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Development of a Nontyphoidal *Salmonella* Controlled Human Infection Model

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Department of Infectious Disease

06 December 2023

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Outline

- **Rationale for an NTS CHIM**
- **Clinical Study Design**
- **GMP Manufacture and Characterisation**
- **Study Objectives and Progress**
  - Dose-escalation and safety
  - Clinical and microbiological responses
  - Immunological responses
- **Exploratory sub-studies**

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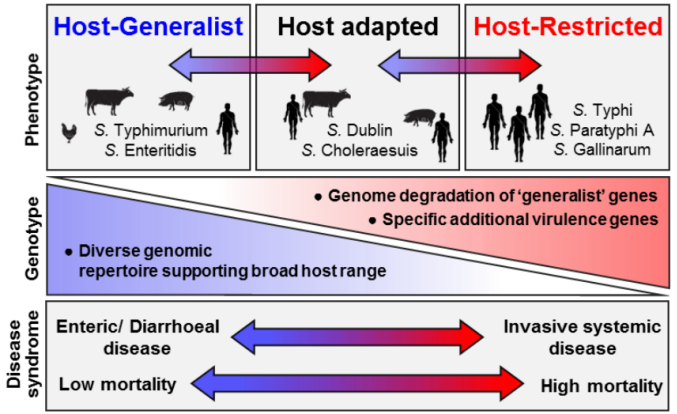
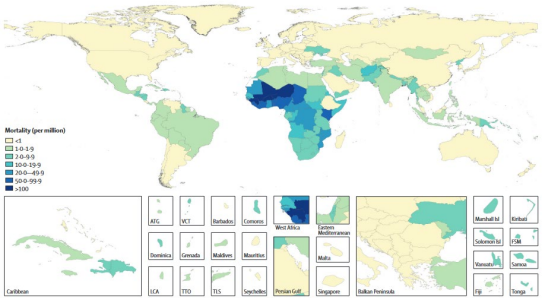
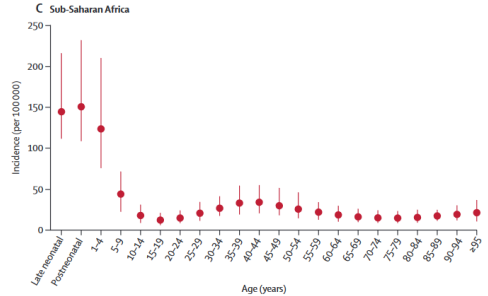
Nontyphoidal *Salmonella* (NTS)

- Spectrum of disease
  - iNTS vs. dNTS
- iNTS serovars dominant in Africa
- High case-fatality
  - 15-20%
- Vaccine development in progress







Feasey et al., Lancet 2012; 379: 2489–99

Stanaway et al., Lancet Infect Dis 2019; 19: 1312–24

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Susceptible Host vs. Pathogen

Host

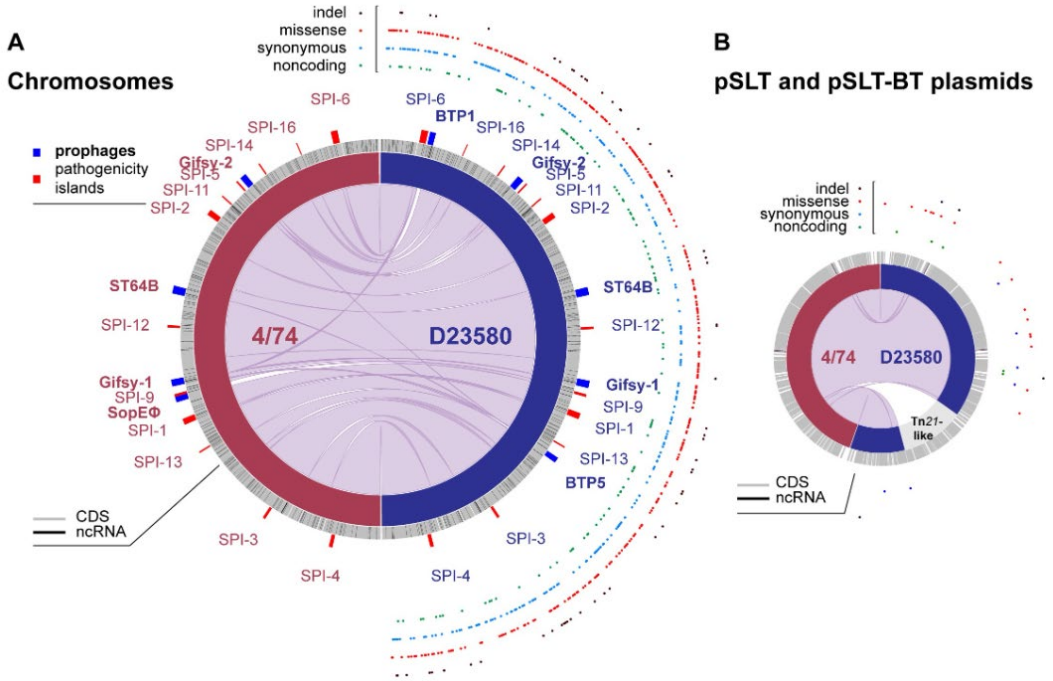
Primary immunodeficiency
- MSMD
- CGD

Adults
- HIV

Children
- Malaria
- Malnutrition
- Sickle cell disease
- Anaemia

Pathogen

- Host-adaptation
- **ST313 Lineage II D23580**
- Loss of multicellular behaviour and adaptation to extra-intestinal lifestyle



Canals et al., PLoS Biol 17(1): e3000059

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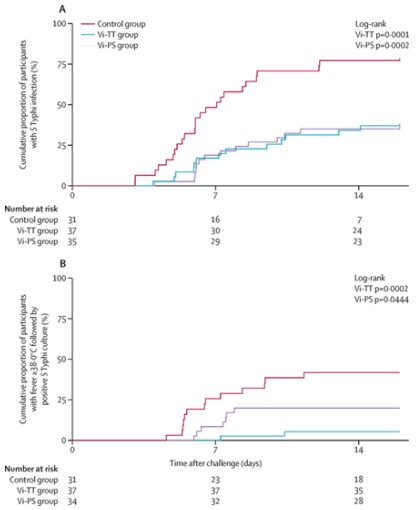
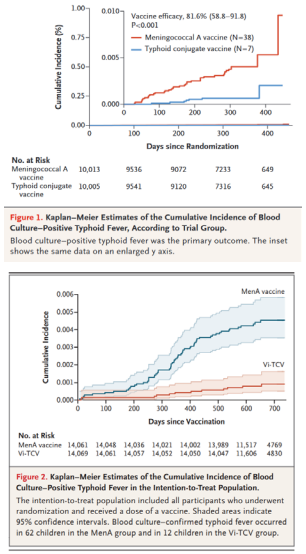
Controlled Human Infection Models

- Unique opportunity to obtain early efficacy data of candidate vaccines or therapeutics
- Opportunity to dissect physiological and immunological responses to infection

Efficacy and immunogenicity of a Vi-tetanus toxoid conjugate vaccine in the prevention of typhoid fever using a controlled human infection model of *Salmonella* Typhi: a randomised controlled, phase 2b trial

Celina Jin, Malick M Gibani, Maria Moore, Helene B Juel, Elizabeth Jones, James Meiring, Victoria Harris, Jonathan Gardner, Anna Nebykova, Simon A Kerridge, Jennifer Hill, Helena Thomaides-Brears, Christoph J Blohmke, Ly-Mee Yu, Brian Angus, Andrew J Pollard





Jin et al., Lancet 2017; 390: 2472–80

Patel et al., N Engl J Med 2021;385:1104-15

Shakya et al., N Engl J Med 2019;381:2209-18

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The CHANTS Consortium

- Overarching aim to develop a first-in-human NTS CHIM
- Support and accelerate NTS vaccine development

PATH · University of Liverpool · WRAIR Walter Reed Army Institute of Research · MRC Weatherall Institute of Molecular Medicine · Ragon Institute of MGH, MIT and Harvard · Oxford Vaccine Group · University of Oxford · MRC Centre for Molecular Bacteriology and Infection · Imperial College London

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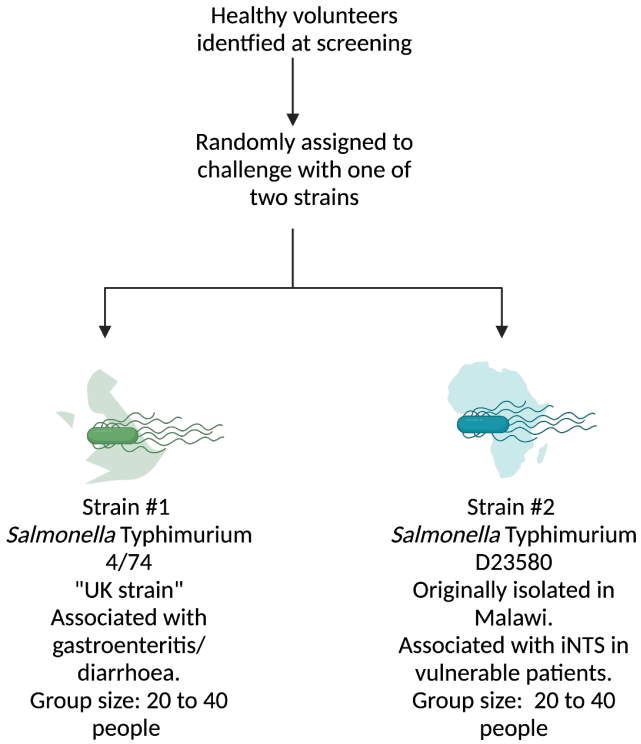
Study design

- Phase 1, randomised, double-blind, first-in human
- *Salmonella* Typhimurium (GMP)
  - ST19 (4/74) vs. ST313 (D23580)
  - NaHCO3 pre-treatment
- Healthy, *Salmonella* naïve, UK resident adult volunteers, aged 18-50 (n = 40-80)
- Primary Outcome > Systemic Salmonellosis
  - Sustained fever >38C >12hrs **and/or** bacteraemia
- Dose-escalation model – target attack rate 60-75%
  - 1-5 x 10^3 to 10^6 CFU



Development of non-Typhoidal *Salmonella* controlled human infection model

4/74 (ST19)

D23580 (ST313)

Genomes: 95% identical

	4/74	D23580
Genome degradation	some	↑ Increased compared to 4/74
Invasiveness of epithelial cells	↑	↓
Uptake in macrophages	↓	↑
Survival & replication in macrophages	↓	↑
Apoptosis and inflammatory response in macrophages	↓	↑
Resistance to killing by macrophages	↓	↑
Amount of complement required for antibody mediated bactericidal activity	↓	↑

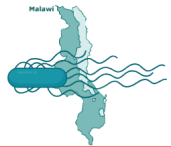
Healthy volunteers identified at screening

↓

Randomly assigned to challenge with one of two strains

Strain #1
Salmonella Typhimurium
4/74
"UK strain"

Associated with gastroenteritis/ diarrhoea.



Strain #2
Salmonella Typhimurium
D23580
Originally isolated in Malawi.

Associated with iNTS in vulnerable patients.

R. Canals, 2019

THE CHANTS STUDY

Development of non-Typhoidal *Salmonella* controlled human infection model

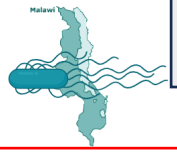
Healthy volunteers identified at screening

↓

Randomly assigned to challenge with one of two strains

Strain #1
Salmonella Typhimurium
4/74
"UK strain"
Associated with gastroenteritis/ diarrhoea.

ST19 ←



Strain #2
Salmonella Typhimurium
D23580
Originally isolated in Malawi.
Associated with iNTS in vulnerable patients.

→ ST313

Partnered with Walter-Reed Army institute for research (WRAIR) to generate GMP quality stocks of both strains **4/74 & D23580**

1) **Manufacture of GMP stocks**
2) **Characterization of GMP stocks for challenge**
3) **Preparation of Challenge Agent dose**

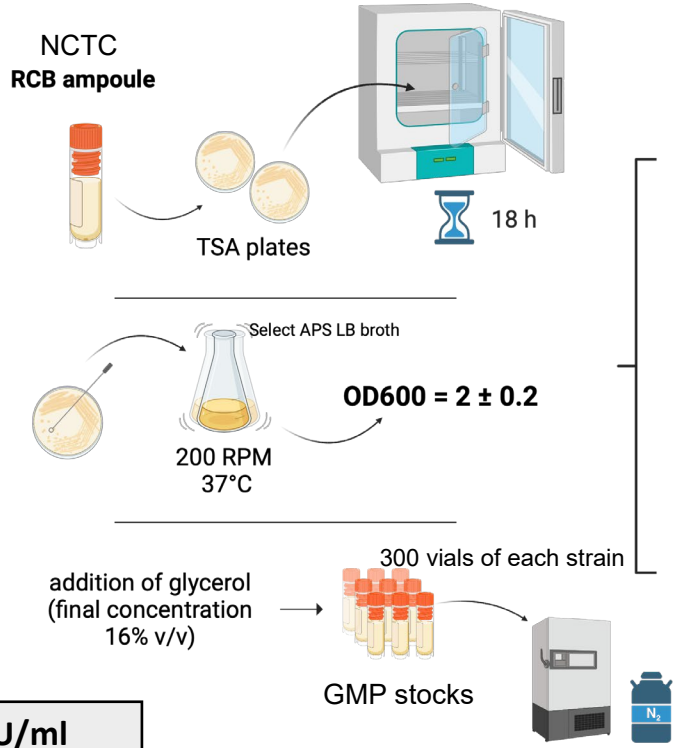
Eddie Suvarnapunya

1) MANUFACTURE OF GMP STOCKS (and quality control)

Walter-Reed Army institute for research (WRAIR)

PILOT BIOPRODUCTION FACILITY
WRAIR
Walter Reed Army Institute of Research
Soldier Health • World Health

Generation of Master Cell Bank from Research Cell Bank (RCB) ampoule



verification of purity

gram staining

pre-freeze viability

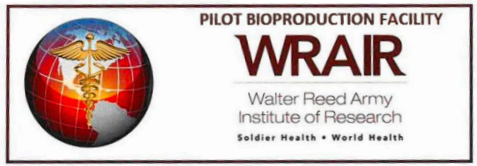
PRE-FREEZE VIABILITY NUMBERS

4/74: **1.24 x 10^9 CFU/ml**

D23580: **9.95 x 10^8 CFU/ml**

Eddie Suvarnapunya

2) CHARACTERISATION OF GMP STOCKS



Prof Jay Hinton's team

PATH

Specified Contaminant	Enrichment Broth	Selective Agar
Escherichia coli	Fluid Lactose Medium	MacConkey Agar Medium
Pseudomonas aeruginosa	Fluid Lactose Medium Fluid Soybean-Casein Digest Medium	Cetrimide Agar Medium
Bacillus cereus	Fluid Soybean-Casein Digest Medium	Bacillus cereus Agar
Staphylococcus aureus	Fluid Soybean-Casein Digest Medium	Baird-Parker Agar Medium
Aspergillus niger	N/A	Acidified Potato Dextrose Agar Medium
Candida albicans	N/A	Sabouraud Dextrose Agar Medium + Kanamycin

2) CHARACTERISATION OF GMP STOCKS

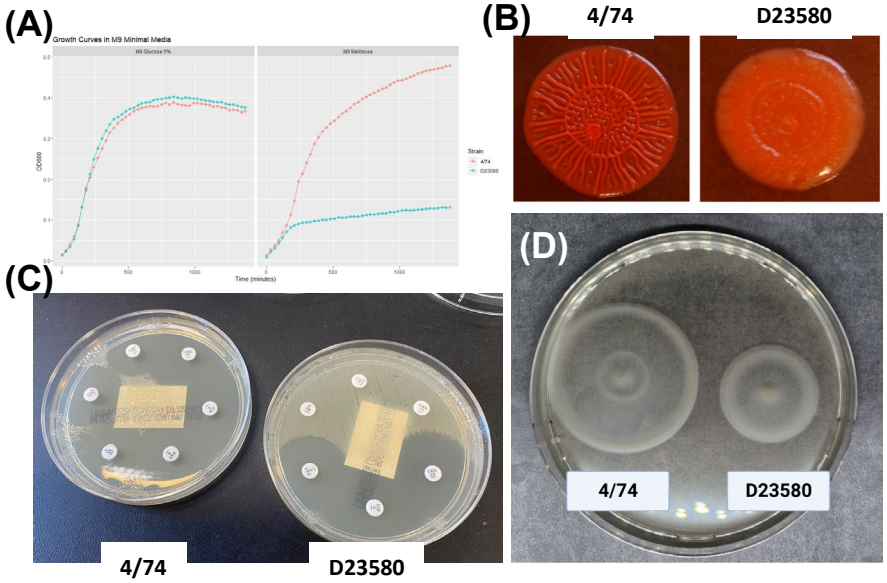
PILOT BIOPRODUCTION FACILITY
WRAIR
Walter Reed Army Institute of Research
Soldier Health • World Health

UNIVERSITY OF LIVERPOOL
Prof Jay Hinton's team

PATH

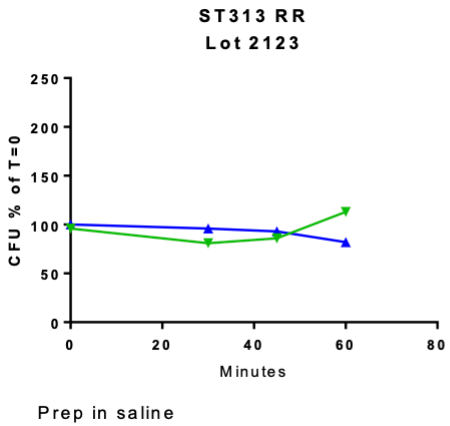
NO UNEXPECTED RESULTS

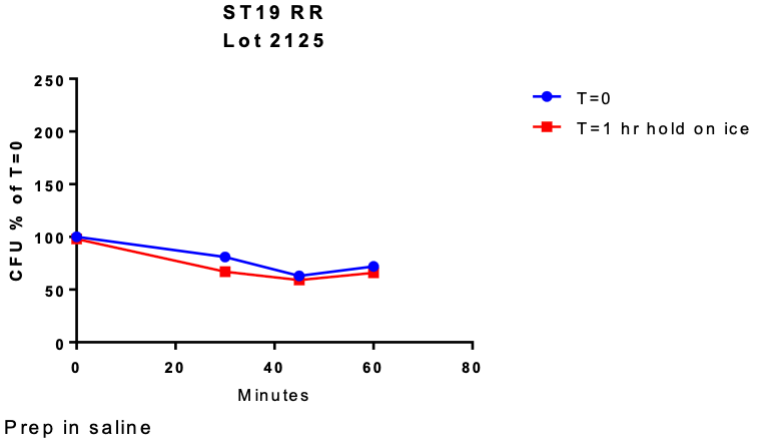
- biochemical ID
- growth curves **(A)**
- RDAR phenotype **(B)**
- antimicrobial sensitivity testing **(C)**
- motility **(D)**
- whole genome sequencing



2) CHARACTERISATION OF GMP STOCKS

STABILITY TESTING – USING ROSSETT-RICE MODEL





TAKEAWAY numbers of *Salmonella* bacteria remain relatively stable for at least an hour (at RT and on ice)

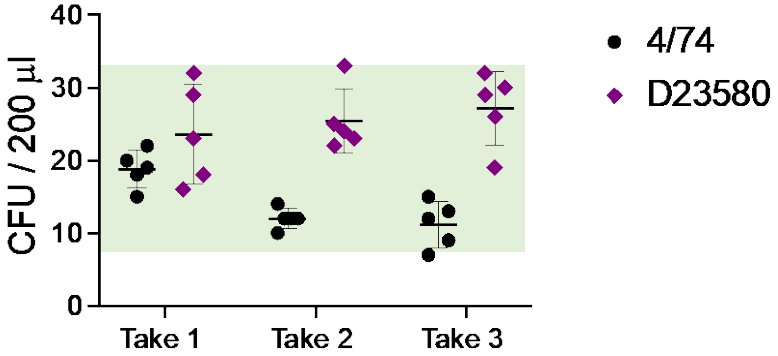
ASSESSING POST-THAW VIABILITY

PRE-FREEZE VIABILITY NUMBERS

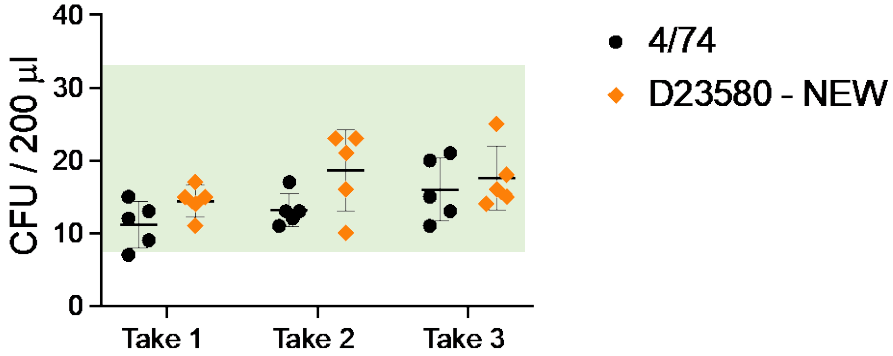
4/74: **1.24 x 10^9 CFU/ml**

D23580: **9.95 x 10^8 CFU/ml**

1. Dilutions based on official BPR stock concentrations of 4/74 and D23580 GMP vials



2. Adjusting D23580 stock concentration estimates:



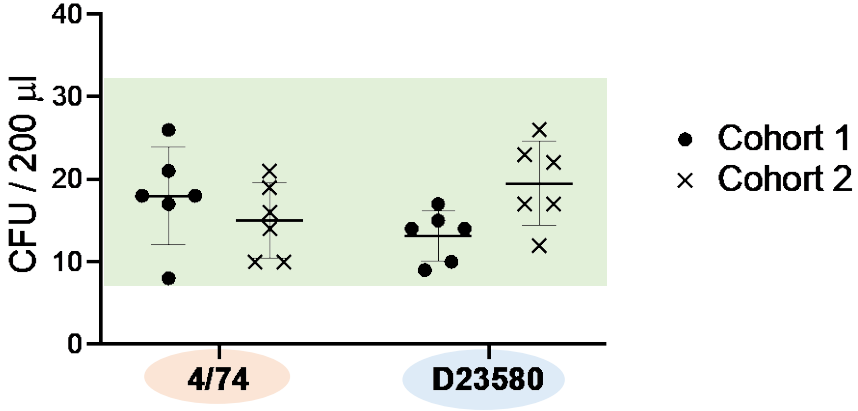
TAKEAWAY

- Some variability in concentrations of GMP master stocks (within strains)
- D23580 GMP master stocks have higher than expected number of CFUs (between 20-50% higher)

OUR AIM WAS / IS TO ADMINISTER DOSE IN THE RANGE **1 – 5 x 10^3 CFU / 30 ml**

(after dose escalation x 10^4 etc.)

THE CHANTS STUDY



	CFU / 30 ml	CFU in GMP vial
4/74	2.7 x 10^3	1.7 x 10^9
	2.3 x 10^3	1.7 x 10^9
D23580	2.0 x 10^3	1.9 x 10^9
	2.9 x 10^3	2.1 x 10^9

TAKEAWAY

- Very low level of variability of the GMP stock concentrations
- We are able to successfully administer doses within a very narrow range.

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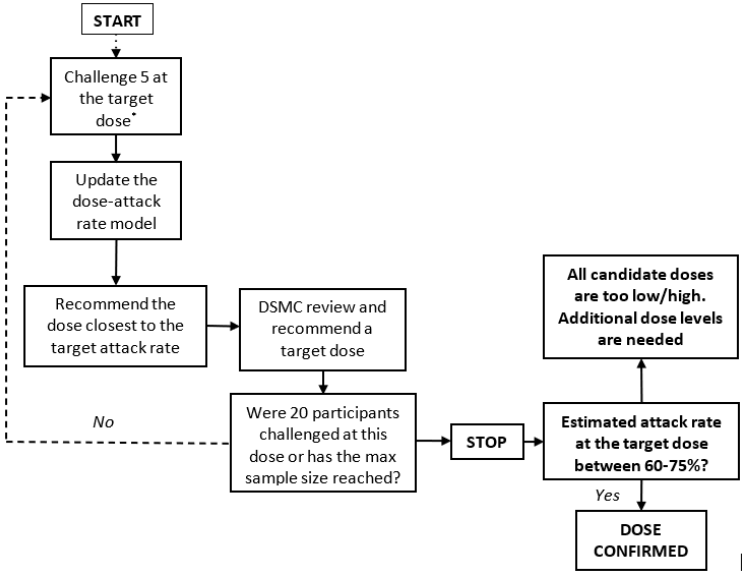
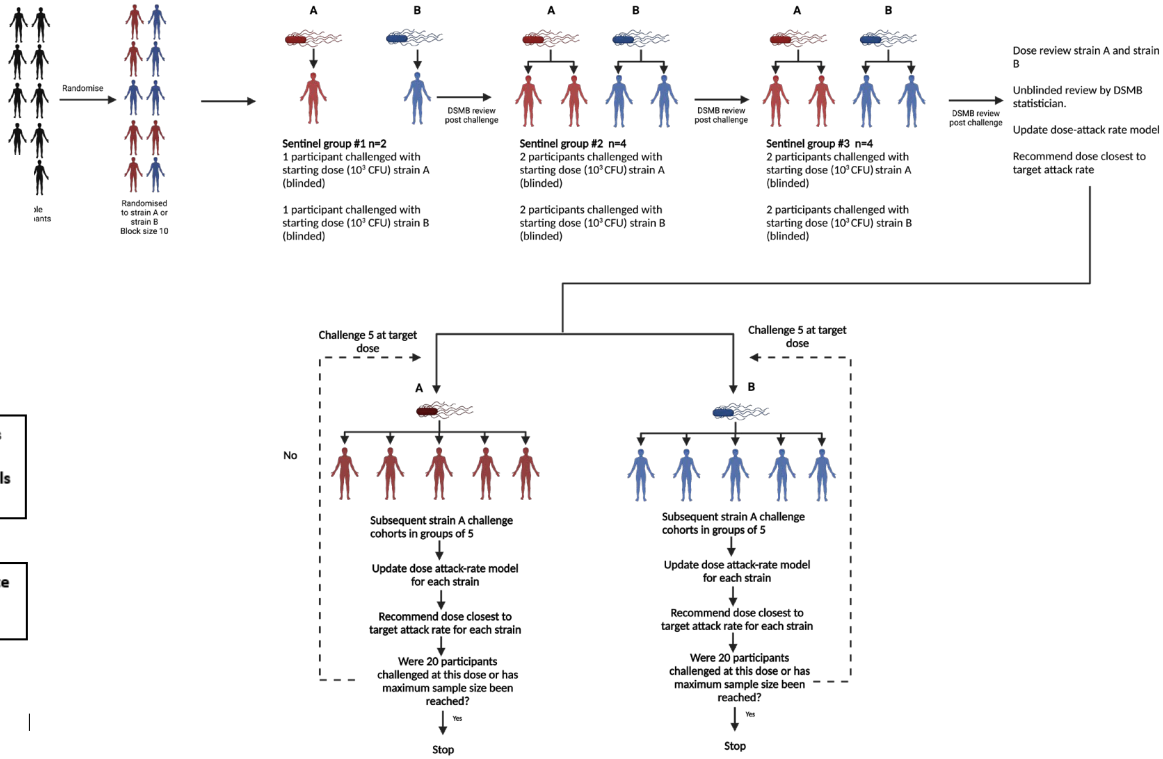
Dose-escalation





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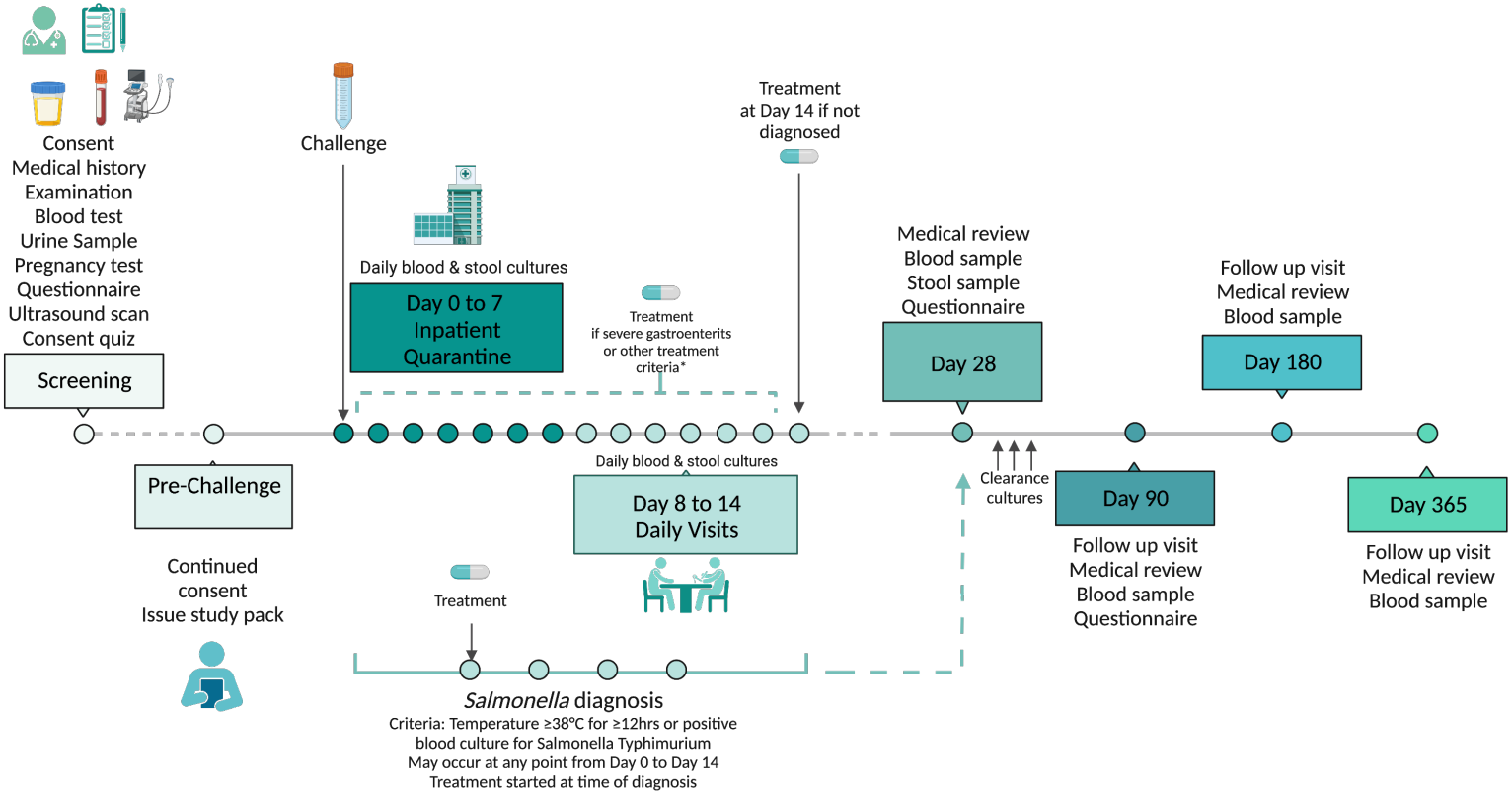

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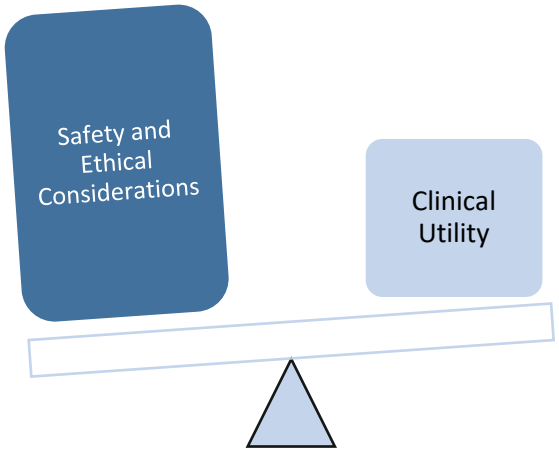
Safety



Possible risks and how we minimise them

1 Gastroenteritis

Very common risk:
Developing Gastroenteritis

How is it minimised?
Participants are:
- Treated with antibiotics to shorten duration.
- Monitored for dehydration and given fluids to rehydrate.

2 Invasive Infection

Uncommon risk:
Invasive *Salmonella* infection

How is it minimised?
- High risk people are excluded from the study.
- Daily blood samples to test for bacteria in the blood.
- Immediate treatment to prevent severe symptoms.

3 Antibiotic side effects

Common risk:
Antibiotic side effects

How is it minimised?
- People with known allergies are excluded.
- Treatment is as short as possible.
- We monitor for and treat any side effects.
- We can use alternative antibiotics if side effects occur.

4 Irritable bowel syndrome

Common risk:
Post infectious irritable bowel syndrome (IBS)

How is it minimised?
- Screening questionnaire for symptoms of IBS.
- If symptoms don't resolve, refer to a specialist.

5 Reactive arthritis

Uncommon risk:
Reactive arthritis (joint pain/ swelling)

How is it minimised?
- A screening blood test (HLA-B27).
- If symptoms occur, refer to a specialist.

6 Transmission & Shedding

Uncommon risk:
Transmission or shedding

How is it minimised?
- Quarantine until no longer infectious.
- Participants given information and soap/disposable towels for good handwashing.
- Testing at follow up visits.
- Close contacts can be tested for *Salmonella.*

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Clinical and Microbiological Responses

Clinical Response

- Gastroenteritis Rate
- Severe gastroenteritis
- Persistent fever
- Safety
- Clinical features – symptom diaries
- Haematological and biochemical parameters

Microbiological Response

- Colonisation
- Stool shedding
- Stool culture/PCR (Quantitative)
- Blood culture

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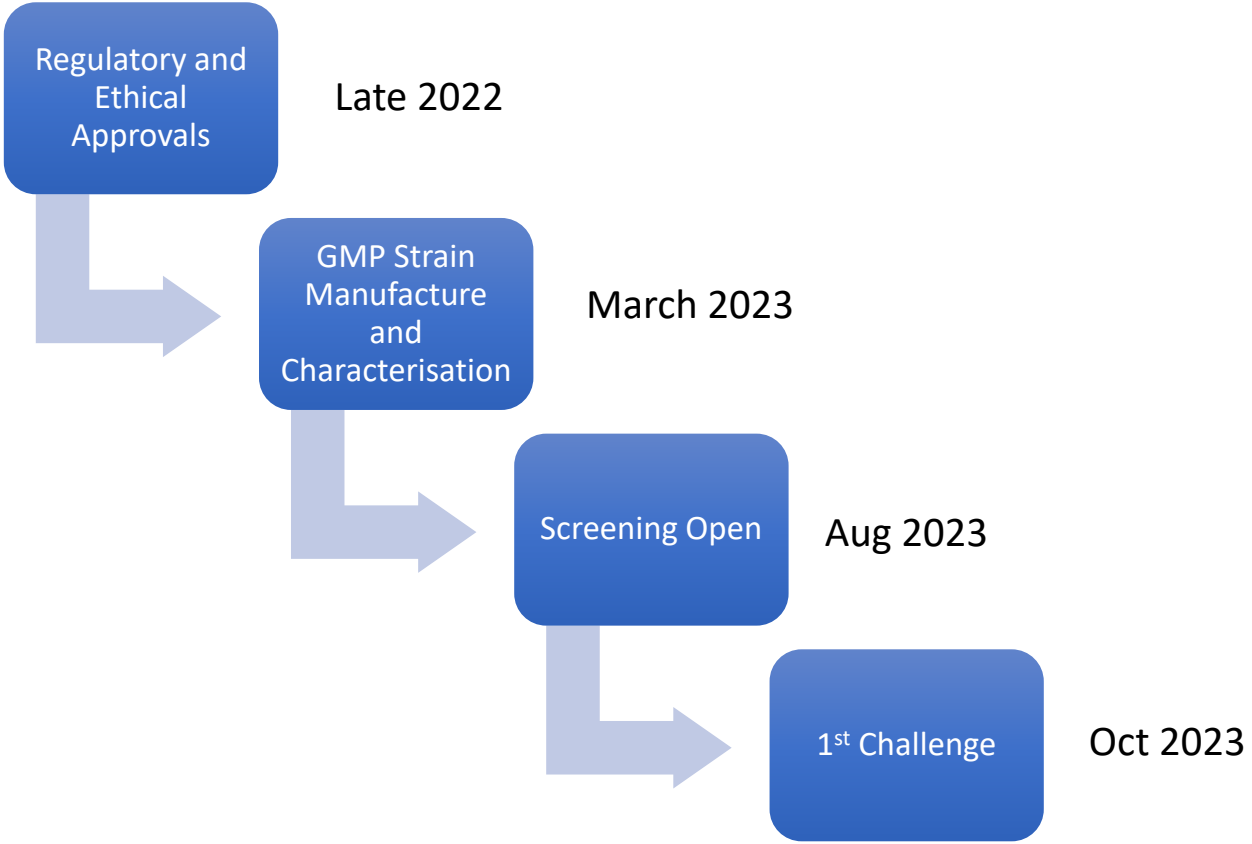

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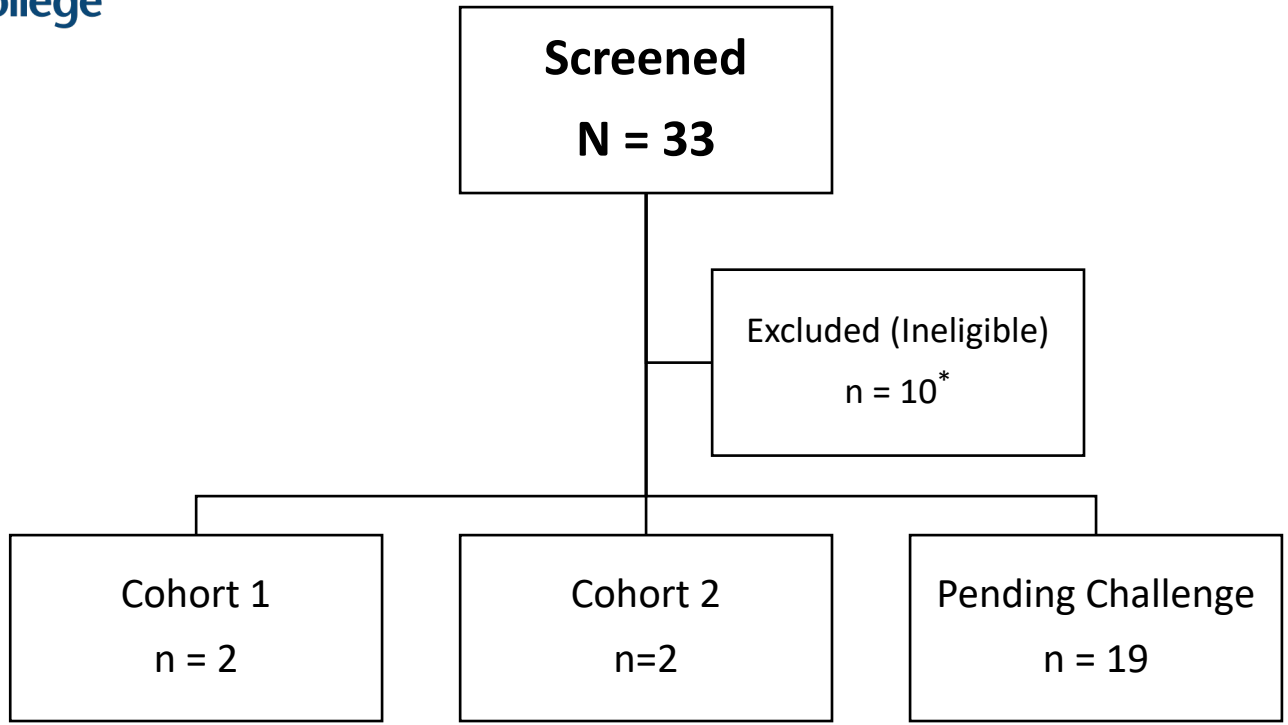
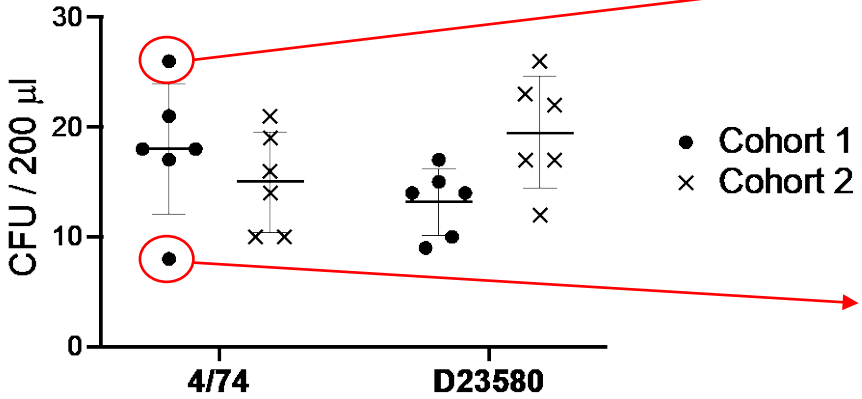


*Gallstones; Sickle cell trait; HLA-B*27 positive; Orthopaedic prostheses; Valvular murmur; SARS-CoV-2 unvaccinated

OUR AIM WAS / IS TO ADMINISTER DOSE IN THE RANGE **1 – 5 x 10^3 CFU / 30 ml**

(after dose escalation x 10^4 etc.)



26 CFU (if average) corresponds to **3.9 x 10^3 CFU / 30 ml**

8 CFU (if average) corresponds to **1.2 x 10^3 CFU / 30 ml**

… all within range

TAKEAWAY

- Very low level of variability of the GMP stock concentrations
- We are able to successfully administer doses within a very narrow range.

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Challenge Summary

- Colonisation Achieved
- 0/4 primary outcome (Systemic Salmonellosis)
- 0/4 gastroenteritis
- No safety signals/SAEs (clinically or by blood parameters)

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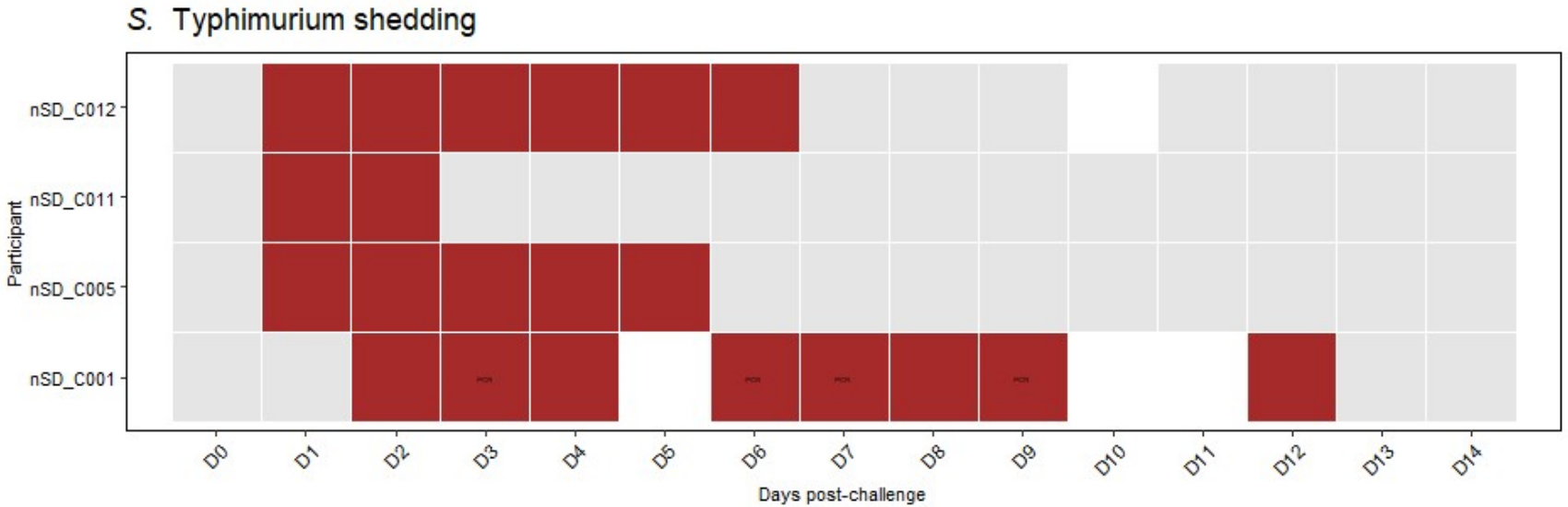

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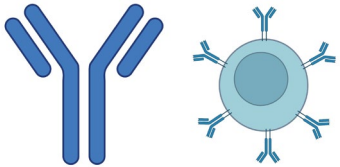
Next Steps – Immunological Responses

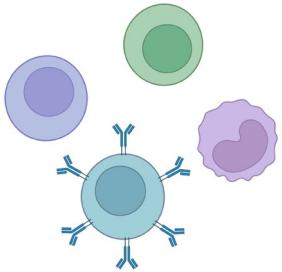
Mucosal Immunity

Saliva
Stool
Secretory IgA/IgG



Humoral Immunity

Serum LPS O-Ag IgG/IgA
Serum bactericidal activity
ALS Assay
ASC and memory B-cell



Cell-Mediated Immunity

Lymphocyte populations
Intracellular cytokine staining

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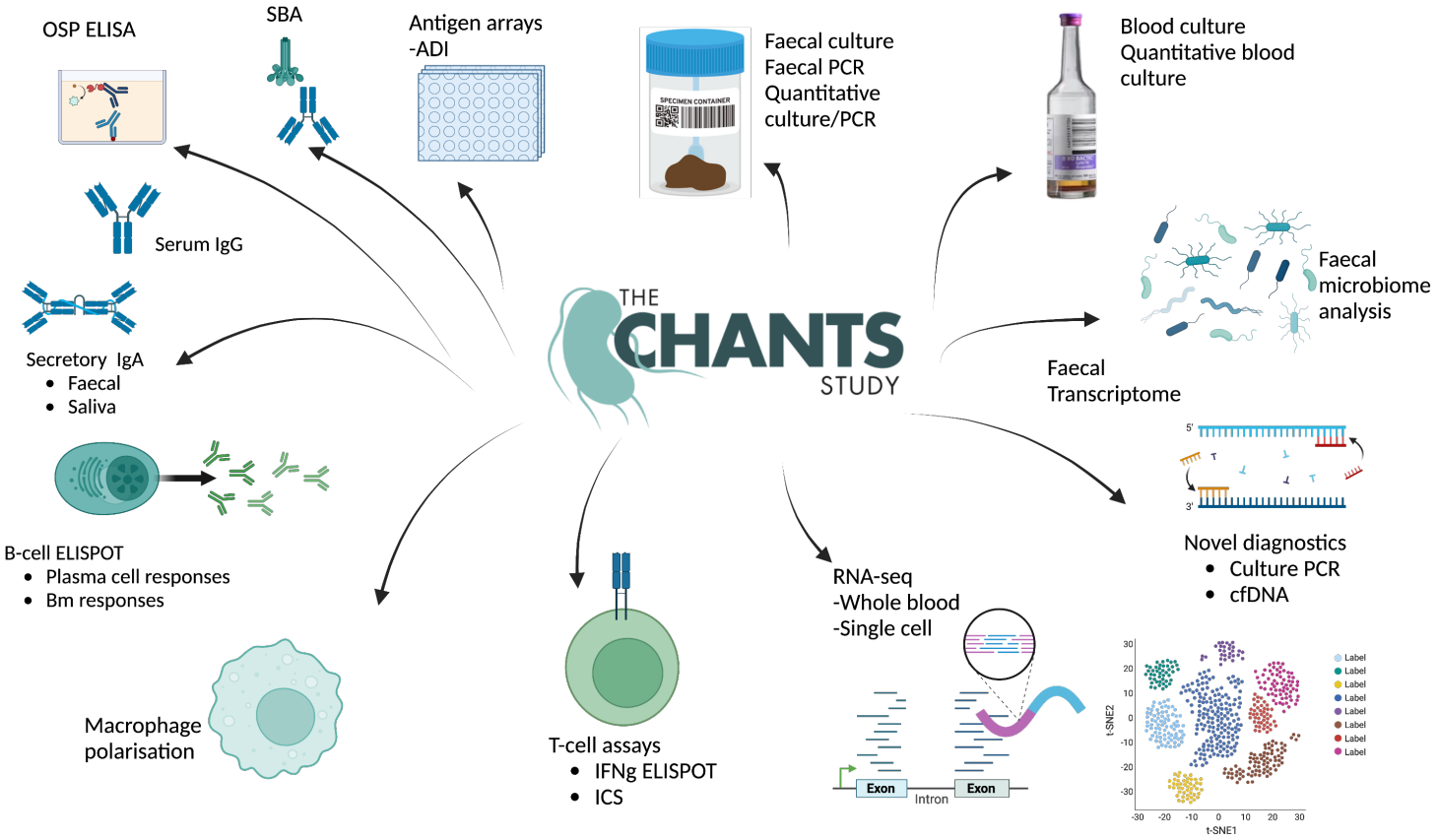

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Summary

- **Progress thus far**
  - First NTS CHIM
  - Evidence of reliable colonisation > dose-escalation pending for disease endpoints
  - No safety signals

- **Future studies**
  - Vaccine efficacy
  - Novel therapeutics
  - Heterologous and homologous re-challenge
  - Transfer model to endemic setting

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Acknowledgements

CHANTS Study Team

Malick Gibani

Emma Smith

Anna Rydlova

Robert Varro

Graham Cooke

Imperial College London

Amanda Bravery

Abinithya Udayakumaran

Smita Das

Christopher Chiu

Polly Fox

Lydia Taylor

Peter Hill

Jacob Lee

Sonia Vidal

NWLP Microbiology Staff

University of Liverpool

Jay Hinton

Melita Gordon

Simon Zhu

Ada Liu

Blanca Perez Sepulveda

Modupeh Betts

University of Oxford

Andrew Pollard

Xinxue Liu

PATH/WRAIR

Robert Choy

Eddie Suvarnapunya

wellcome

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Rossett-Rice model

- Rossett-Rice model is in vitro measure to mimic the passage through the stomach on the bench top.
- Allows for monitoring of the simulated stomach pH after antiacid dosing and samples to be testing for viability of the challenge organism at various timepoints after dosing.
- Image on the left shows the image from GSK's rota patent for the Baby Rossett-Rice model.
    - The volumes and secretion rates are adjusted for adults. We have substituted simulated gastric fluid for HCl.