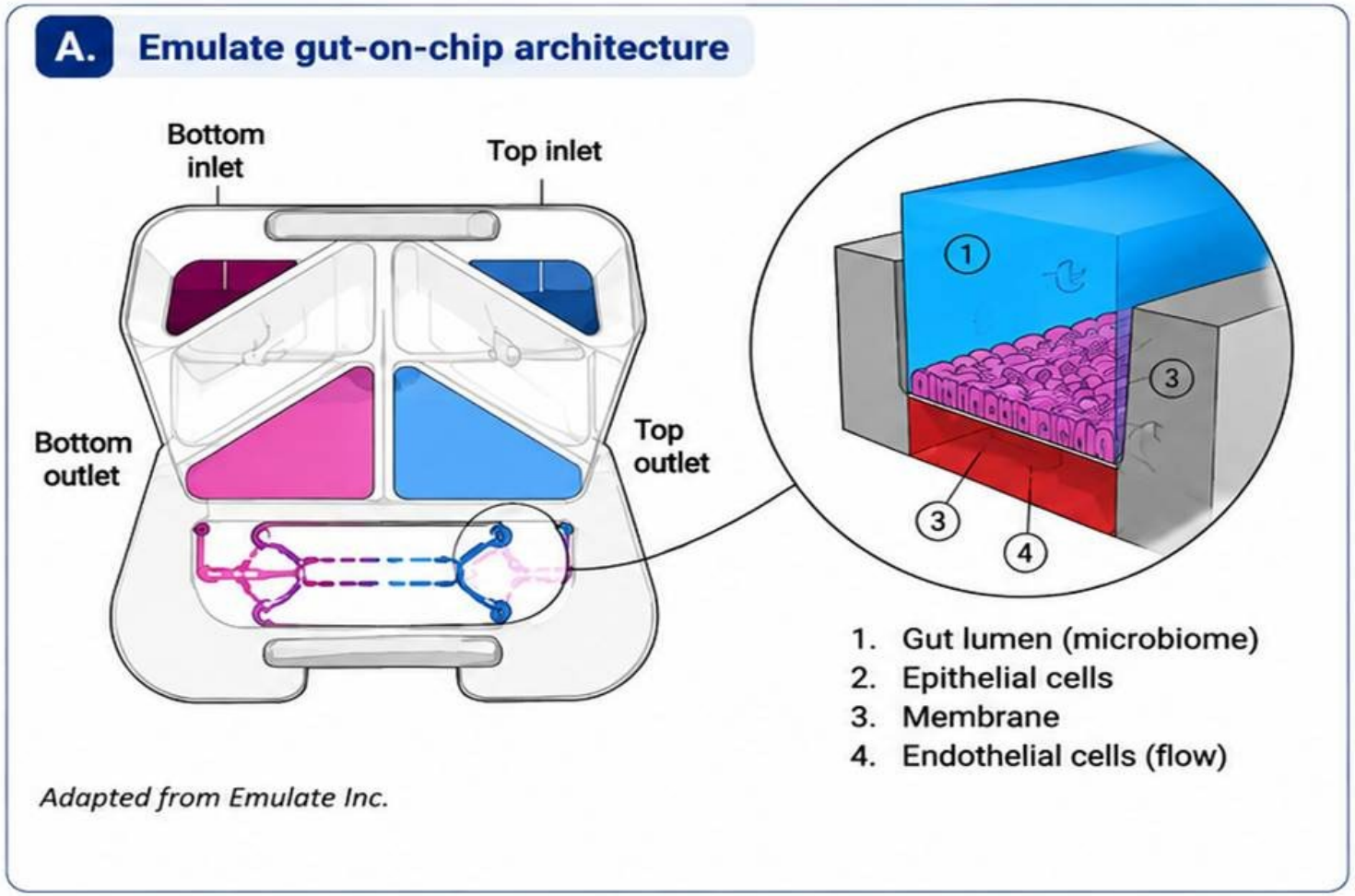
GenEpi group

Jonathan Jensen

Probiotic-Induced Pathogen Modulation in *In Vitro* Gut on Chip Model Under Varying pH Conditions

Introduction

- A common infection of *Salmonella enterica* can normally be treated using antimicrobial agents.
- We seek to understand if the intensity of an *S. enterica* infection, can be reduced with the introduction of a probiotic.
- We also seek to see if the changes in intensity are based on environmental factors such as pH.
- We are using Gut-on-chip technology to allow mimicry of the gut, giving us an authentic environment to that of the gut.

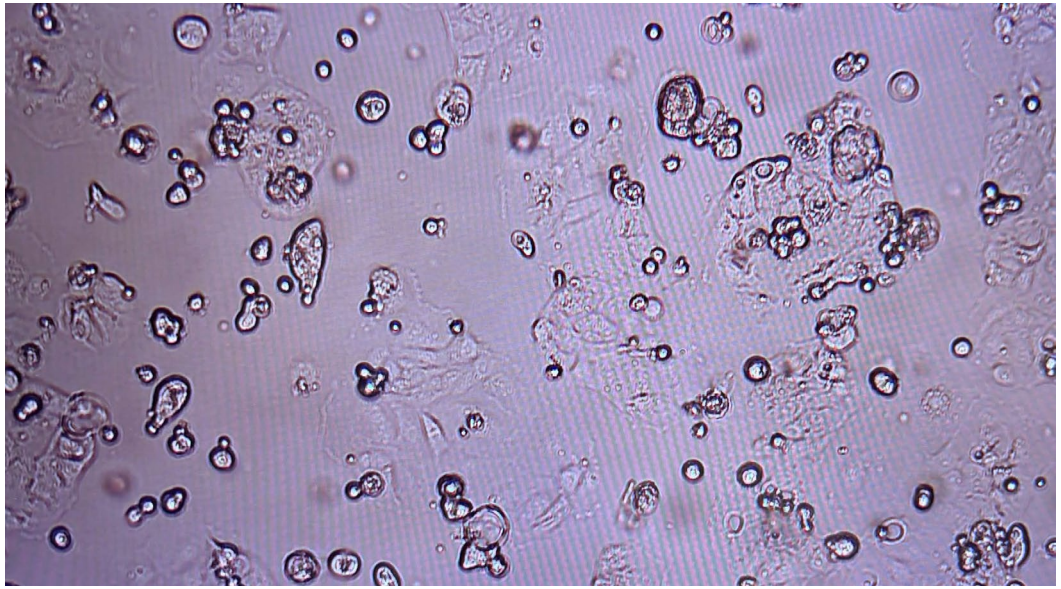


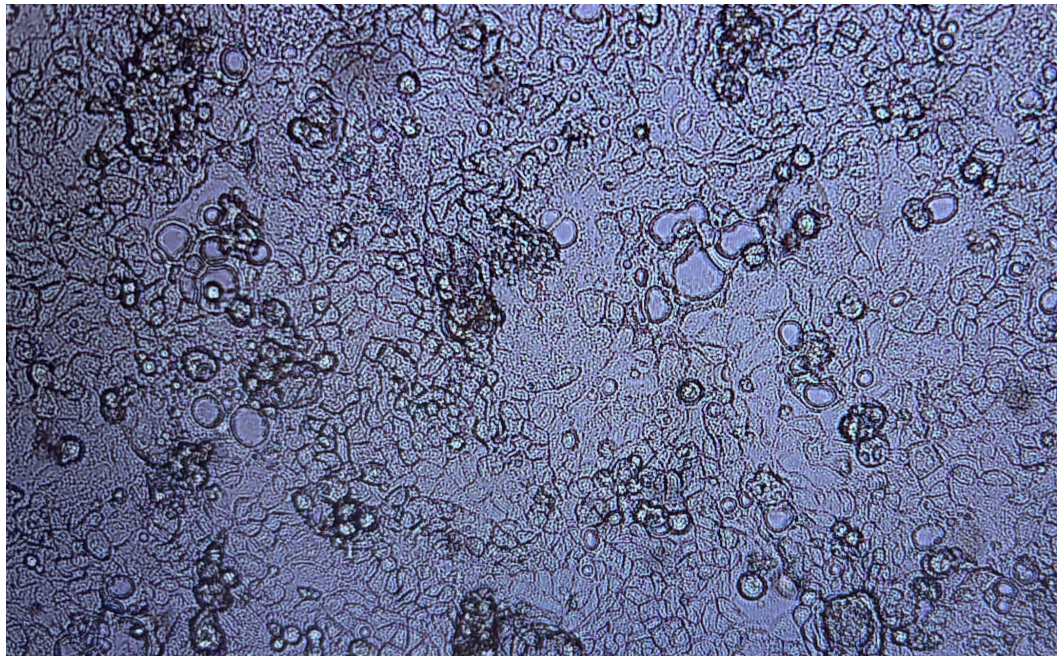
Timeline – Cell growth

24th August – 9th of October

Caco2 cell growth stage

10 passages for high density culture.





Timeline – Chip assembly

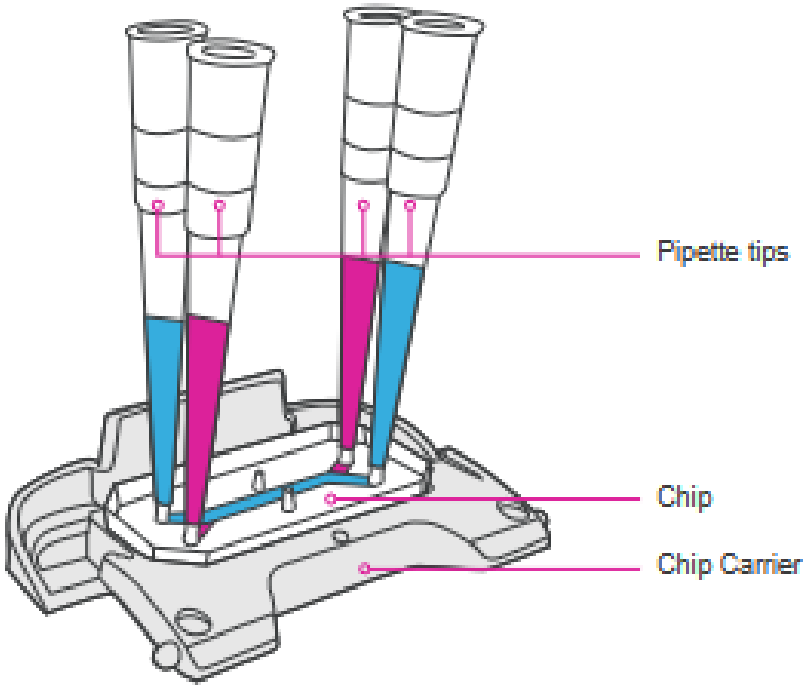
24th August – 9th of October

Caco2 cell growth stage

10 passages for high density culture.

October 12th

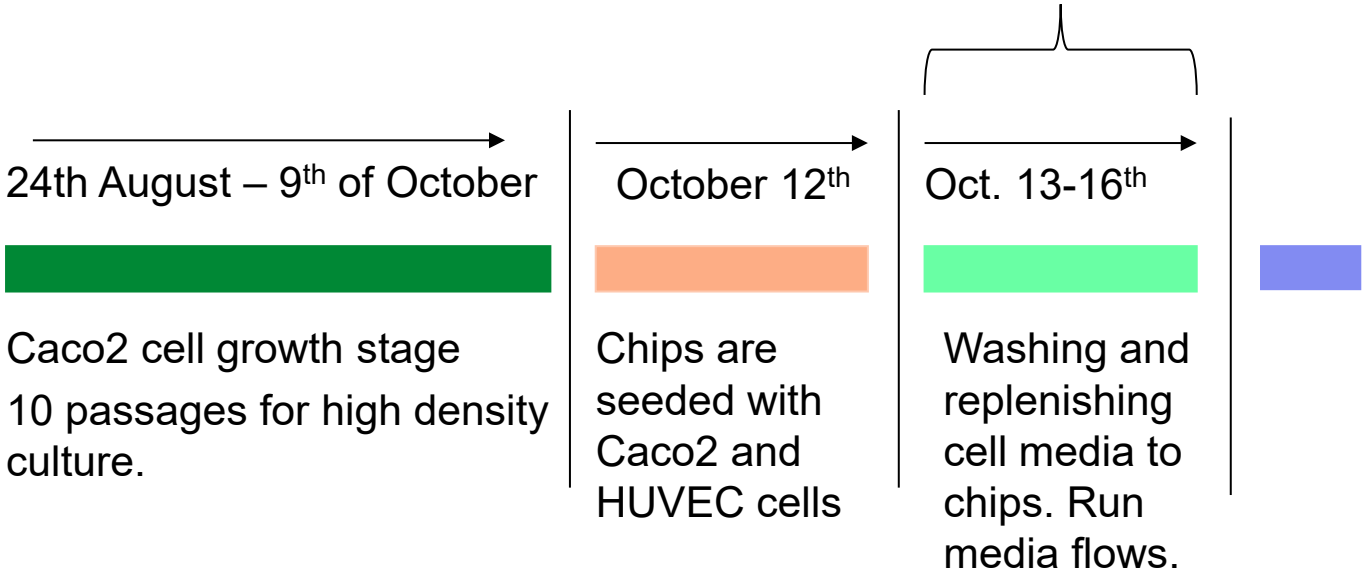
Chips are seeded with Caco2 and HUVEC cells



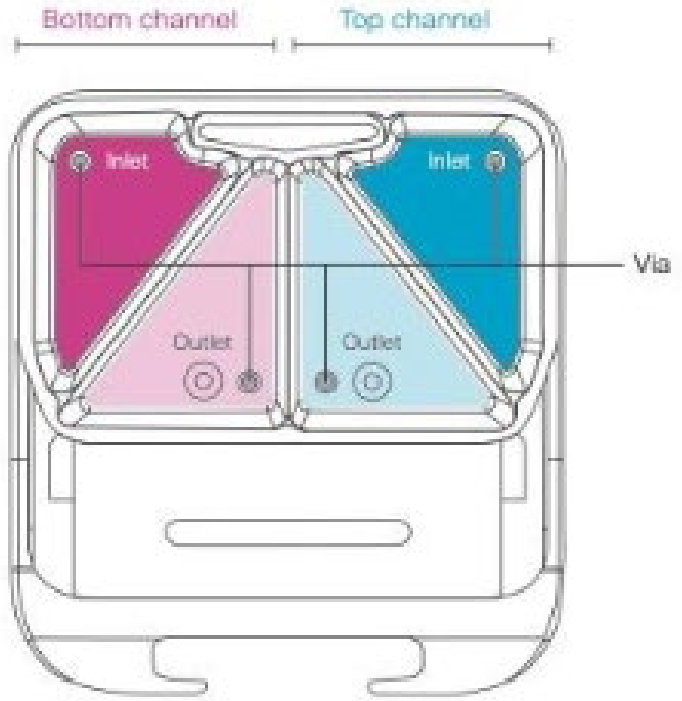
Emulate protocol – Day 0 seeding.

HUVEC and Caco2 cells are seeded into their respective channels, after the chip has been coated with a collagen layer to allow surface binding.

Timeline – Chip maintenance phase



- Initial introduction of media along with via washing to avoid bubble formation.
- Time is now given to cells over the course of 1-2 weeks to increase in density, daily washing.



Emulate protocol – Day 1+ chip maintenance.

Introduction of Caco2 and HUVEC media into chip, of high importance bubbles are not introduced as they will impede function of chip severely.

Timeline – Experiment phase

Community taxa

- *Bifidobacterium*
- *Collinsella*
- *E. coli*
- *E. faecium*

Probiotic

- *Lactobacillus lactis*

Pathogen

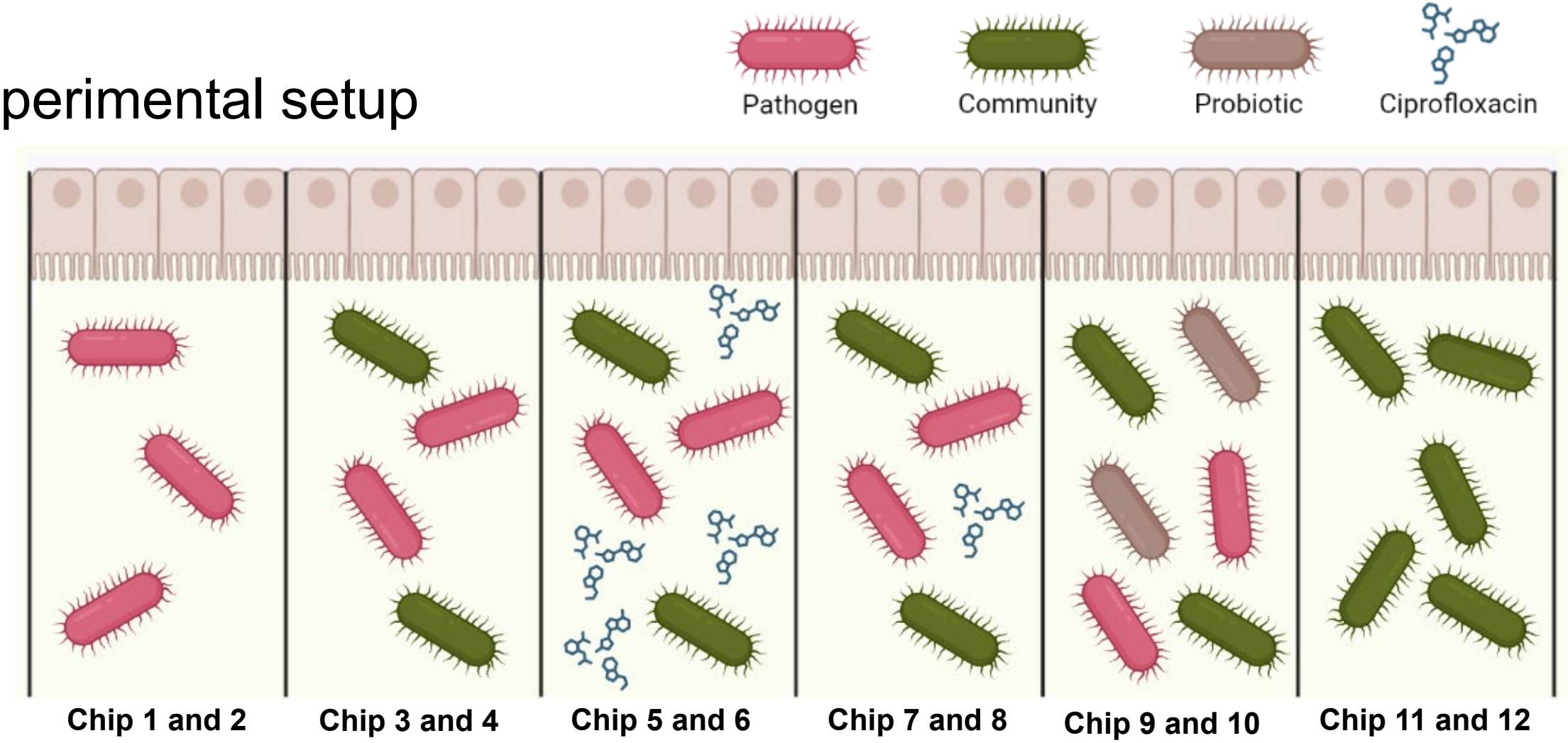
- *Salmonella enterica*

October 27-30th

Infection begins, introduction of both commensal community, pathogen and probiotic into respective chips.

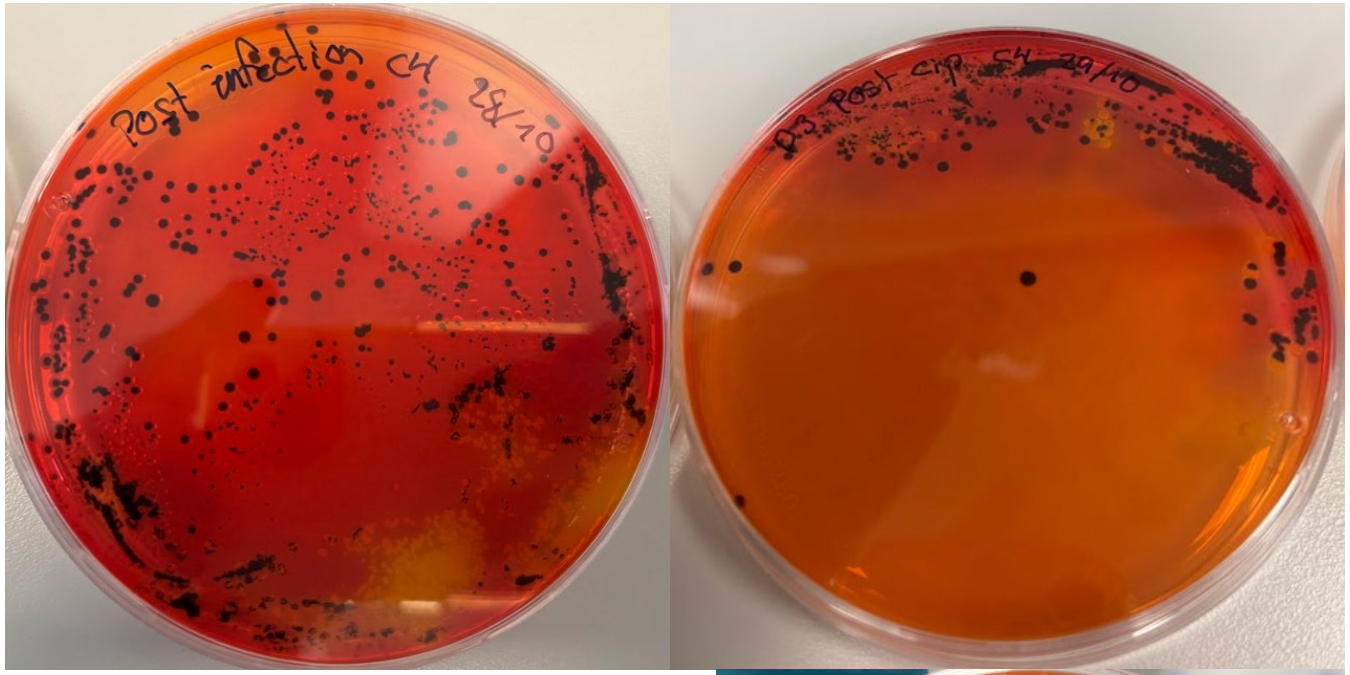
- Day 1 - Community
  - Community of 4 commensal taxa is added to chips, simulating a gut microbiome
- Day 2 - Infection
  - Infection stage and treatment, *S. enterica* is introduced into the chips to simulate the initial stage of infection.
- Day 3 - Treatment
  - An antibiotic (Ciprofloxacin) is added to some of the chips; this is to simulate the stage at which treatment is occurring.
- Day 4 - Recovery
  - All media is removed from chips, then flushed and replenished with new media. Giving us a recovery stage to see if the infection bounces back.

Experimental setup

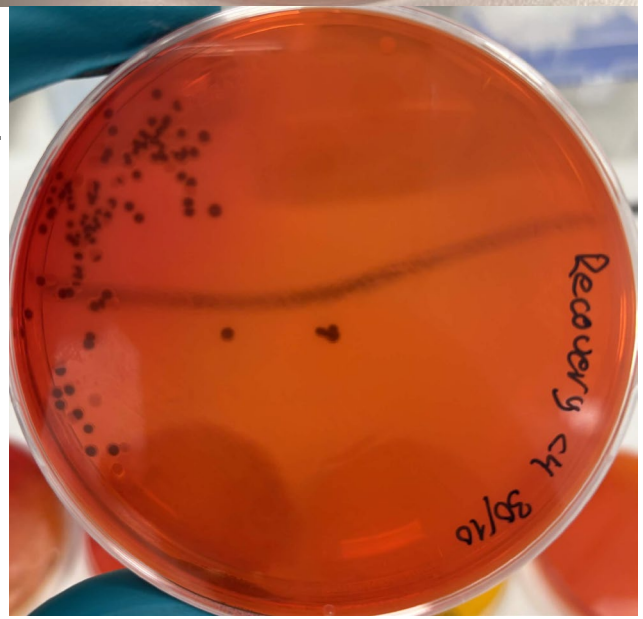


Preliminary results

- We are currently extracting and sequencing the samples to gain final results.
- We have performed *Salmonella* colony counting to get some preliminary results.



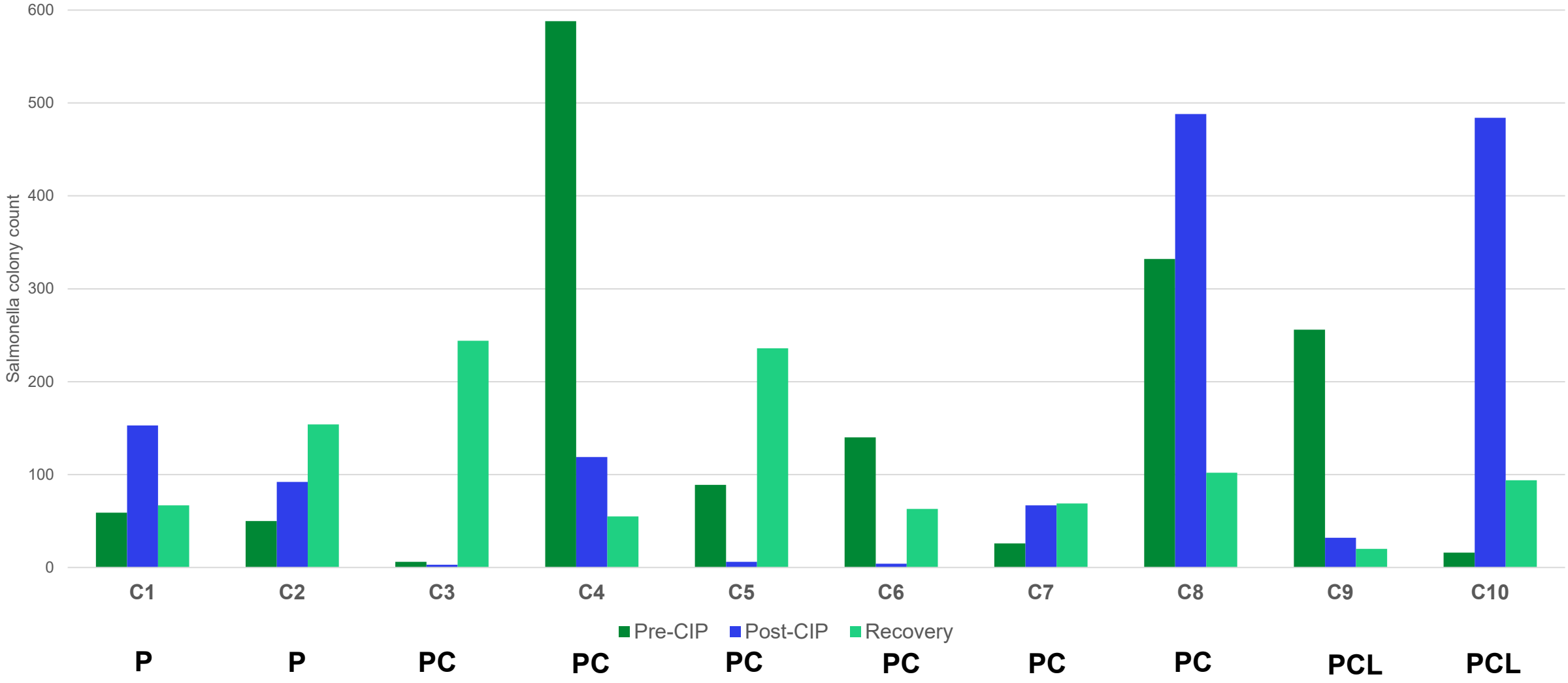
XLD plates containing salmonella from chip 4 (Community + pathogen).



Preliminary results

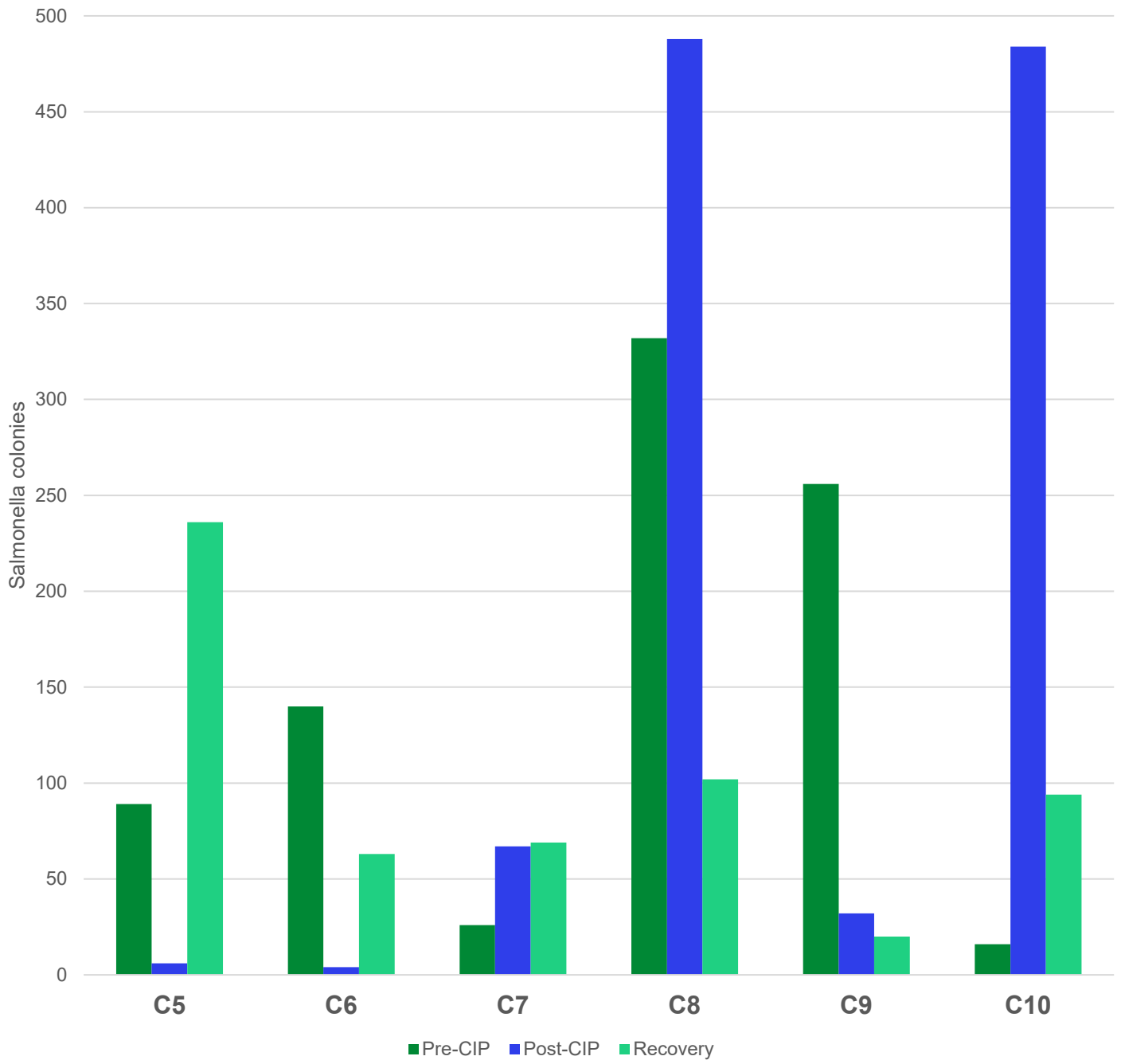
P – Pathogen, C – Community, L - Lacto

Chip 1, 3, 5, 7, 9. – Media pH 5.5
Chip 2, 4, 6, 8, 10 – Media pH 7.4



Preliminary results

- *Salmonella* is seen to favour a mixed community for growth.
- At pH 5 the *Salmonella* count decreases with the addition of *Lactobacillus.*
- With *Lactobacillus* there seems to be an anti-synergy at pH 7 resulting in high *Salmonella* growth.




DTU