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BRIEF REPORT

Manufacturing Process of SER-109, a Purified Investigational Microbiome Therapeutic, Reduces Risk of Coronavirus Transmission From Donor Stool

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) may present risk to patients treated with donor-derived microbiome therapies when appropriate manufacturing controls and inactivation processes are lacking. We report that the manufacturing steps for SER-109, a purified investigational microbiome therapeutic developed to reduce risk of *Clostridioides difficile* recurrence, inactivate porcine epidemic diarrhea virus, a model coronavirus for SARS-CoV-2.

Keywords. *Clostridioides difficile* infection; microbiome; microbiome therapeutic.

Over the past several years, fecal microbiota transplantation (FMT) has been utilized following antibiotics to achieve a sustained clinical response in patients with recurrent *Clostridioides difficile* infection (CDI), with varied clinical efficacy [1]. There are inherent safety concerns associated with FMT, including reliance on donor screening alone to detect pathogens. FMT-related transmission of pathogens, not detected during donor screening, has led to hospitalizations and deaths [2]. In March 2020, 2 months after coronavirus disease 2019 (COVID-19) was first reported in the United States, the US Food and Drug Administration warned of potential risk of transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a human respiratory coronavirus, through contaminated stool, leading to an extended quarantine of FMT [3].

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FMT carries predictable risk of infectious hazards [4]; thus, improved risk mitigation is necessary for donor-derived products. SER-109, an investigational, donor-derived oral microbiome therapeutic comprised of purified Firmicutes spores, was observed to be superior to placebo in prevention of recurrent CDI in a randomized controlled phase 3 trial [5, 6]. The manufacturing process includes inactivation of potential pathogens, such as viruses, parasites, fungi, and vegetative bacteria, including drug-resistant pathogens, as previously reported [6]. We evaluated whether the manufacturing process for SER-109 inactivates SARS-CoV-2 utilizing a model coronavirus with gastrointestinal tropism.

METHODS

Selection of Specific Model Organism

Porcine epidemic diarrhea virus (PEDV), a mammalian, non-human coronavirus with native enteric tropism, was selected as a specific model organism representing SARS-CoV-2 based on the following criteria: (*i*) well-characterized virus-cell system to provide reproducible results [7]; (*ii*) similarity to the target viruses of interest, including inactivation mechanisms, stability, and detectability in the relevant matrix favoring native enteric organisms [8]; (*iii*) cultivability to prepare virus stock solutions of sufficient titer to demonstrate robust inactivation; and (*iv*) safety of operators performing the inactivation studies [9].

Challenge Virus, Host Cells, and Test Articles

PEDV stock (Iowa State University, Ames, Iowa) was maintained by Microbac (Sterling, Virginia). Vero cells (American Type Culture Collection CCL-81) were used as the PEDV host during both preparation of virus stocks and for virus quantitation. Two independent test articles made from different donors' stool and representative of the SER-109 manufacturing intermediate were provided by Seres Therapeutics (Cambridge, Massachusetts).

Spiking and Inactivation

Virus stock was spiked into each test article at a volumetric ratio of 1 mL stock per 25 mL spiked test article. Dehydrated ethanol was added to a final concentration of 70% v/v for inactivation and well mixed into the spiked test article.

Sample Preparation

Sample preparation methods were determined using cytotoxicity and viral interference studies to determine effects of the test articles on host cells. At the appropriate time, samples were diluted 3-fold in dilution medium (Earle's balanced salt solution [EBSS]) to quench inactivation and were centrifuged at 2000*g*

for 5 minutes, and supernatant liquid was filtered (0.45-µm syringe filter).

Virus Quantitation

Virus titer was determined using a 50% tissue culture infectious dose ($TCID_{50}$) assay utilizing 10-fold dilutions (EBSS) with 8 replicates per dilution and the Spearman-Karber method for calculation of titer and 95% confidence interval. Vero cells were seeded into 24-well plates with culture medium (Minimum Essential Medium, 0.3% tryptose phosphate broth, 0.02% yeast extract, 2.0 µg/mL trypsin). Cells were inspected microscopically for cytopathic effect on day 10 postinoculation. Controls included virus stock, Vero cell only (no test article, no virus), test article control (no virus), spiked test article (no ethanol, immediate quench), held spiked test article (no ethanol, quench after final timepoint), and a control test series without test article. The limit of detection (LOD) was 0.7 $TCID_{50}$/mL.

Calculation of Virus Inactivation

Total virus inactivation was calculated as a $\log_{10}$ reduction factor (LRF) using the measured virus amount at the target timepoint and the measured initial virus amount in the spiked test article before ethanol addition.

RESULTS

The inactivation results (LRF) versus incubation time for PEDV in ethanol are presented in Figure 1, including data for 2 independent test articles and for the control (virus plus buffer and ethanol with no process intermediate test article). Virus was detected immediately after ethanol addition in the 0- to 1-minute sample, but then reached LOD for all subsequent samples. All controls were within predefined criteria suitable for a valid assay, including the nonethanolic controls, which collectively ensured specificity of the inactivation. PEDV was inactivated by >4.2 $\log_{10}$ (LOD) within 4 minutes (Figure 1). The d-value (time required to achieve 1 $\log_{10}$ reduction) was therefore <1 minute.

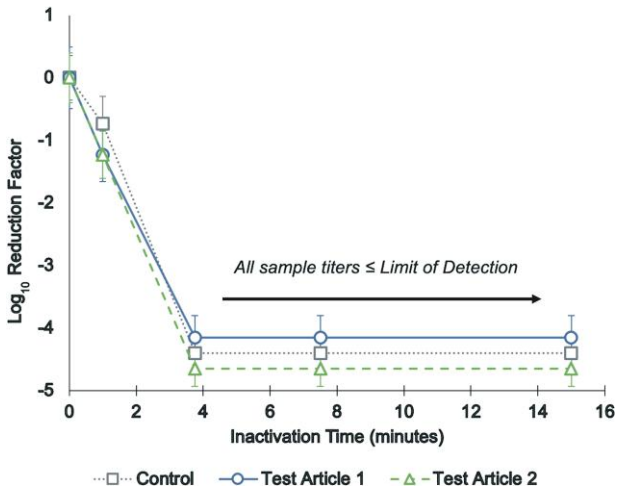

Figure 1. Inactivation of porcine epidemic diarrhea virus, a model coronavirus for severe acute respiratory syndrome coronavirus 2, presented as $\log_{10}$ reduction factor versus time as measured for the SER-109 manufacturing process. Error bars represent 95% confidence interval based on 50% tissue culture infectious dose in-sample replicates.

DISCUSSION

The manufacturing process for SER-109, an investigational oral microbiome therapeutic, is enriched for Firmicutes spores, while inactivating potential bacterial, viral, parasitic, and fungal pathogens via ethanolic exposure [5]. Ethanol inactivation of coronaviruses, including SARS-CoV-2, is well-established to be effective in hand disinfectants and environmental surface decontamination [10]. The experiments described in this article uniquely demonstrate substantial and rapid solvent inactivation of a coronavirus within the manufacturing process of a donor-derived therapeutic and were completed with representative process intermediates. Ethanol and the background aqueous solution are in excess during the inactivation step with residual fecal solids constituting <1%, ensuring complete ethanolic exposure. Reported titers for shedding of SARS-CoV-2 in stool range from 10^2 to 10^8 copies per gram [11, 12], suggesting that a 10 $\log_{10}$ reduction, achieved via a 10-minute exposure based on the d-value calculated above, provides meaningful risk mitigation. The manufacturing process for SER-109 ensures an exposure time of >10 minutes. These results support the potential benefit of SER-109 manufacturing processes to mitigate risk to patients while providing the spore-forming Firmicutes bacteria needed for therapeutic efficacy. In a phase 3 randomized placebo-controlled trial, oral administration of SER-109 was superior to placebo in reducing the risk of recurrent infection, the primary endpoint [6].

FMT products will always be vulnerable to undetected pathogens because they lack manufacturing procedures capable of selective pathogen inactivation. Donor screening is an important first step toward the development of a purified product. However, pathogens may go undetected via donor screening due to inappropriate assay selection [3], LODs of the assay, interfering substances in the stool matrix, or new viral variants that may escape detection. FMT products are particularly susceptible to emerging pathogens due to delay in clinical recognition and development and validation of sensitive screening assays [4]. Emergent infections occur regularly and are not "black swan" events, as evidenced by outbreaks of monkeypox virus [13], Zika virus, hantavirus, severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus, and others over the last few decades. Occult infection with human immunodeficiency virus, hepatitis B virus, and hepatitis C virus are examples of pathogen

transmission through donor-derived biologic products that affected millions of persons for years before they were clinically recognized and identified [14].

Several of these emerging infectious agents are shed in stool [15]. Although SARS-CoV-2 transmission through the fecal route has yet to be proven, the high levels of virus found in wastewater support possible risk of infectious bioaerosols related to drainage systems as historically reported in 2003 with SARS-CoV [16] and more recently for SARS-CoV-2 [17]. The feasibility of oral transmission of SARS-CoV-2 has been established [18]. FMT guidelines have recommended screening of donors for SARS-CoV-2 through nasopharyngeal swabbing [19]. However, fecal shedding of SARS-CoV-2 is of longer duration than upper respiratory shedding [20], and a small proportion of individuals continue to shed viral RNA in the feces up to 7 months postinfection [21]. Therefore, nasopharyngeal detection of SARS-CoV-2 is a suboptimal risk mitigation measure for patients [22]. Assays for detection of SARS-CoV-2 in stool matrix were not reported until several months after the beginning of the COVID-19 pandemic [23].

There are several limitations and strengths to this report. Inactivation experiments did not use SARS-CoV-2 for direct measurement of manufacturing process capability. Instead, PEDV, a model coronavirus, was utilized in these experiments to safeguard laboratory workers. Strengths of the methodology include use of a related coronavirus natively stable in a fecal matrix to ensure selective measurement of the effect of ethanol exposure, execution of spiking studies directly into representative process intermediates, and selection of a well-researched virus/host cell assay with historical precedence and laboratory practice. Furthermore, studies were completed within a Good Laboratory Practice environment and associated quality system oversight.

In conclusion, the SER-109 manufacturing processes are an essential complementary strategy beyond donor screening alone to mitigate viral transmission risk to patients.

Notes

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Potential conflicts of interest. C. M., A. A., M.-J. L., B. H., J. G. A., B. H. M., and D. S. E. are all employees and shareholders of Seres Therapeutics. S. S. Z. is the Director of Virology and Toxicology at Microbac Laboratories, Inc, a contract research laboratory paid by Seres Therapeutics for the completion of this research.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Tariq R, Pardi DS, Bartlett MG, etal. Low cure rates in controlled trials of fecal microbiota transplantation for recurrent *Clostridium difficile* infection: a systematic review and meta-analysis. Clin Infect Dis **2019**; 68:1351–8.
2. DeFilipp Z, Bloom PP, Soto MT, et al. Drug-resistant *E. coli* bacteremia transmitted by fecal microbiota transplant. N Engl J Med **2019**; 381:2043–50.
3. US Food and Drug Administration. Fecal microbiota for transplantation—safety alert: risk of serious adverse events likely due to transmission of pathogenic organisms. https://www.fda.gov/safety/medical-product-safety-information/fecal-microbiota-transplantation-safety-alert-risk-serious-adverse-events-likely-due-transmission. Accessed 5 September 2022.
4. Blaser MJ. Fecal microbiota transplantation for dysbiosis—predictable risks. N Engl J Med **2019**; 381:2064–6.
5. McGovern BH, Ford CB, Henn MR, et al. SER-109, an investigational microbiome drug to reduce recurrence after *Clostridioides difficile* infection: lessons learned from a phase 2 trial. Clin Infect Dis **2020**; 72:2132–40.
6. Feuerstadt P, Louie TJ, Lashner B, et al. SER-109, an oral microbiome therapy for recurrent *Clostridioides difficile* infection. N Engl J Med **2022**; 386:220–9.
7. Antas M, Szczotka-Bochniarz A, Woźniakowski G. Effective inactivation of porcine epidemic diarrhea virus on contaminated surgery masks by low-concentrated sodium hypochlorite dispersion. Pol J Vet Sci **2020**; 23:647–50.
8. Brian DA, Baric RS. Coronavirus replication and reverse genetics. Curr Top Microbiol **2005**; 287:1–30.
9. Chen Q, Li G, Stasko J, et al. Isolation and characterization of porcine epidemic diarrhea viruses associated with the 2013 disease outbreak among swine in the United States. J Clin Microbiol **2014**; 52:234–43.
10. Kampf G, Brüggemann Y, Kaba HEJ, et al. Potential sources, modes of transmission and effectiveness of prevention measures against SARS-CoV-2. J Hosp Infect **2020**; 106:678–97.
11. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. Nature **2020**; 581:465–9.
12. Zheng S, Fan J, Yu F, et al. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January–March 2020: retrospective cohort study. BMJ **2020**; 369:m1443.
13. Safety alert regarding use of fecal microbiota for transplantation and additional safety protections pertaining to monkeypox virus. News release. US Food and Drug Administration. https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/safety-alert-regarding-use-fecal-microbiota-transplantation-and-additional-safety-protections-0. Accessed 23 August 2022.
14. Hecht GA, Blaser MJ, Gordon J, et al. What is the value of a Food and Drug Administration investigational new drug application for fecal microbiota transplantation to treat *Clostridium difficile* infection? Clin Gastroenterol Hepatol **2014**; 12:289–91.
15. Sklenovská N, Van Ranst M. Emergence of Monkeypox as the Most Important Orthopoxvirus Infection in Humans. Front Public Health **2018**; doi:10.3389/fpubh.2018.00241.
16. McKinney K, YY G, Lewis T. Environmental transmission of SARS at Amoy Gardens. J Environ Health **2006**; 68:26–30; quiz 51–2.
17. Kang M, Wei J, Yuan J, et al. Probable evidence of fecal aerosol transmission of SARS-CoV-2 in a high-rise building. Ann Intern Med **2020**; 173:974–80.
18. Guo M, Tao W, Flavell RA, Zhu S. Potential intestinal infection and faecal–oral transmission of SARS-CoV-2. Nat Rev Gastroenterol **2021**; 18:269–83.
19. Ianiro G, Mullish BH, Kelly CR, et al. Reorganisation of faecal microbiota transplant services during the COVID-19 pandemic. Gut **2020**; 69:1555–63.
20. Zhang Y, Cen M, Hu M, et al. Prevalence and persistent shedding of fecal SARS-CoV-2 RNA in patients with COVID-19 infection: a systematic review and meta-analysis. Clin Transl Gastroenterol **2021**; 12:e00343.
21. Natarajan A, Zlitni S, Brooks EF, et al. Gastrointestinal symptoms and fecal shedding of SARS-CoV-2 RNA suggest prolonged gastrointestinal infection. Med (N Y) **2022**; 3:371–87.e9.
22. Khanna S, Pardi D. Fecal microbiota transplantation for recurrent *Clostridioides difficile* infection: the COVID-19 era. Am J Gastroenterol **2020**; 115:971–4.
23. Coryell MP, Iakiviak M, Pereira N, et al. A method for detection of SARS-CoV-2 RNA in healthy human stool: a validation study. Lancet Microbe **2021**; 2:e259–66.