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Manufacturing Processes of SER-109, a Purified Investigational Microbiome Therapeutic, Reduce Risk of Transmission of Emerging and Undetected Infections in Donor Stool

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Background

- *Clostridioides difficile* infection (CDI) is a two-hit process characterized by disruption of the microbiome and exposure to *C. difficile* spores. The leading risk factor for CDI is exposure to broad spectrum antibiotics, which cause collateral damage to beneficial microbes that normally reside in the GI tract.[1,2,3]
- Studies of fecal microbiota transplantation (FMT) in patients with recurrent CDI, have demonstrated proof-of-concept that a sustained clinical response is linked to the recovery of Firmicutes bacteria in the GI tract. However, due to reliance on donor screening alone for risk mitigation, safety concerns persist about transmission of infectious pathogens.[3,4,5]
- In March 2020, these concerns were realized in a Food and Drug Administration (FDA) safety report of enteropathogenic *Escherichia coli* (EPEC) and Shigatoxin-producing *Escherichia coli* (STEC) infections following the investigational use of FMT.[4,5] Later that same month, another FDA safety alert highlighted that SARS-CoV-2 was isolated in stool of infected patients and warned of potential risk of SARS-CoV-2 transmission via the fecal route,[6] leading to an extended quarantine of FMT.

Fecal shedding of SARS-CoV-2 in infected patients exceeds respiratory shedding in duration[8]

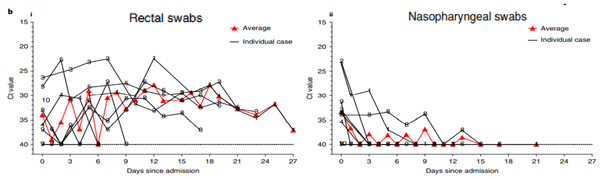


Fig. 1 | Chronology of major epidemiological events and molecular testing results of *n* = 10 independent pediatric patients confirmed with SARS-CoV-2 infection. a, Dates of exposure, illness onset and sampling and real-time RT–PCR results of nasopharyngeal swabs and rectal swabs. The total number of patients was *n* = 10 and real-time RT-PCR was assayed only once for one type of sample at one time point from one independent patient. Colors in the figure represent individual patients. **b**, Chronological changes in Ct values of *Orf1ab* and *N* genes using real-time RT–PCR after hospital admission. The Ct values of *Orf1ab* and *N* genes on real-time RT–PCR detected in rectal swabs obtained from *n* = 10 independent patients (i) and Ct values in nasopharyngeal swabs from *n* = 10 independent cases (ii). The Ct value is supposed to be inversely related to viral RNA copy numbers and a value of 40 means the virus is molecularly undetectable.

Pathogenic *E. coli* have a very low infectious dose

- Pathogenic *E. coli* include a variety of toxin-producing species including Shiga toxin-producing *E. Coli* (STEC), also called Enterohemorrhagic *E. coli* (EHEC)
- Exposures that result in illness include consumption of contaminated food or water, contact with cattle or with the feces of infected persons.
- Can tolerate acidic environments and are associated with a very low infectious dose (<10 cells), which could lead to missed detection in a donor.

Etcheverria AI, Padola NL. Shiga toxin-producing *Escherichia coli. Virulence.* 2013 Jul 1; 4(5): 366–372.

ECOSPOR-III

Phase 3 Double-blind, Placebo-Controlled Trial of SER-109 for Recurrent CDI

281 adult subjects screened

182 toxin+ adult subjects with symptom resolution after 10-21 days of antibiotics

10 oz magnesium citrate administered prior to randomization to minimize residual antibiotic

Subjects stratified by age and antibiotic received

4 Capsules once daily for 3 Days

Primary endpoint Recurrence at 8 weeks

Safety through 24 weeks

SER-109

Placebo

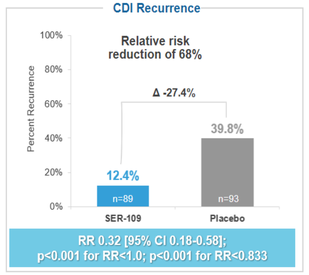
Recurrences evaluated through 24 weeks

Adult Study Participants ≥18 Years

- Toxin testing required at study entry and at suspected recurrence to ensure enrollment of patients with active disease and accurate assessment of endpoint
- All subjects had acute infection
- No chronic suppressive antibiotics allowed

ClinicalTrials.gov Identifier: NCT03183128

SER-109 was superior to placebo in reducing recurrence at week 8



- A qualifying episode at study entry was defined as:
  a) ≥3 unformed bowel movements over 2 consecutive days
  b) a positive *C. difficile* toxin test and
  c) symptomatic resolution on 10-21 days of standard-of-care antibiotics
- SER-109 met the primary endpoint of superiority compared to placebo
- By the alternative metric of sustained clinical response, 87.6% of the SER-109 recipients achieved this benchmark compared to 60.2% on placebo
- Number Needed to Treat for SER-109 = 3.6

Manufacturing Processes of SER-109 lead to a purified consortia of Firmicutes spores

Why Firmicutes?

- Spores are resistant to gastric acid allowing formulation into oral capsules
- Key role of spore-forming Firmicutes in modulating bile acid metabolism and inhibition of *C. difficile* spore germination

Mitigation of risk

- Comprehensive donor screening is an essential first step
- Manufacturing processes are designed to inactivate and eliminate pathogenic bacteria, parasites, fungi and viruses
- Product testing for contaminants adds a third layer of protection
- Rigorous manufacturing process represents a necessary redundancy to mitigate risk to patients.

McGovern B et al. *Clin Infect Dis* 2020; ciaa387

Objective

In the development of SER-109, we have previously evaluated whether the manufacturing processes could inactivate organisms of potential concern.

Herein we present relevant data on pathogens, which were highlighted by recent FDA safety alerts on FMT.

Approach to the selection of model viruses for inactivation studies and companion detection assays

The following inclusion criteria were evaluated for selection of a representative virus for inactivation studies:

- Well-characterized virus-cell system to provide reproducible results
- Similarity to the target viruses of interest, including inactivation mechanisms
- Stability in the relevant matrix (*favoring native enteric organisms*)
- Cultivability to prepare virus stock solutions of sufficient titer
- Detectability of virus activity to determine quantitative inactivation potential
- Safety of operators performing the inactivation studies.

Methods

- Model organisms were selected based on biological relevance and suitability, detectability, and laboratory safety.
- Spiking experiments into stool-derived process intermediates were completed to measure inactivation of model organisms by the selective unit operation
- Log-reduction factors (LRF) were calculated based on the drop in organism titer during inactivation
- Hold controls in non-ethanolic test matrices were used to confirm specificity of the ethanol inactivation

Candidate viruses for inactivation testing, and selection of Porcine Epidemic Diarrhea Virus (PEDV)

Virus	Family	Genus	Host	Primary Target	Biosafety Risk Group
229E	Coronaviridae	Alpha	Human	Respiratory	2
TGEV	Coronaviridae	Alpha	Porcine	Enteric	1 (non-human)
PEDV	Coronaviridae	Alpha	Porcine	Enteric	1 (non-human)
SARS-1	Coronaviridae	Beta	Human	Respiratory	3
SARS-2	Coronaviridae	Beta	Human	Respiratory	3
BVDV	Flaviviridae	Pestivirus	Bovine	Enteric	1 (non-human)

PEDV was selected as a representative model organism based on the following rationale:

- Biosafety Risk Group 1 (non-human).
- Native enteric system virus likely to be stable for spiking studies into donor stool derived matrices
- Long development history: high capacity for cultivation and use of well-established Vero cell line for quantitation

The selected system consists of cultured PEDV and a viral detection assay using Vero cells in a tissue culture infectious dose ($TCID_{50}$) assay using the Spearman-Karber calculation method.

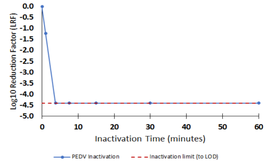
Candidate bacteria for inactivation testing, and selection of *Salmonella enterica*

Bacteria Type	Organism	Biosafety Risk Group	Unique Detectability
Gram negative bacilli	***E. coli* (pathogenic)**	2	Low
	***E. coli* (non-pathogenic)**	1	Low
	Salmonella enterica	2	High
	Klebsiella pneumoniae	2	Medium

S. enterica was selected as a representative model organism based on biological similarity, risk group, and unique cultivability to promote detection after spiking.

S. enterica detection on MacConkey lactose agar plus rifampicin

Spike of PEDV and Inactivation by SER-109 Process

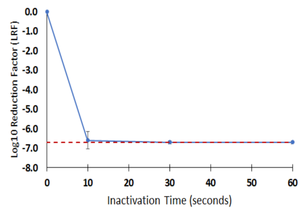


The ethanolic exposure achieved complete inactivation of PEDV to LOD within the target processing window.

PEDV was inactivated by more than **4.2 log_{10}** (to limit of detection, LOD) within **4 minutes.**

Figure 2: Inactivation of Porcine Epidemic Diarrhea Virus (PEDV), log_{10} reduction factor (LRF) versus time.
Average of two experiments shown. Also shown is the maximum achievable inactivation based on the limit of detection (LOD).

Spike of *S. enterica* and Inactivation by SER-109 Process



Rapid inactivation to limit of detection (LOD) is demonstrated by the scale-down process, with all replicates quickly achieving LOD.

The inactivation of *S. enterica* was more than **6.5 log_{10}** within **30 seconds.**

Figure 1: Inactivation of *S. enterica*, log_{10} reduction factor (LRF) versus time. Average of three experiments with error bars represent 95% CI. Also shown is the maximum achievable inactivation based on the limit of detection (LOD).

Summary

FMT and FMT-like products are vulnerable to pathogens, which may go undetected during donor screening due to:

- Inappropriate assay selection or its limits of detection
- Interfering substances that may thwart detection in stool
- Occult emerging pathogens that may be present years before clinical detection, as seen in the past with HIV, hepatitis B and C viruses

The manufacturing processes for SER-109, an investigational oral microbiome therapeutic, are designed to enrich for beneficial Firmicutes spores, while inactivating potential pathogens. Product testing for contaminants adds a third layer of protection.

These experiments demonstrate substantial and rapid inactivation of the model organisms including PEDV, a coronavirus with gastrointestinal tropism and *Salmonella enterica*, a representative enteric Gram-negative bacterium

- Lends insight into the impact of these manufacturing processes on a wide range of potential pathogens, including SARS-CoV-2 and multidrug resistant organisms (e.g, E.coli, CRE).

Conclusions

The manufacturing processes and product testing for SER-109 are designed to afford a manufacturing "safety net" to mitigate risk to patients, beyond donor screening alone.

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