Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

Myeloperoxidase-mediated solution demonstrates rapid virucidal properties against the delta variant of SARS-CoV-2

Sir,

Since coronavirus SARS-CoV-2 was first discovered as the causative agent of the COVID-19 pandemic, there have remained unmet infection prevention needs. Despite many mitigation efforts and discovery of new variants, COVID-19 cases continue to increase [1]. As such, we investigated the in-vitro antiviral efficacy of a myeloperoxidase-mediated solution (MPMS) as a potential oral rinse and nasal spray against coronavirus.

MPMS is a first-in-class tissue-safe topical myeloperoxidase-mediated formulation developed to reduce the microbial burden of pathogens on mucosal and epithelial surfaces. The active ingredients in MPMS are two enzymes: porcine myeloperoxidase (pMPO) and glucose oxidase (GO) in an aqueous solution. The enzymes are activated by the addition of a glucose solution. Once activated, the reactive species hydrogen peroxide, hypochlorous acid, and singlet oxygen are generated [2].

MPMS at concentrations 300 GU/mL (0.744 mg pMPO/mL) and 50 GU/mL (0.124 mg pMPO/mL) were tested against severe acute respiratory syndrome-related coronavirus-2 lineage B.1.617.2 (SARS-CoV-2, delta variant, BEI Resources #NR-55611) and human coronavirus, strain OC43, respectively. Stock solutions of MPMS enzyme solution containing pMPO and GO and MPMS substrate solution containing glucose were prepared at Exoxemis, Inc. (Omaha, NE, USA). The final concentrations of enzyme solutions were prepared just prior to use. The enzyme and substrate solutions were mixed together to activate the system. The activated formulation was held at room temperature for 20 ± 5 min for oxidant generation before the addition of mucin and target viruses. Mucin (Sigma–Aldrich, St Paul, MO, USA) was prepared in Dulbecco's phosphate-buffered saline (Gibco, Billings, MT, USA) to achieve a final concentration of 0.1%, 0.15%, or 0.32%.

A virucidal suspension test (in-vitro time-kill method) based on ASTM E1052-11 was used [3]. In the virucidal suspension test, a 0.5 mL aliquot of test virus was added to 4.5 mL MPMS with and without mucin at a 90% (v/v) concentration. The mixtures were exposed for 1 or 15 min. After each exposure time, the mixture was neutralized to stop viricidal activity and plated in four replicates. The plates were incubated in CO_2 for 7–21 days at 33 ± 2 °C and monitored for cytopathic/cytotoxic effect. Viral titre, 50% tissue culture infectious dose ($TCID_{50}$), was calculated by the Spearman–Karber method [4]. All testing was performed at Bioscience Laboratories, LLC, Bozeman, MT, USA.

The results of the virucidal suspension test (in-vitro time-kill method) for SARS-CoV-2 delta variant and coronavirus OC43 are shown in Table I. For SARS-CoV-2 delta variant, MPMS tested at 300 GU/mL pMPO reduced infectivity by 3.25 $\log_{10}$ (99.94%) in the absence of mucin and by 2.75 $\log_{10}$ (99.82%) in the presence of 0.1% mucin after a 1 min exposure. A 1.50 $\log_{10}$ cytotoxicity to host tissue culture cells was observed at 300 GU/mL pMPO. For coronavirus OC43, MPMS tested at 50 GU/mL reduced infectivity by $\geq$4.25 $\log_{10}$ (>99.99%) in the absence of mucin, $\geq$4.25 $\log_{10}$ (>99.99%) in the presence of 0.15% mucin, and $\geq$4.00 $\log_{10}$ (>99.99%) in the presence of 0.32% mucin after 15 min exposure. No cytotoxicity to host tissue culture cells was observed at 50 GU/mL pMPO.

SARS-CoV-2 utilizes a pathogenic process that involves human mucosa as the infection entry gate followed by virus dissemination into susceptible organs. The mechanism of cellular entry by SARS-CoV-2 is through the binding to angiotensin-converting enzyme 2 (ACE2) receptor. Elevated ACE2 receptor protein in the upper respiratory tract provides evidence for the initial site of SARS-CoV-2 entry and replication [5]. With high concentrations of virus in the throat and oral cavity, the viral replication cycle promotes further infectivity or dissemination of virus, thus advocating the use or oral antiseptics. Several commercially available oral rinses have been described to have inactivating properties *in vitro* against SARS-CoV-2 [6]. MPMS differs from these commercial rinses in that it is a formulated cell-free oxidant-generating enzyme system which mimics the intrinsic in-situ functions of the phagolysosome [7]. We found that SARS-CoV-2 delta variant can be efficiently inactivated by MPMS within 1 min of exposure. At a low concentration, MPMS continued to demonstrate activity at 15 min against coronavirus OC43. The presence of mucin at concentration for healthy humans and stage III chronic obstructive pulmonary disorder patients did not interfere with the activity of MPMS [8]. The rapid rate of viral inactivation induced by MPMS is consistent with the combustive oxygenation mechanism of action, which is advantageous in the treatment of new coronavirus variants. Furthermore, a Phase III clinical trial showed no safety differences between MPMS at 300 GU/mL and saline (Clinical Trials.gov NCT01297959).

Our findings support the use of MPMS to reduce the epithelial viral load of SARS-CoV-2 in the upper respiratory tract as a topical additive, thereby providing a treatment benefit to an

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Table I
Virucidal suspension test results for MPMS against SARS-CoV-2, delta variant and coronavirus strain OC43

Variable	SARS-CoV-2 delta variant			Coronavirus strain OC43		
	Virus control	MPMS$^+$	MPMS$^+$	Virus control	MPMS$^+$	MPMS$^+$
	Range	No mucin	0.1% mucin	Range	0.15% mucin	0.35% mucin
Exposure time	1 min	1 min	1 min	15 min	15 min	15 min
$TCID_{50}$ (log_{10})	6.50–6.75	3.50	3.75	6.50–6.75	≤2.50	≤2.50
Log_{10} reduction	N/A	3.25	2.75	N/A	≥4.25	≥4.00
% reduction	N/A	99.94	99.82	N/A	>99.99	>99.99

Delta variant: myeloperoxidase-mediated solution (MPMS) concentration 300 GU/mL; neutralization control = 6.25 log_{10}; cytotoxicity control = 1.50 log_{10}; cell culture negative control = 0000.
Coronavirus OC43: MPMS concentration 50 GU/mL; neutralization control = 6.50 log_{10}; cytotoxicity control = 0000; cell culture negative control = 0000.
GU, guaiacol unit, i.e. the amount of MPO enzyme that catalyses the conversion of 1 μmol of hydrogen peroxide per minute at 25 °C.
$TCID_{50}$, 50% tissue culture infectious dose.

infected or exposed individual and preventing dissemination of COVID-19 within the population. A randomized clinical trial remains to be investigated.

Conflict of interest statement
G.D. serves as microbiology consultant for Exoxemis, Inc. R.A. serves as a general consultant for Exoxemis, Inc. K.B. and J.D. are employees of Bioscience Laboratories, LLC. J.S. is president and CEO of Exoxemis, Inc.

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G.A. Denys[a,*]
K. Burningham[b]
J. Dyba[b]
R.C. Allen[c]
J.T. Stephens Jr.[d]
[a]*Indiana University School of Medicine, Indianapolis, Indiana, USA*
[b]*Bioscience Laboratories, LLC, Bozeman, Montana, USA*
[c]*Creighton University School of Medicine, Omaha, Nebraska, USA*
[d]*Exoxemis, Inc., Little Rock, Arkansas, USA*

* Corresponding author. Address: Indiana University School of Medicine, Indianapolis, IN 46202, USA. Tel.: +1 317 698-0329.
E-mail address: gdenys@att.net (G.A. Denys)

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