

## Introduction

Open-label studies suggest that fecal microbiota transplant (FMT) may be effective in preventing *Clostridium difficile* infection (CDI) in patients with multiple recurrences by restoring the ecology of a healthy microbiome [1,2,3].

However, stool is minimally processed so potential transmission of undetected pathogens or emerging infections is still a concern [4]. This is a particular problem for microbes acquired through diet, like *Listeria*, which can lead to transient, asymptomatic colonization [5]. An alternative treatment for recurrent CDI with an improved risk profile is urgently needed.

SER-109 is an investigational, Ecobiotic® drug, which contains a purified ecology of approximately 50 unique bacterial spore-forming anaerobic Firmicutes fractionated from rigorously screened stool donors [6]. This microbiome-based drug product represents <0.1% of whole stool, facilitating convenient delivery as 4 oral capsules. A randomized, double-blind, placebo-controlled Phase 2 trial evaluating the efficacy and safety of SER-109 for patients with multiple recurrences of CDI has completed enrollment.

The SER-109 manufacturing process includes multiple steps of purification and ethanol inactivation of vegetative bacteria (e.g. *Listeria*, *Salmonella*, *Staphylococcus*, or *Enterococcus*), which can serve as pathogens if not detected during screening. Ethanol is widely known to inactivate vegetative bacteria [7].

## Aim

To evaluate inactivation of vegetative bacteria in the SER-109 manufacturing process

## Methods

### Inactivation kinetics

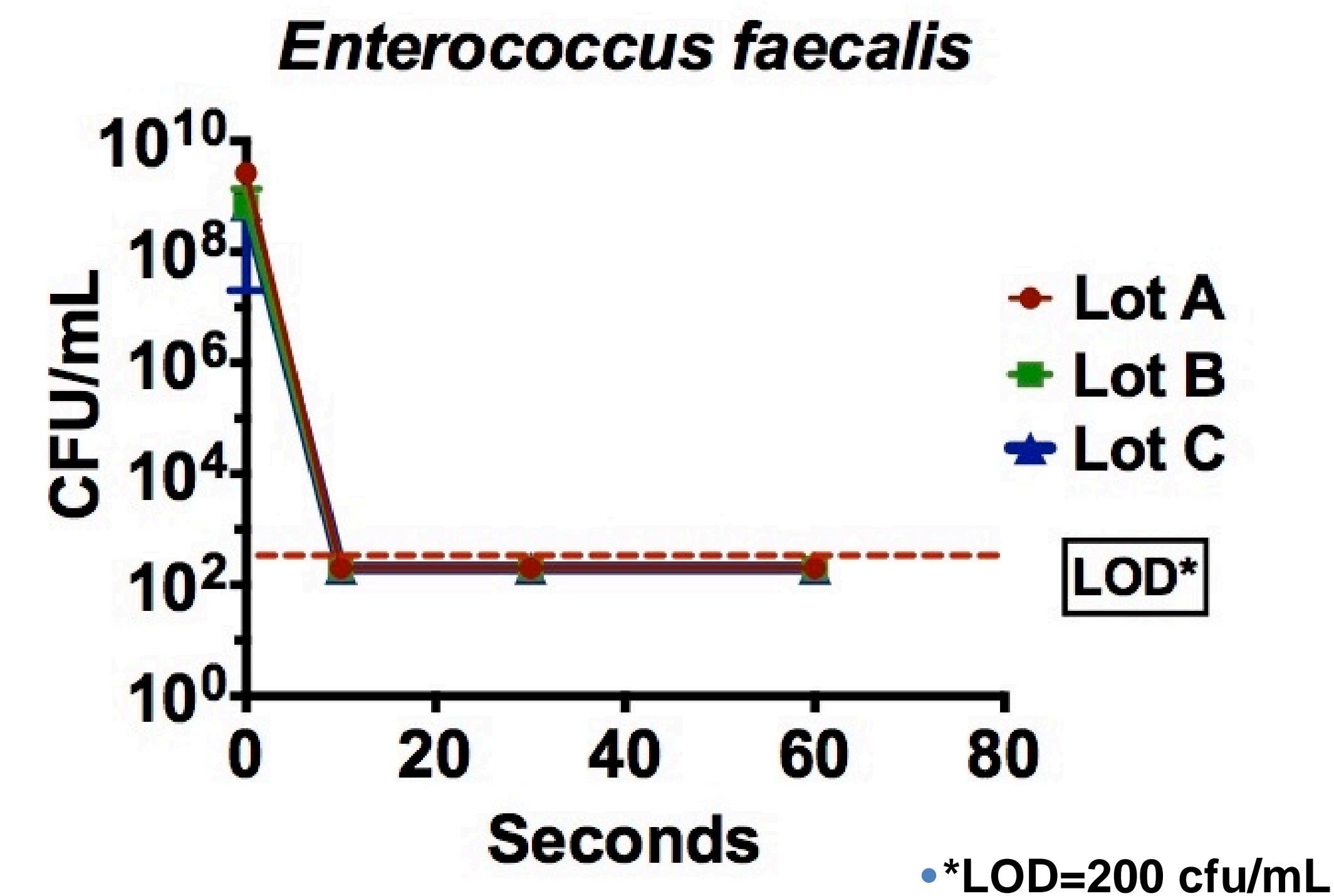
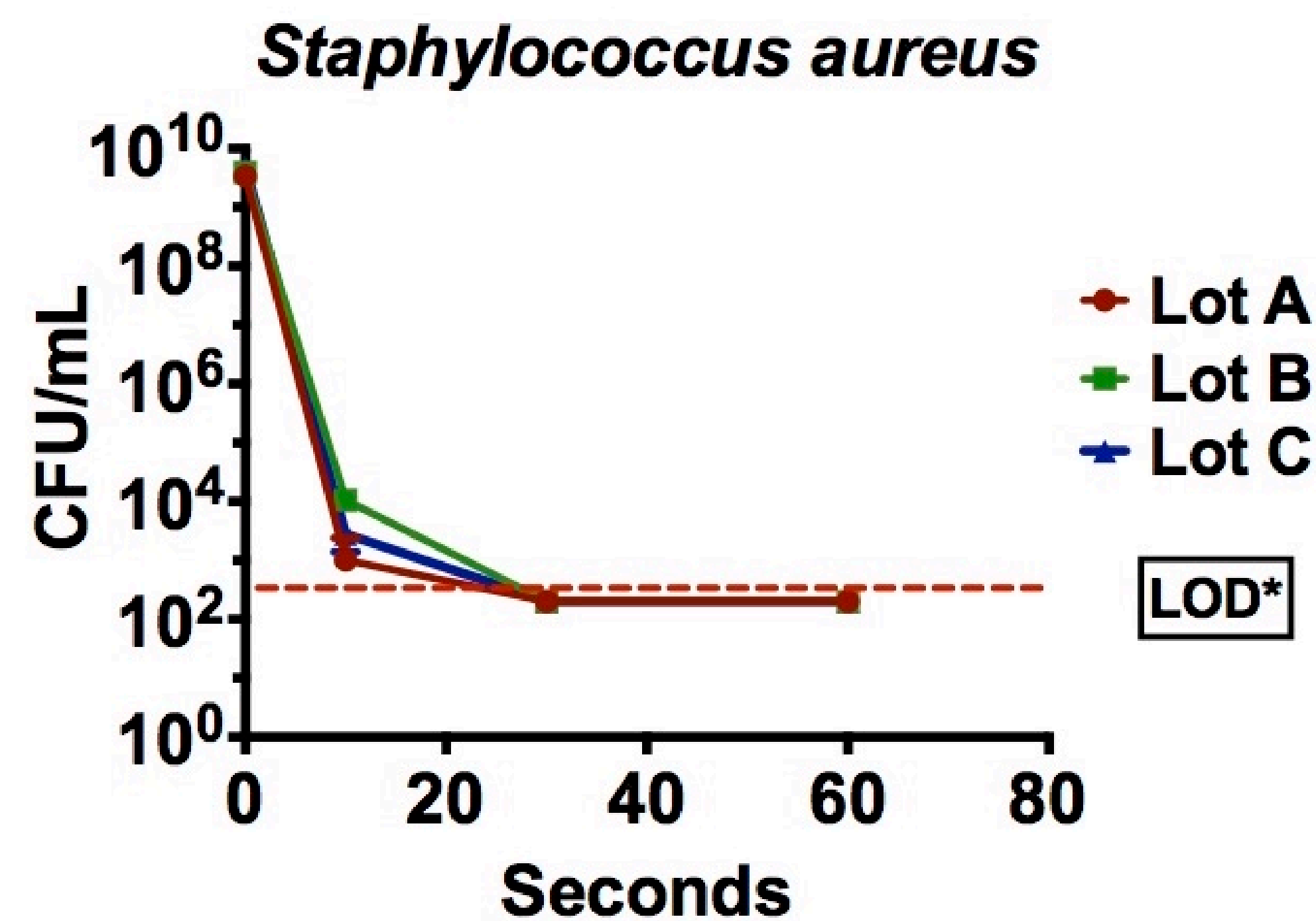
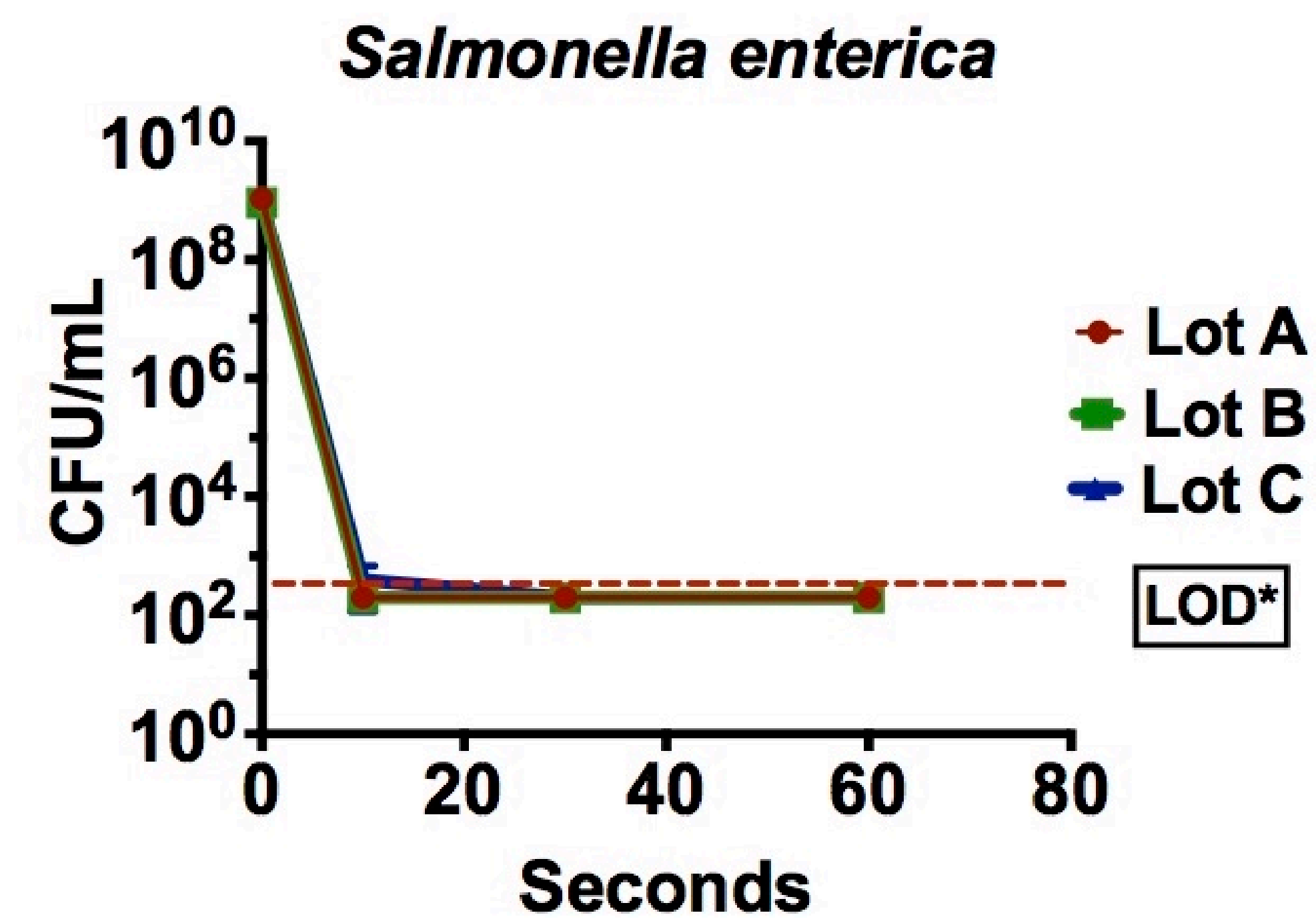
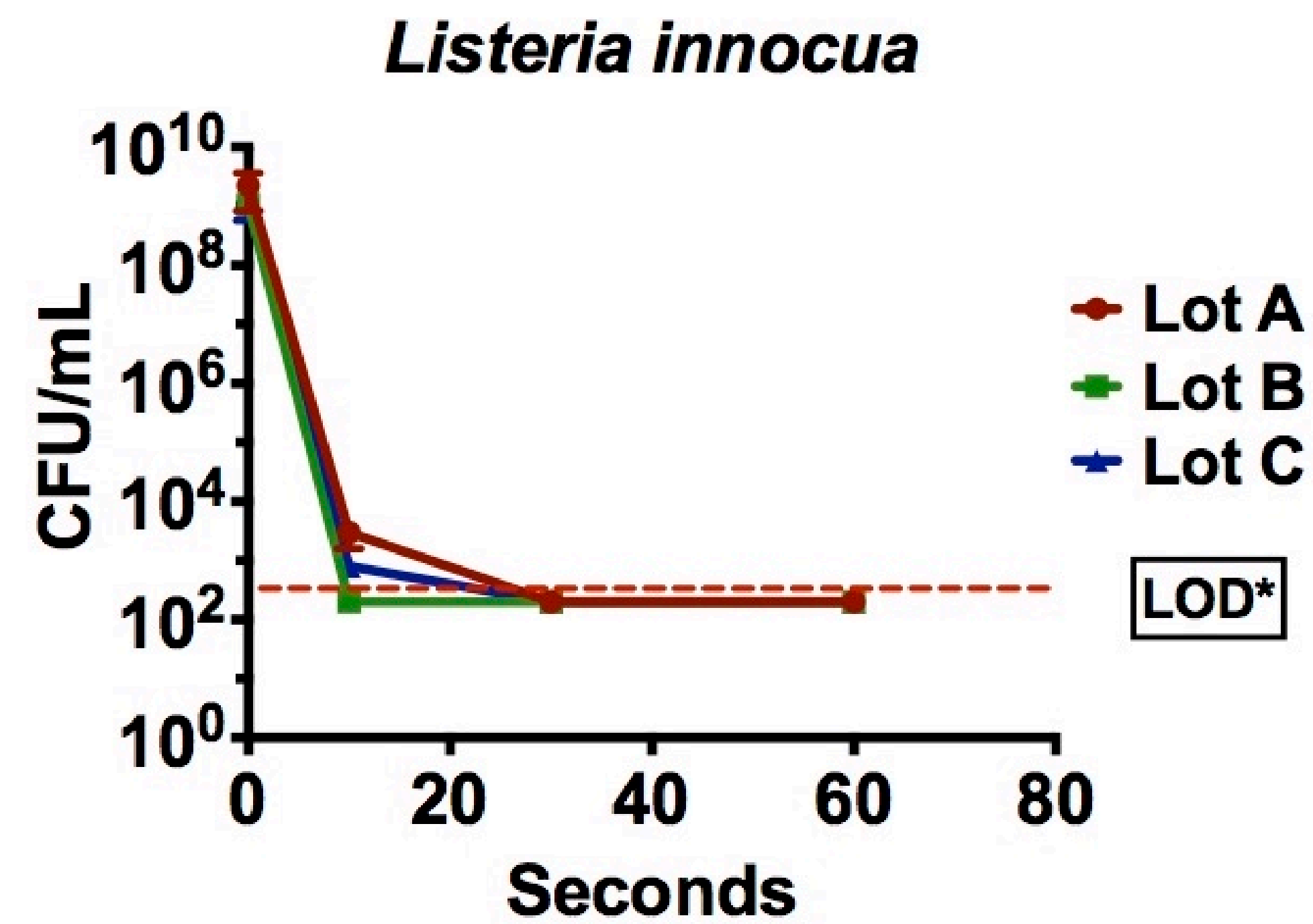
- Cell banks of *Listeria innocua* SLCC 3379, *Salmonella enterica* LT2, *Staphylococcus aureus* Wichita, and *Enterococcus faecalis* NCTC 775 were added to three separate lots of SER-109 intermediates immediately prior to ethanol inactivation.
- Ethanol was added up to 50% v/v. Inactivations were stopped by diluting 1:10 to reduce the concentration of ethanol to 5% v/v (demonstrated to not be bacteriocidal, data not shown).
- Titers of these samples were taken within 60 minutes of dilution on media selecting for the appropriate spiked organism. Titers in CFU/mL were plotted over time.

### Identification of cultivatable anaerobes in SER-109

- Samples of SER-109 lots were cultured using standard anaerobic culturing methods. Single colonies were randomly sampled and characterized via sequencing of the 16S rRNA gene.

### Inactivation kinetics demonstrate that ethanol processing leads to inactivation below the limit of detection in seconds

- Measuring vegetative bacteria in a complex mixture is complicated by the presence of bacterial spores. Three different lots of SER-109 were processed up to the ethanol inactivation step.
- These lots were spiked with cells banks of the indicated organisms. Ethanol was added to 50% v/v, and titers were determined post-ethanol inactivation.
- All samples show inactivation below the limit of detection within seconds



## Results

### SER-109 consists of spore-forming organisms of the phylum Firmicutes

- Samples of SER-109 lots were cultured using standard anaerobic culturing methods.
- Single colonies were randomly sampled and characterized via sequencing of the 16S rRNA gene.
- A total of 16,810 colonies from a number of SER-109 lots were sampled.
- All colonies were classified as spore-forming organisms belonging to the phylum Firmicutes.
- Taxonomic families detected are listed.

Family
Clostridiaceae
Erysipelotrichaceae
Eubacteriaceae
Lachnospiraceae
Oscillospiraceae
Peptostreptococcaceae
Ruminococcaceae
Unclassified Clostridiales
Total colonies sampled: 16,810

## Conclusions

- These spike recovery studies demonstrate >6 Log of inactivation of vegetative cells by ethanol within seconds. When considering the full length of ethanol exposure that occurs within the inactivation process, greater than 10-Log of vegetative bacteria would be expected to be inactivated.
- Determination of the identity of 16,810 recovered viable anaerobes from SER-109 lots demonstrates that the ethanol inactivation step is effective at removing vegetative bacterial cells that would otherwise be present.
- The manufacturing process of SER-109 reduces the risk of pathogen transmission to a level that cannot be achieved by donor screening alone.

## References

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