Inactivation of Vegetative Bacteria During Production of SER-109, a Microbiome-Based Therapeutic for Recurrent Clostridium difficile Infection

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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**

**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 7755 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 lots were cultured using standard anaerobic culturing methods. Single colonies were randomly sampled and characterized via sequencing of the 16S rRNA gene.

**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**

1. Van Nostrand Inactivation of Donor Feces for Recurrent Clostridium difficile infection NEm JCI 2013

**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 selected.
- All colonies were classified as spore-forming organisms belonging to the phylum Firmicutes.
- Taxonomic families detected are listed.