DOI: 10.1002/jmv.26722

LETTER TO THE EDITOR

JOURNAL OF MEDICAL VIROLOGY WILEY

Overinterpretation of the antiviral results for human coronavirus strain 229E (HCoV-229E) relative to severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)

Dear Editor,

Published reports provide evidence that the antiviral activity of products against human coronavirus (HCoV) 229E, severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), or other viruses may not be generalized to the antiviral activity against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2).

The recent article by Meyers et al.[1] suffers from several interpretation issues that are not supported by the results and thereby it has the potential to mislead readers regarding health claims related to the current SARS-CoV-2 pandemic. The aforementioned article already received some criticism from the scientific community and mainstream print media.[2] We provide additional observations on this article and on research pertaining to use of oral care products in reducing SARS-CoV-2 viral load.

OVERSTATEMENT OF SCOPE AND METHODOLOGY SHORTCOMING

The title overstates the investigation. The "Lowering the transmission and spread" of an infectious virus can be claimed only after proper in vivo or clinical studies have been conducted.

HCoV strains 229E, NL63, OC43, and HKU1 account for approximately one third of the cases of the common cold, are not as highly contagious, and do not manifest mortality rates comparable to SARS-CoV, SARS-CoV-2, or MERS-CoV.[3–5] Meyer et al.[1] studied the HCoV strain 229E as surrogate for SARS-CoV-2 claiming same as a "rapid test" since studies with SARS-CoV-2 require a Level 3 Biocontainment Laboratory. HCoV 229E cannot serve as a legitimate surrogate SARS-CoV-2 without comparison and calibration of the results, which we believe unlikely due to the high rate of infection and contagion exhibited by SARS-CoV-2 which is substantiated by published studies (discussed later).

Meyers et al.[1] used Huh7 cells, an epithelia-like, tumorigenic cell, often used in hepatitis C and dengue virus research. MRC-5 and Vero E6 cells are used by ISO accredited laboratories for antiviral tests against HCoV 229E and SARS-CoV-2 and SARS-CoV, respectively. Furthermore, the Huh7 cells used in the investigation were obtained from another researcher; not from an authenticated source, for example, American Type Culture Collection (ATCC). Cell line authentication is required to ensure validity and reproducibility of data and for publication in reputable scientific journals.[6]

DISCUSSION OF THE FINDINGS, RESULTS, AND CONCLUSIONS

Meyers et al.[1] conclude that the results of HCoV 229E are applicable to all strains of human coronavirus including in particular, SARS-CoV-2. This conclusion contradicts extant literature without substantiation. While Meyers et al.[1] note that the coronaviruses known to infect humans have a common structure, they fail to acknowledge that there are also differences in surface protein and lipid structure that may play a critical role in the severity of infection, response to antibodies, and inactivation by antiviral products. There are clear differences in the antiviral activity of a compound against different viruses, strains of HCoV; *even among different strains of SARS-CoV-2*.[7–9] Singstam et al.[10] found that investigations of disinfection kinetics and mechanisms are important in understanding whether particular compounds or products may reduce the transmission of the SARS-CoV-2. Notably, the SARS-CoV-2 spike protein is 10–20 times more likely to bind to the angiotensin converting enzyme 2 receptor on human cells than the spike protein of SARS-CoV, thereby enabling SARS-CoV-2 to spread more easily from person to person. Furthermore, three different antibodies against SARS-CoV do not bind successfully to the SARS-CoV-2 spike protein, emphasizing that the differences in the chemical structure of each of these unique spike proteins is critically important to the physiologic and pathologic response by the human host.[11]

We find more robust studies of the reduction of SARS-CoV-2 viral load by oral care products emerging in the literature. Meister et al.[7] reported that the antiviral activity of eight different products varied when each was applied to *three different strains* of SARS-CoV-2. Oral

TABLE 1 Antiviral activity of CloSYS® ultra sensitive rinse

Virus	Log reduction of viral load	
	30 s	60 s
SARS-CoV-2[12]	1.96	1.39
SARS-CoV[13]	0.19	0.56–1.06
Influenza A H3N2[14]	4.13	4.38–4.63

Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.

rinses containing dequalinium chloride, benzalconium chloride, polyvidone-iodine, ethanol and essential oils exhibited excellent antiviral activity against SARS-CoV-2. However, each oral rinse provided different log reductions in viral load for the three strains; ≥ 3.11, ≥ 2.78, and ≥ 2.61 for Strain 1, Strain 2, and Strain 3 of SARS-CoV-2, respectively. Similarly, hydrogen peroxide rinse exhibited poor antiviral activity, showing 0.78, 0.61, and 0.33 log reduction of SARS-CoV-2 Strain 1, Strain 2, and Strain 3, respectively. Eggers et al.[8] observed variations in the log reduction of the Influenza A subtype H1N1, SARS-CoV, MERS-CoV, and Non-enveloped human rotavirus strain Wa by a povidone-iodine solution. Sanekata et al.[9] reported a wide variation in the antiviral activity of chlorine dioxide gas in solution and sodium hypochlorite against feline calicivirus, human Influenza virus, Measles virus, canine distemper virus, human herpesvirus, human adenovirus, canine adenovirus, and canine parvovirus. These studies support the observation that the effect of a single composition may vary by virus and virus strain.

ISO accredited infectious disease laboratories have shown CloSYS Ultra Sensitive Oral Rinse to reduce the viral load of SARS-CoV-2, SARS-CoV and Influenza A H3N2 to varying extent (Table 1).[12–15] The data show that the viral load reduction of SARS-CoV-2 by Ultra Sensitive rinse was 10-fold more than reduction of SARS-CoV in 30 s. Log reduction of SARS-CoV-2 viral load by CloSYS Ultra Sensitive rinse, CloSYS Sensitive rinse and CloSYS Oral Spray was 1.96, 1.81, and 2.98, respectively, in 30 s.[12,16,17] While we believe these results to be promising, we also acknowledge that clinical trials (now underway) are required to determine if the transmission and spread of SARS-CoV-2 virus can be reduced by an oral rinse or mouth wash. Lastly, Bidra et al.[18] reported that an oral rinse containing 0.5%–1.5% of povidone-iodine exhibited a 3.3 log reduction of SARS-CoV-2 in 30 s.

Taken collectively, these reports provide mounting evidence that the antiviral activity of products against coronaviruses 229E, SARS-CoV, MERS-CoV, or other viruses *may not be generalized* to the antiviral activity against SARS-CoV-2. The first question that clinical professionals ask oral care product manufacturers is whether or not the product is specifically tested against SARS-CoV-2.

Well-conceived, scientifically sound research on the effect of oral care products on reducing the viral load of SARS-CoV-2 is underway. As SARS-CoV-2 largely infects and transmits through the oral and nasal cavity, future research requires soundly constructed investigations and clinical studies that do not conflate this virus with others and that do not overestimate the effect of antiviral agents applicable to one human coronavirus to another. Meyers et al.[1] fail to account for the subtle differences in viral composition that affects disinfection kinetics and mechanisms. At best, conclusions should have been limited to the HCoV 229E and the common cold, not conflated with SARS-CoV-2 and the COVID-19 pandemic.

CONFLICT OF INTERESTS

James L. Ratcliff is Chairman and CEO of Rowpar Pharmaceuticals, Inc. Jaiprakash G. Shewale is Director of Research and Technical, Rowpar Pharmaceuticals, Inc.

Jaiprakash G. Shewale PhD
James L. Ratcliff PhD

Rowpar Pharmaceuticals, Inc., Scottsdale, Arizona, USA

Correspondence
Jaiprakash G. Shewale, PhD, Rowpar Pharmaceuticals, Inc., 16100 N. Greenway-Hayden Loop, Suite 400, Scottsdale, AZ 85260, USA.
Email: jshewale@rowpar.com

ORCID

Jaiprakash G. Shewale https://orcid.org/0000-0002-4934-1581

REFERENCES

1. Meyers C, Robison R, Milici J, et al. Lowering the transmission and spread of human coronavirus. *J Med Virology*. 2020:1-8. https://onlinelibrary.wiley.com/doi/10.1002/jmv.26514
2. Wu KJNo. Mouthwash will not save you from the coronavirus. *New York Times*. October 21, 2020. https://www.nytimes.com/2020/10/21/health/covid-mouthwash.html. Accessed November 16, 2020.
3. Chen B, Tian EK, He B, et al. Overview of lethal human coronaviruses. *Sig Transduct Target Ther*. 2020;5:89. https://doi.org/10.1038/s41392-020-0190-2. Accessed October 29 2020.
4. Sampson V, Kamona N, Sampson A. Could there be a link between oral hygiene and the severity of SARS-CoV-2 infections? *British Dental J*. 2020;228:971-975.
5. Centers for Disease Control and Prevention. Human coronavirus types. 2020; Feb 15 (page last reviewed). https://www.cdc.gov/coronavirus/types.html. Accessed October 29, 2020.
6. American Type Culture Collection. Cell line authentication publication requirements. https://www.atcc.org/en/Services/Testing_Services/Cell_Authentication_Testing_Service/Cell_Line_Authentication_Publication_Requirements.aspx. Accessed October 29, 2020.
7. Meister TL, Brüggemann Y, Todt D, et al. Virucidal efficacy of different oral rinses against severe acute respiratory syndrome coronavirus 2. *J Infect Dis*. 2020;222:1289-1292.
8. Eggers M, Koburger-Janssen T, Eickmann M, Zorn J. In vitro bactericidal and virucidal efficacy of povidone-iodine gargle/mouthwash against respiratory and oral tract pathogens. *Infect Dis Ther*. 2018;7:249-259.
9. Sanekata T, Fukuda T, Miura T, et al. Evaluation of the antiviral activity of chlorine dioxide and sodium hypochlorite against feline calicivirus, human Influenza virus, Measles virus, canine distemper virus, human herpesvirus, human adenovirus, canine adenovirus and canine parvovirus. *Biocontrol Sci*. 2010;15:45-49.
10. Sigstam T, Gannon G, Cascella M, Pecson BM, Wigginton KR, Kohn T. Subtle differences in virus composition affect disinfection kinetics and mechanisms. *Appl Environ Microbiol*. 2013;79:3455-3467.

11. National Institutes of Health. Novel coronavirus structure reveals targets for vaccines and treatments. 2020; March 3. https://www.nih.gov/news-events/nih-research-matters/novel-coronavirus-structure-reveals-targets-vaccines-treatments. Accessed October 29, 2020.
12. MRIGlobal. Assessment of CloSYS Ultra Sensitive Rinse (CloSYS Unflavored Rinse) against SARS-CoV-2, Final Report, November 3, on file at Rowpar Pharmaceuticals, Inc.; 2020. https://closys.com/pages/closys-reduced-covid-19-virus. Accessed November 16, 2020.
13. Microbac Laboratories. Final Report, Virucidal Efficacy Suspension Test, SARS-associated Coronavirus (SARS-CoV), July 28, 2020 on file at Rowpar Pharmaceuticals, Inc.; 2020.
14. Microbac Laboratories. Final Report, Virucidal Efficacy Suspension Test, Influenza A virus (H3N2), September 20, 2020 on file at Rowpar Pharmaceuticals, Inc.; 2020. https://www.closys.com/pages/closys-reduced-influenza-virus. Accessed November 16, 2020.
15. Microbac Laboratories. Final Report, Virucidal Efficacy Suspension Test, Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2) (COVID-19 virus), July 29, on file at Rowpar Pharmaceuticals, Inc.; 2020. https://closys.com/pages/closys-reduced-covid-19-virus. Accessed November 16, 2020.
16. MRIGlobal. Assessment of CloSYS Oral Spray against SARS-CoV-2, Final Report, November 3, report on file at Rowpar Pharmaceuticals, Inc.; 2020. https://closys.com/pages/closys-reduced-covid-19-virus. Accessed November 16, 2020.
17. MRIGlobal. Assessment of CloSYS Sensitive Rinse (CloSYS GentleMint Flavored Rinse) against SARS-CoV-2, Final Report, November 3, on file at Rowpar Pharmaceuticals, Inc.; 2020. https://closys.com/pages/closys-reduced-covid-19-virus. Accessed November 16, 2020.
18. Bidra AS, Pelletier JS, Westover JB, Frank S, Brown SM, Tessema B. Rapid in-vitro inactivation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) using povidone-iodine oral antiseptic rinse. *J Prosthodont*. 2020;29:529-533.