peroxide, nucleic acids were extracted and reverse transcribed with Superscript IV and a primer (P1) that hybridizes to the 3' end of the HA segment [(-) sense]. qPCR was performed using DyNAmo Flash SYBR Green with primers P2 and P3 to amplify a 522 bp sequence corresponding to the 5' end of the HA segment. Specificity of the signal was assessed by running a melt curve.



CONCLUSIONS

- Conditions were evaluated for inactivation of influenza virus with hydrogen peroxide, both tissue culture and egg-grown viruses.
- Catalase removed toxicity of treated virus to enable innocuity testing in tissue culture.
- Peroxide treatment did affect subsequent binding of antibodies, including enhanced binding by some monoclonal antibodies.
- Retention of HA activity following H_2O_2 treatment allowed evaluation in hemagglutination inhibition (HAI) assays.
- Peroxide-treated viruses performed similarly to BPL-treated viruses in HAI assays.
- RT-qPCR analysis showed a reduction in detection of intact viral nucleic acids.
- Peroxide may provide a useful alternative to BPL for generating inactivated influenza viruses.

ACKNOWLEDGEMENTS

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AFS 10801 University Boulevard, Manassas, VA 20110-2209 **Phone:** 800.359.7370 **Email:** federalsolutions@atcc.org **Web:** https://www.atcc.org/federal-solutions