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## A Protocol for a Cross Sectional Observation Study Exploring the Gut Microbiota of Infants with Complex Congenital Heart Disease Undergoing Cardiopulmonary Bypass (the GuMiBear study)

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**Title:** A Protocol for a Cross Sectional Observation Study Exploring the Gut Microbiota of Infants with Complex Congenital Heart Disease Undergoing Cardiopulmonary Bypass (the GuMiBear study)

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## Abstract

### Introduction

The gut microbiota develops from birth and matures significantly during the first 24 weeks of life, playing a major role in infant health and development. The composition of the gut microbiota is influenced by several factors including mode of delivery, gestational age, feed type and treatment with antibiotics. Alterations in the pattern of gut microbiota development and composition can be associated with illness and compromised health outcomes.

Infants diagnosed with 'Congenital Heart Disease' (CHD) often require surgery involving cardiopulmonary bypass (CPB) early in life. The impact of this type of surgery on the integrity of the gut microbiome is poorly understood. In addition, these infants are at significant risk of developing the potentially devastating intestinal condition Necrotising Enterocolitis (NEC).

### Methods and Analysis

This study will employ a prospective cross-sectional observational methodology to investigate the gut microbiota and urine metabolome of infants with CHD undergoing surgery involving CPB. Stool and urine samples, demographic and clinical data will be collected from eligible infants based at the national centre for paediatric cardiac surgery in

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2  
3 Ireland. Shotgun metagenome sequencing will be performed on stool samples and urine  
4 metabolomic analysis will identify metabolic biomarkers. The impact of the underlying  
5 diagnosis, surgery involving CPB, and the influence of environmental factors will be  
6 explored. Data from healthy age matched infants from the INFANTMET study will serve as a  
7 control for this study.  
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## 10 Ethics and Dissemination

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13 This research study is ethically approved (REC REF No: GEN/826/20). Study results will be  
14 available to patients with CHD and their families, carers, support networks, paediatric  
15 cardiology and microbiome societies and other researchers. Study findings will provide a  
16 deeper understanding of the gut microbiota of infants with CHD and inform perioperative  
17 management options including strategies to prioritise the integrity of the gut microbiota.  
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### 23 Abbreviations:

24  
25 APGAR Score Newborn assessment score rating: Appearance, Pulse, Activity, Grimace,  
26 Respiration  
27  
28

29 AVSD Atrioventricular Septal Defect  
30

31 CHD Congenital Heart Disease  
32

33 CHC Children's Heart Centre  
34

35 CHI Children's Health Ireland  
36

37 CPB Cardiopulmonary Bypass  
38

39 CRF Case Report Form  
40

41 EBD Epithelial Barrier Dysfunction  
42

43 GDPR General Data Protection Regulation  
44

45 HLHS Hypoplastic Left Heart Syndrome  
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47 HRHS Hypoplastic Right Heart Syndrome,  
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49 HSE Health Service Executive  
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51 ICF Informed Consent Form  
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3	IV	Intravenous
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6	NEC	Necrotising Enterocolitis
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8	PICU	Paediatric Intensive Care Unit
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11	PIM3	Paediatric Index of Mortality
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14	RBB	Repeated Beat Beating
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16	TGA	Transposition of the Great Arteries
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19	TP	Time Point
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**Keywords:** Congenital Heart disease, Microbiota, Infant, Antibiotic, Development, Cardiac Surgery, Cardiopulmonary Bypass

Word Count: 3,184

## Article Summary

### Strengths and Limitations of this Study

- GuMIBear is the first study to investigate the gut microbiome of infants with CHD undergoing surgery with CPB over a 24 week timeframe
- Comparison with age matched healthy controls allow deeper insight into clinically relevant microbiome alterations throughout the surgical course
- The study involves urine metabolomic analysis providing novel insight into the metabolite profile of study participants compared with healthy age matched infants
- The primary limitation of GuMIBear is that it is a single centre study limiting the generalisability of the findings

## Introduction

### What is currently known

The establishment of gut microbiota begins at birth and continues over the first years of life. Continued evolution of the gut microbiome after birth is governed by host factors such as both the adaptive and innate immune system, as well as external factors such as diet,

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3 medication and toxin exposure, and illness.[1] Understanding the role of the gut  
4 microbiome in metabolism, immune function and nutrition is gaining increasing recognition,  
5 as it is accepted that an altered colonisation has been associated with a higher risk of  
6 diseases later in life.[2] In the critical first weeks and months of life, perturbations to the  
7 infant gut microbiome have implications for growth development and health.[3]  
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#### 14 The Microbiome and Systemic Inflammation

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17 It is evident that under certain conditions, disruption of the normal microbiota that colonise  
18 the intestinal tract can occur.[4] These conditions include systemic inflammatory processes,  
19 which can result in intestinal inflammation, where proinflammatory bacteria can flourish,  
20 interacting with the intestinal epithelium to cause cytokine release, activating key  
21 inflammatory pathways increasing morbidity and prolonging critical illness.[5] The pattern  
22 of cytokine release in patients undergoing CPB is described as comparable to those released  
23 in systemic inflammation such as trauma and sepsis.[6] However, the nature of gut  
24 microbiota compositional changes in infants undergoing surgery with CPB remains  
25 understudied. This research aims to address this knowledge gap to enhance our  
26 understanding and inform care practices.  
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#### 38 CHD and Necrotising Enterocolitis (NEC)

39 CHD affects approximately 1 in every 100 babies born throughout the US every year,[7] and  
40 is the most common congenital defect worldwide.[8] A diagnosis of 'Complex Congenital  
41 Heart Disease' can include conditions such as Hypoplastic Left Heart Syndrome (HLHS);  
42 Hypoplastic Right Heart Syndrome (HRHS), Transposition of the Great Arteries (TGA)  
43 requiring intervention in the first week of life while other cardiac conditions such as  
44 Atrioventricular Septal Defect (AVSD), Tetralogy of Fallot may require corrective surgery in  
45 the first few months of life. Complex CHDs such as those requiring surgery involving  
46 cardiopulmonary bypass (CPB) present a greater risk to patients. This increased risk is not  
47 limited directly to the surgery, compromised ventricular function or low cardiac output  
48 state, but includes the risk of developing NEC.  
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3 There is a well established connection between CHD and NEC, a potentially devastating  
4 intestinal condition of infancy.[9] NEC carries a reported incidence of between 3 and 9% in  
5 infants with congenital heart disease (CHD) with all-cause mortality rates as high as 38% in  
6 children with 'cardiogenic' NEC.[10] While CHD remains one of the most common risk  
7 factors for NEC, the underlying pathophysiology of this association is complex.[11] Growing  
8 evidence suggests that perturbations in the early-life gut microbiota composition increase  
9 the risk for NEC.[3,12] Given the likelihood that haemodynamic and intrinsic gut factors  
10 predispose this group to NEC, acute and chronic circulatory changes have been investigated.  
11 An association between hypoperfusion and episodes of shock and diastolic flow reversal in  
12 the abdominal aorta causing impaired mesenteric blood flow has been identified.[13]  
13 Furthermore, a significant association between episodes of low cardiac output and shock in  
14 the development of NEC was identified.[14] It is reported that infants with certain types of  
15 CHD, mainly HLHS, may possess abnormal systemic vasculature contributing to the  
16 increased risk for NEC.[15]. Whether it is those, or other causes of impaired perfusion to the  
17 gut, the resulting damage to the mucosal barrier can provide an entry point to bacteria  
18 provoking an inflammatory cascade, and the devastating consequences that can ensue.[16]  
19 The vulnerability of infants with CHD is enhanced during the course of surgical intervention  
20 involving CPB, and the role of the gut microbiome has received little research focus in this  
21 context.  
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#### 40 Surgery involving CPB

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43 Infants diagnosed with CHD are at risk of alterations to their intestinal homeostasis, a  
44 further threat is presented in the context of surgery involving CPB. There is evidence to  
45 suggest intestinal ischaemia reperfusion injury occurs after CPB and contributes to epithelial  
46 barrier dysfunction (EBD) potentially exposing the bloodstream to bacteria or bacterial  
47 products.[17] Although alterations in gut barrier integrity and resident microbiota have  
48 been demonstrated,[3] it is not fully understood what changes to the microbiome occur  
49 following CPB, and the nature and severity of EBD. While the gut microbiota in infants with  
50 CHD following CPB remains understudied, a small single centre case control study recently  
51 identified significant gut microbiota perturbations in patients with CHD.[18] This case-  
52 control study highlighted that children with CHD had a disrupted gut microbiome at baseline  
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3 with an over-representation of pro-inflammatory bacteria, this was further exacerbated by  
4 CPB. Samples were collected pre-operatively and in a limited 24 and 48 hour time frame  
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6 postoperatively. The significance of intraoperative variables including aortic cross clamp  
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8 time and duration of CPB was not determined.  
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12 Our study proposes to address the knowledge gap and advance existing research by  
13 examining the gut microbiome of infants with CHD pre-operatively, and at defined time  
14 points up to 24 weeks postoperatively. This timeline will account for the recovery phase  
15 post cardiac surgery, including time to re-establish full feeds, wean from mechanical  
16 ventilation and circulatory support, and allow for surveillance of NEC postoperatively.  
17  
18 Comparisons will be made with healthy age-matched infants recruited as part of the  
19 INFANTMET study.[19] As well as collecting intraoperative variables such as duration of CPB,  
20 and aortic cross clamp time, a novel aspect of this research will be to profile the metabolites  
21 in urine to assess potential metabolic biomarkers and pathway changes. Our research will  
22 recruit patients in a National Centre for Paediatric Cardiac Surgery, where 40 open cardiac  
23 surgeries are performed on infants annually. We therefore anticipate active recruitment will  
24 ensure the proposed target sample of 50 participants is achievable. No additional invasive  
25 procedures will be required for sample collection, enhancing the acceptability of the  
26 research for consenting parents or carers. This project will investigate the subdivisions of  
27 the gut microbiota of infants with CHD, and environmental factors such as the influence of  
28 mode of delivery, pre-operative fasting states and mode of feeding, and use of pre-  
29 operative antibiotics. Understanding the status of the intestinal microbiome of infants with  
30 CHD and the effects of undergoing surgery with CPB is vital in informing best care practices  
31 to enhance patient outcomes.  
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## 50 **Methods**

### 51 **Study design**

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55 This study is a cross-sectional observational study of infants with Complex CHD undergoing  
56 CPB at the National Centre for Paediatric Cardiac Surgery at Children's Health Ireland at  
57 Crumlin, Dublin, Ireland. This single-site study will investigate the differences in the  
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microbiome, metabolomics readouts, and stress levels between infants with CHD undergoing CPB and healthy age matched controls.

### Participant selection

This study will involve the collection of demographic and clinical maternal and infant data from those meeting eligibility criteria with complex CHD scheduled for surgery involving CPB (see Table 1 for participant inclusion and exclusion criteria). Infant stool and urine samples will be collected at defined time points set out in the protocol (See Appendix 1 for Case Report Form).

### Public and Patient Involvement Statement

The mother of a child who had cardiac surgery as a baby was involved in reviewing the study literature.

### Inclusion and exclusion criteria

To be eligible for the study, the participants must meet the terms of the inclusion and exclusion criteria as presented in Table 1.

Table 1. Inclusion and Exclusion Criteria.

Inclusion criteria	Exclusion criteria
Infants born full term ( $\geq 37$ weeks) weeks gestation)	Stillbirth or live birth where the baby is born alive but dies shortly after
Infants diagnosed with *CHD and scheduled for surgery involving CPB	Infants who are born healthy with no underlying illness, syndrome, or chronic disease
Infants born in Ireland to allow sample follow up	Participation in another study
Ability of the participant's parent/carers (in the investigator's opinion) to comprehend the full nature and purpose of the study	Infants not undergoing surgery involving cardiopulmonary bypass

Consent to participate in the study and willingness to comply with the protocol and study restrictions by the participant's parent/carers	Infants where parents/carers do not give consent to participate in the study
Upper age limit of less than 6 months	Gastrointestinal pathology or intestinal surgery, excluding gastrostomy tube

\*CHD includes Hypoplastic left heart syndrome (HLHS); Hypoplastic Right Heart Syndrome (HRHS), Transposition of the Great Arteries (TGA), Atrioventricular Septal Defect (AVSD), Tetralogy of Fallot.

### Recruitment

Participants meeting inclusion criteria will be selected after admission to the hospital, outpatient clinic or cardiac day unit. Study-related information will be given in written form as well as explained by a member of the project team. No study-related activities will begin before the potentially eligible participants' parents/carers have signed the Informed Consent Form (ICF). Participants parents/carers will be asked to refer to the Privacy Notice on the hospital website or they can receive a hardcopy if they wish. Signed ICFs will be stored safely in a locked cabinet in the research office.

### Compensation

No compensation will be provided to the participants. There are no cost implications for the Health Service Executive (HSE) or to the participants. The management of patients and investigative tests will comply with current standards of care.

### Study timeline

After completing recruitment procedures, i.e., determining whether the patient meets the study inclusion criteria, discussing the study with the parents/caregivers and obtaining informed consent, clinical and demographic data will start to be collected.

The study will be undertaken for a period of 24 weeks after the infant is initially recruited.

### Demographic Data

The infants' diagnosis, co-morbidities, date of birth, gestational age, sex, mode of delivery, APGAR scores, birth weight, head circumference and antibiotics administered post-delivery and antenatal events will be recorded.

### Maternal Data

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3 Maternal history including age, antibiotics received, smoking status and significant antenatal  
4 events will be recorded.  
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### 7 **Surgical course**

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10 The type of surgery performed, duration of cardiopulmonary bypass and cross-clamp time  
11 will be recorded. Antibiotic use and any intraoperative events will be recorded as well as  
12 clinical information including arterial blood gas data.  
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### 15 **Post-operative Data**

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18 Paediatric Index of Mortality (PIM) score, duration of mechanical ventilation, renal and  
19 cardiovascular support and duration of stay in paediatric intensive care unit will be  
20 recorded.  
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### 23 **Feeding Information Data**

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26 Feeding information including the type of feed and duration of the feed prior to surgery will  
27 be recorded. The date the patient is established on full feeds will be recorded. Full feeding is  
28 defined as when the patient no longer requires parenteral nutrition or intravenous fluids.  
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### 31 **Discharge Information Data**

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34 Discharge information data: This will include the patient's status on discharge from PICU, as  
35 well as length of PICU and hospital stay.  
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### 38 **Complications**

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41 The occurrence of complications will be recorded, for example the development of NEC. The  
42 timeline for recording NEC onset will be based on the initiation of triple antibiotic therapy,  
43 based on a full surgical review including clinical presentation, radiological and laboratory  
44 data.  
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### 47 **Subject withdrawal/exclusion**

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50 Under the Declaration of Helsinki, the research nurse will explain to the consenting adult  
51 that they have the right to withdraw from the study at any time and that this will in no way  
52 prejudice their future treatment. The reason for withdrawal will be recorded in the source  
53 documents and on the appropriate CRF. Consenting adults will be made aware that stored  
54 samples from individuals withdrawing from the study may have undergone processing and  
55 may be analysed in the study.  
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### 59 **Regulatory procedures**

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3 The study is conducted following the version Fortaleza, Brazil, October 2013 of the  
4 Declaration of Helsinki 1964. The Protocol and the ICF have been approved by the Clinical  
5 Research Ethics Committee of Children's Health Ireland GEN/826/20. As biological samples  
6 will be procured in one institution and sent to another, a data sharing agreement is in place  
7 between The Cardiology Department, Children's Health Ireland at Crumlin Hospital, and APC  
8 Microbiome in Cork. This research is fully compliant with the guidelines as set out in The  
9 General Data Protection Regulation (GDPR), the Irish Data Protection Acts 1988 to 2018  
10 including Protection Act 2018 (Section 36(2)) (Health Research) Regulations 2018.  
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#### 15 Data Statement

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18 Once collected, the anonymised demographic, clinical and laboratory analysis data as well  
19 as statistical codes will be uploaded to the open access Research Repository University  
20 College Dublin.  
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#### 23 Objectives and Outcomes

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26 The primary study objectives and outcomes are-

- 27  
28 • To characterise the gut microbiota composition of infants with specified CHD  
29 undergoing surgery with CPB at specific time points perioperatively.
- 30  
31 • To determine any differences in gut microbiota composition of infants who take part  
32 in this study compared with the microbiota of healthy babies from the INFANTMET  
33 study at matching time points.[19]
- 34  
35 • To characterise the urine metabolite profile of infants with specified CHD undergoing  
36 surgery with CPB and compare with healthy infants from the INFANTMET study.[19]  
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40 Secondary objectives and outcomes

- 41  
42 • To explore the influence of maternal and environmental factors on gut microbiome  
43 composition.  
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#### 45 Sample collection and analysis

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48 Faecal samples

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50 Stool samples will be collected at the following time points: within 24 hours after birth (TP  
51 1), within 24 hrs pre surgery (TP 2) 1 week post-surgery (TP 3) 4 weeks post-surgery (TP 4),  
52 24 weeks post-surgery (TP 5). Information about antibiotic therapy administered before or  
53 during the stool collection will be recorded.  
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57 The sample will be collected by the bedside nurse or the parent/carers and transferred to  
58 the laboratory upon receipt of the sample during the weekdays or weekends. At night, the  
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3 sample will be kept in the dedicated fridge prior to transfer to the laboratory the following  
4 day by the bedside nurse. Samples will be stored at -80°C until further analysis.  
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## 7 Urine Samples 8

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10 Urine samples will be collected at 4-8 weeks post-surgery for metabolomic analysis using  
11 Sterisets Uricol Urine Collection Pack (MedGuard, Ireland). The urine sample will be  
12 collected from the urinary catheter if the participant is catheterised. Alternatively, a pad will  
13 be placed in the diaper and used to collect an unsoiled urine sample from the infant. The  
14 pad will then be placed in a biohazard bag and frozen immediately at -80 °C prior to  
15 processing. After all the sample collections are complete, they will be shipped to Teagasc  
16 Food Research, Moorepark, Ireland, using DHL overnight service for microbiome and  
17 metabolomics analyses. Styrofoam Saf-T-Pak STP-309 shipper box or equivalent will be used.  
18 DNA extraction will be performed on stool samples using the modification of the Repeated  
19 Bead Beating Plus Column (RBB+C) method.[20] LC-MS will be utilised for metabolomics  
20 analysis of urine.[19] Sample collection for discharged participant  
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26 Parents/carers will receive a sample collection discharge pack and a parent diary/instruction  
27 served as reminder to collect due samples at different time-point prior to discharge home.  
28 They will receive a follow up text message or phone call to remind them on the due sample.  
29 The sample collection discharge pack consists of urine/stool collection containers with study  
30 code, sterile pad, syringe, zip-lock bag, gloves, biohazard bag and cooler bag. Parents/carers  
31 are asked to keep the collected sample at the dedicated section of their home freezer. They  
32 will transport the collected sample in the cooler bag provided when attending out-patient  
33 department for appointments. They will ask a member of the project team to transfer the  
34 collected sample to the laboratory upon arrival at the hospital. The study researcher is  
35 available at the dedicated contact number for any queries.  
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## 41 **Adverse events and participant well-being** 42

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44 There are no expected safety concerns related to the study. All study participants will be  
45 under the care of the cardiology team at the hospital with access to psychological support,  
46 as well as nursing and medical professionals, social workers and chaplaincy.  
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## 50 **Data collection and management** 51

52 The study diaries, study dataset, and paper/digital CRF systems will be used for recording  
53 data from each study participant. All the data collected in this study is pseudonymised, as  
54 each of the participants will be assigned a specific study code and upon receipt, data will be  
55 referred to the study code. All study staff responsible for entering data into the CRF system  
56 received training in advance of the study commencement. This training included familiarity  
57 with the study diaries, study dataset, and paper/digital CRF system and have completed  
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3 good clinical practice in research training. They have individual access to the password  
4 protected study shared drive within the hospital. The study team will monitor the  
5 data/sample collection process. Any inconsistencies identified during the study will be  
6 presented as queries at the regular project team meeting.  
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### 9 10 **Comparison Data**

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12 Data collected as part of the INFANTMET Study will serve as a healthy control comparison  
13 for this study. INFANTMET compared the gut microbiota development of breastfed infants  
14 born via C-section or vaginally at full-term or preterm at Cork University Maternity  
15 Hospital. Ethical approval for sample collection by Cork University Hospital Research Ethics  
16 Committee (reference number ECM(w) 07/02/2012). One hundred and ninety two infants  
17 were recruited to the INFANTMET study and stratified according to delivery mode and  
18 gestational age at birth. Faecal samples were collected from the infants at 1, 4, 8 and 24  
19 weeks of age and stored under controlled conditions. Urine samples were collected at 4  
20 weeks of age for metabolomic analysis and stored in a freezer at -80°C prior to processing.  
21 Samples were analysed in accordance with the analysis proposed for the GuMIBear study.  
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### 28 **Bioinformatics and Statistical analysis**

#### 29 30 Sample size justification

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33 Published research in this area is lacking. However one case control study by Salomon et al  
34 (2021) included 17 cases and 12 control participants and was sufficiently powered to  
35 determine a statistically significant difference in beta diversity in cases versus controls  
36 ( $F=5.6$ ,  $p<0.001$ ). Our study proposes to include 50 patients with CHD undergoing surgery  
37 with CPB and age matched controls, almost three times the Solomon et al (2021) study. We  
38 therefore anticipate that the proposed sample size is justified.  
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#### 43 Demographic and Clinical Data

44  
45 Demographic and clinical data and laboratory information will be tested for normality using  
46 the Shapiro-Wilk test. Descriptive statistics will be used to describe normally distributed  
47 data, and expressed as mean  $\pm$  SD. Continuous data not normally distributed will be  
48 reported as median and interquartile ranges. Categorical variables will be expressed as  
49 counts and percentages. Groups will be compared using chi square tests for categorical  
50 variables and independent-samples student's  $t$ -tests for normally distributed continuous  
51 variables. For variables not normally distributed, the Mann-Whitney  $U$  test will be used.  
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#### 56 Microbiome analysis

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3 Metagenomic shotgun sequencing data will be analysed using bioBakery suite of tools  
4 ([https://huttenhower.sph.harvard.edu/biobakery\\_workflows/](https://huttenhower.sph.harvard.edu/biobakery_workflows/)). Trimmed and human reads  
5 filtered using KneadData (v0.7.2) with the default parameters. Quality controlled data will  
6 be taxonomically profiled at the species level with relative abundance by MetaPhlan2.  
7 Functional profiling will be performed using HUMAnN and strain profiling using StrainPhlan.  
8  
9

10  
11 For alpha diversity analysis, samples will be rarefied to even depth and phyloseq::estimate  
12 richness will be used to calculate Chao1, Shannon and Simpson indices. Alpha diversity  
13 indices between groups will be univariately compared using the Wilcoxon rank sum test. A  
14 beta-diversity ordination will be generated using the Aitchison distance and visualised using  
15 Principal Component Analysis (PCA) plot. The Adonis function in the vegan package will be  
16 used to implement a permutational multivariate analysis of variance to test whether  
17 samples cluster beyond that expected by sampling variability. Differentially abundant  
18 features will be identified using MaAsLin2.  
19  
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## 23 **Discussion**

24  
25 Despite an increasing awareness of the early-life critical window of microbiome  
26 development on the health and wellness of infants, there remains much to learn about the  
27 interactions of the microbiome with the infant host with CHD undergoing surgery involving  
28 CPB. This study is designed to address this knowledge gap, and incorporates a sound  
29 methodology with particular strengths enhancing the value of its findings. The specimen  
30 collection strategy occurring at multiple time points over a 24 week period in the proposed  
31 study will deepen our understanding of the temporal dynamics of the colonising microbiota,  
32 and their interactions with host physiology. The study design will account for maternal and  
33 perioperative variables to determine changes to the microbiome. Access to existing  
34 microbiome data from healthy age matched infants provides a valuable opportunity to  
35 present high quality comparative information. While multi-centre trials capturing sufficient  
36 case numbers of NEC cases will offer robust conclusions, this study will offer valuable  
37 evidence to support the influence of CHD and CPB on the microbiome and intestinal EBD.  
38 Future research can build on existing studies, and explore treatment strategies including  
39 recommendations for efficacious probiotic strain administration, including the supplements  
40 to promote a diverse gut microbiota to improve outcomes for this vulnerable population.  
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## 52 **Status of Study**

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54 The trial is ongoing and as of July 20th 1<sup>st</sup> 2022, 70 % of the participants have been  
55 recruited. Laboratory analysis has been carried out on 20% of study samples.  
56  
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1  
2  
3 This research received no specific grant from any funding agency in the public, commercial  
4 or not-for-profit sectors  
5  
6

### 7 **Competing interests**

8  
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10 The authors declare no association with commercial entities, either financial or non-  
11 financial.  
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### 14 **Author contributions**

15  
16 CM, CJMcM, CS, KM devised the project, and the main conceptual ideas. CJMcM, CM, CS,  
17 RPR, FK, DJ, MHT were involved in the study design and writing of the manuscript. MOT, JB,  
18 NM, SD, CJMcM are involved in consenting participants, collecting samples and acquiring  
19 data. DJ, CS, FK and RPR are responsible for analysing study samples. All authors read  
20 manuscript revisions, approved the final manuscript and accept accountability for the  
21 accuracy and integrity of the work.  
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### 36 **Appendix 1: Case Report Form PDF Version**

### 37 **References**

- 38  
39 1. Skillington O MS, Gupta A, Mayer EA, Gill CIR, Del Rio D, O'Riordan KJ, Cryan JF, Ross RP and  
40 Stanton C The contrasting human gut microbiota in early and late life and implications for host  
41 health and disease. *Nutrition and Healthy Ageing*. 2012;6:157-78.  
42 2. Cilieborg MS, Boye M, Sangild PT. Bacterial colonization and gut development in preterm  
43 neonates. *Early human development*. 2012;88 Suppl 1:S41-9.  
44 3. Healy DB, Ryan CA, Ross RP, Stanton C, Dempsey EM. Clinical implications of preterm infant  
45 gut microbiome development. *Nature microbiology*. 2022;7(1):22-33.  
46 4. Ohland CL, Jobin C. Microbial activities and intestinal homeostasis: A delicate balance  
47 between health and disease. *Cellular and molecular gastroenterology and hepatology*. 2015;1(1):28-  
48 40.  
49 5. Cabrera-Perez J, Badovinac VP, Griffith TS. Enteric immunity, the gut microbiome, and sepsis:  
50 Rethinking the germ theory of disease. *Experimental biology and medicine*. 2017;242(2):127-39.  
51 6. Halter J, Steinberg J, Fink G, Lutz C, Picone A, Maybury R, et al. Evidence of systemic cytokine  
52 release in patients undergoing cardiopulmonary bypass. *The journal of extra-corporeal technology*.  
53 2005;37(3):272-7.  
54 7. Centers for Disease Control and Prevention (CDC). Data and Statistics on Congenital Heart  
55 Defects. 2022 [updated January 24th 2022;26th July 2022]. Available from:  
56 <https://www.cdc.gov/ncbddd/heartdefects/data.html> Accessed 26<sup>th</sup> July 2022.  
57 8. van der Linde D, Konings E, Slager M, Witsenburg M, Helbing W, Takkenburg J, Roos-



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2  
3 Hesselink J. Birth Prevalence of Congenital Heart Disease Worldwide: A Systematic Review and  
4 Meta-Analysis. *Journal of the American College of Cardiology*. 2011;58(21):2241-2247.
- 5 9. Martin R, Makino H, Cetinyurek Yavuz A, Ben-Amor K, Roelofs M, Ishikawa E, et al. Early-Life  
6 Events, Including Mode of Delivery and Type of Feeding, Siblings and Gender, Shape the Developing  
7 Gut Microbiota. *PloS one*. 2016;11(6):e0158498.
- 8 10. Siano E, Lauriti G, Ceccanti S, Zani A. Cardiogenic Necrotizing Enterocolitis: A Clinically  
9 Distinct Entity from Classical Necrotizing Enterocolitis. *European journal of pediatric surgery : official  
10 journal of Austrian Association of Pediatric Surgery*. 2019;29(1):14-22.
- 11 11. Kelleher ST, McMahon CJ, James A. Necrotizing Enterocolitis in Children with Congenital  
12 Heart Disease: A Literature Review. *Pediatric cardiology*. 2021;42(8):1688-99.
- 13 12. Murphy K, O'Donovan AN, Caplice NM, Ross RP, Stanton C. Exploring the Gut Microbiota and  
14 Cardiovascular Disease. *Metabolites*. 2021;11(8).
- 15 13. Carlo WF, Kimball TR, Michelfelder EC, Border WL. Persistent diastolic flow reversal in  
16 abdominal aortic Doppler-flow profiles is associated with an increased risk of necrotizing  
17 enterocolitis in term infants with congenital heart disease. *Pediatrics*. 2007;119(2):330-5.
- 18 14. McElhinney DB, Hedrick HL, Bush DM, Pereira GR, Stafford PW, Gaynor JW, et al. Necrotizing  
19 enterocolitis in neonates with congenital heart disease: risk factors and outcomes. *Pediatrics*.  
20 2000;106(5):1080-7.
- 21 15. Miller TA, Minich LL, Lambert LM, Joss-Moore L, Puchalski MD. Abnormal abdominal aorta  
22 hemodynamics are associated with necrotizing enterocolitis in infants with hypoplastic left heart  
23 syndrome. *Pediatric cardiology*. 2014;35(4):616-21.
- 24 16. Nino DF, Sodhi CP, Hackam DJ. Necrotizing enterocolitis: new insights into pathogenesis and  
25 mechanisms. *Nature reviews Gastroenterology & hepatology*. 2016;13(10):590-600.
- 26 17. Typpo KV, Larmonier CB, Deschenes J, Redford D, Kiela PR, Ghishan FK. Clinical  
27 characteristics associated with postoperative intestinal epithelial barrier dysfunction in children with  
28 congenital heart disease. *Pediatric critical care medicine : a journal of the Society of Critical Care  
29 Medicine and the World Federation of Pediatric Intensive and Critical Care Societies*. 2015;16(1):37-  
30 44.
- 31 18. Salomon J, Ericsson A, Price A, Manithody C, Murry DJ, Chhonker YS, et al. Dysbiosis and  
32 Intestinal Barrier Dysfunction in Pediatric Congenital Heart Disease Is Exacerbated Following  
33 Cardiopulmonary Bypass. *JACC Basic to translational science*. 2021;6(4):311-27.
- 34 19. Hill CJ, Lynch DB, Murphy K, Ulaszewska M, Jeffery IB, O'Shea CA, et al. Evolution of gut  
35 microbiota composition from birth to 24 weeks in the INFANTMET Cohort. *Microbiome*. 2017;5(1):4.
- 36 20. Yu Z, Morrisson M. Improved extraction of PCR-quality community DNA from digesta and  
37 fecal samples. *Biotechniques*. 2004;36(5):808-12.
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^999 – missing data; 0 – no data in medical notes

## CASE RECORD FORM

### Stool Sample Collections

Period/ Time Frame	Projected Date	Sample Date	Sample Collected	Comments
1. Within 24h of Birth			<input type="checkbox"/> YES <input type="checkbox"/> NO	
2. Pre-operatively			<input type="checkbox"/> YES <input type="checkbox"/> NO	
3. Week 1 of life/Post-Op			<input type="checkbox"/> YES <input type="checkbox"/> NO	
4a. Week 4 to 8 life/Post-op			<input type="checkbox"/> YES <input type="checkbox"/> NO	
4b. <b>Urine</b> Sample Week 4 to 8			<input type="checkbox"/> YES <input type="checkbox"/> NO	
5. Week 24 of life/Post-Op			<input type="checkbox"/> YES <input type="checkbox"/> NO	

### PATIENT DEMOGRAPHIC

Date of Enrolment:		Date of Birth:	
Gender:	<input type="checkbox"/> Male <input type="checkbox"/> Female <input type="checkbox"/> Other/Ambiguous	Gestational Age:	Multiple <input type="checkbox"/> Yes <input type="checkbox"/> No
		APGAR at ① ⑤ min	Birth Order:
		<input type="text"/> <input type="text"/>	
Reason for admission:			
Comorbidities:			
Mode of Delivery:		Weight at Birth: _____ KGs	
SVD: <input type="checkbox"/> Yes <input type="checkbox"/> No		Head Circumference: _____ cms	
LSCS: <input type="checkbox"/> Yes <input type="checkbox"/> No, if Yes: <input type="checkbox"/> Elective/ <input type="checkbox"/> Emergency			
Antibiotics to Infant post-delivery; <input type="checkbox"/> Yes <input type="checkbox"/> No			
If Yes, list:			
Significant Antenatal Events:			

### MATERNAL INFORMATION

Maternal Age (years) at Birth:	Gestational Age at Booking Appt:
Antibiotics given Pre-Delivery: <input type="checkbox"/> Yes <input type="checkbox"/> No	List:
Maternal Smoking during Pregnancy: <input type="checkbox"/> Yes <input type="checkbox"/> No	Other Household Members Smoking during Pregnancy: <input type="checkbox"/> Yes <input type="checkbox"/> No



^999 – missing data; 0 – no data in medical notes

**SURGERY INFORMATION**

Surgery Date:			
Surgery Performed:			
Pre-Op Antibiotics <sup>1</sup> ? List:		Post-Op Antibiotics <sup>1</sup> ? List:	
ABG	pH	PO <sup>2</sup>	
First Pre-Op ABG:			Cardiopulmonary Bypass Duration:
First Post-Op ABG:			Aortic Cross Clamp Duration:
Significant Intraoperative Events:			

**POST-OPERATIVE INFORMATION**

PIM3 Score:		No. of days in ICU (1 <sup>st</sup> adm):	
Mechanical Vent:	<input type="checkbox"/> Yes <input type="checkbox"/> No	No of Days on ECLS:	
No of Days Vent:		No of Days on RRT:	
Agent	Day 1	Day 2	Day 3
Milrinone			
Epinephrine			
Norepinephrine			
Vasopressin			
Midazolam			
Morphine			
Clonidine			
Others			

**FEEDING INFORMATION**

Mode of Feeding (note <b>date initiated</b> and <b>date d/c</b> ):	
Breastmilk:	Infant Formula: Other:
Prebiotics given to Infant: <input type="checkbox"/> Yes <input type="checkbox"/> No	Type and Date Given:
Excessive Infantile Crying (cried for $\geq 3$ Hrs for 3 Days in one week): <input type="checkbox"/> Yes <input type="checkbox"/> No	
Date Trophic Feeds Commenced:	Type of Feed Used:
Time to Establishment of full feed <sup>2</sup> :	
Development of NEC <sup>3</sup> :	
Days post-op when developed NEC?	
Gut stasis:	N/A
Management Strategy:	

**DISCHARGE INFORMATION**

Date of Discharge		
Ward:	Home:	RIP:



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^999 – missing data; 0 – no data in medical notes

### READMISSION TO ICU

Total ICU Readmission days:				
	Date of Admission	Date of Discharge	Total ICU stays	Reason for admission
1.				
2.				
3.				
4.				
5.				

### DATA ENTRY BY (NAME)

Admission	Date	Paper	Date	G-Drive
First				
Second				
Third				

<sup>1</sup>Antibiotic treatment at time of stool sampling as below. Important not to include antibiotics which were started post the stool sampling

- 1) Abs < 48hrs pre sampling
- 2) Abx < 72 hrs pre sampling
- 3) Abx in previous 7 days

<sup>2</sup>Full feed – No longer requires parenteral nutrition or intravenous fluids supplement regardless the TFI.

<sup>3</sup>NEC –Initiation of triple IV antibiotic therapy and nil by mouth for at least 5 days, based on a full surgical review including clinical presentation, radiological and laboratory data.

# BMJ Open

## A Protocol for a Prospective Cohort Study Exploring the Gut Microbiota of Infants with Congenital Heart Disease Undergoing Cardiopulmonary Bypass (the GuMiBear study)

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-067016.R1
Article Type:	Protocol
Date Submitted by the Author:	11-Nov-2022
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<b>Primary Subject Heading</b>:	Cardiovascular medicine
Secondary Subject Heading:	Paediatrics
Keywords:	Congenital heart disease < CARDIOLOGY, Cardiac surgery < SURGERY, Paediatric cardiology < CARDIOLOGY, Quality in health care < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, Biochemistry < NATURAL SCIENCE DISCIPLINES

SCHOLARONE™  
Manuscripts

**Title:** A Protocol for a Prospective Cohort Study Exploring the Gut Microbiota of Infants with Congenital Heart Disease Undergoing Cardiopulmonary Bypass (the GuMiBear study)

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## Abstract

### Introduction

The gut microbiota develops from birth and matures significantly during the first 24 months of life, playing a major role in infant health and development. The composition of the gut microbiota is influenced by several factors including mode of delivery, gestational age, feed type and treatment with antibiotics. Alterations in the pattern of gut microbiota development and composition can be associated with illness and compromised health outcomes.

Infants diagnosed with 'Congenital Heart Disease' (CHD) often require surgery involving cardiopulmonary bypass (CPB) early in life. The impact of this type of surgery on the integrity of the gut microbiome is poorly understood. In addition, these infants are at significant risk of developing the potentially devastating intestinal condition Necrotising Enterocolitis (NEC).

### Methods and Analysis

This study will employ a prospective cohort study methodology to investigate the gut microbiota and urine metabolome of infants with CHD undergoing surgery involving CPB. Stool and urine samples, demographic and clinical data will be collected from eligible infants based at the National Centre for Paediatric Cardiac Surgery in Ireland. Shotgun metagenome sequencing will be performed on stool samples and urine metabolomic analysis will identify

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2  
3 metabolic biomarkers. The impact of the underlying diagnosis, surgery involving CPB, and  
4 the influence of environmental factors will be explored. Data from healthy age matched  
5 infants from the INFANTMET study will serve as a control for this study.  
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10  
11 **Abbreviations:**  
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13 APGAR Score Newborn assessment score rating: Appearance, Pulse, Activity, Grimace,  
14 Respiration  
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17 ASD Atrial Septal Defect  
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20 AVSD Atrioventricular Septal Defect  
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22  
23 BUN Blood Urea Nitrogen  
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26 CHC Children's Heart Centre  
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29 CHD Congenital Heart Disease  
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32 CHI Children's Health Ireland  
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35 CPB Cardiopulmonary Bypass  
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38 CRF Case Report Form  
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41 D-TGA Dextro-Transposition of the Great Arteries  
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44 EBD Epithelial Barrier Dysfunction  
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47 ECG Electrocardiogram  
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50 GCP Good Clinical Practice  
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53 GDPR General Data Protection Regulation  
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56 HLHS Hypoplastic Left Heart Syndrome  
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59 HRHS Hypoplastic Right Heart Syndrome  
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62 HSE Health Service Executive  
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65 ICF Informed Consent Form  
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3	IV	Intravenous
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6	IVS	Intra Ventricular Septum
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9	L-TGA	Levo-Transposition of the Great Arteries
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11	LVOT	Left Ventricular Outflow Tract
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14	MaAsLin2	Multivariate Associations with Linear Models
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16	NEC	Necrotising Enterocolitis
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19	PCA	Principal Component Analysis
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21	PICU	Paediatric Intensive Care Unit
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24	PIM3	Paediatric Index of Mortality
25		
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27	PPI	Public and Patient Involvement
28		
29	PS	Pulmonary Valve Stenosis
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31		
32	RBB+C	Repeated Beat Beating Plus Column (RBB+C) method
33		
34		
35	RVOT	Right Ventricular Outflow Tract
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37	sCCA	Sparse canonical correlation analysis
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40	SD	Standard Deviation
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42	SOP	Standard Operating Procedure
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44		
45	TGA	Transposition of the Great Arteries
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48	TP	Time Point
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50	VSD	Ventricular Septal Defect
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**Keywords:** Congenital Heart disease, Microbiota, Infant, Antibiotic, Development, Cardiac Surgery, Cardiopulmonary Bypass

Word Count: 3,824



## Article Summary

### Strengths and Limitations of this Study

- GuMIBear is the first study to investigate the gut microbiome of infants with CHD undergoing surgery with CPB over a 2 year timeframe
- Comparison with age matched healthy controls allow insights into clinically relevant microbiome alterations throughout and beyond the surgical course
- The study involves urine metabolomic analysis providing novel insight into the metabolite profile of study participants compared with healthy age matched infants
- The primary limitation of GuMIBear is that it is a single centre study limiting the generalisability of the findings

## Introduction

### What is currently known

The establishment of gut microbiota begins at birth and continues over the first years of life. Continued evolution of the gut microbiome after birth is governed by host factors such as both the adaptive and innate immune system, as well as external factors such as diet, medication and toxin exposure, and illness.[1] Understanding the role of the gut microbiome in metabolism, immune function and nutrition is gaining increasing recognition, as it is accepted that an altered colonisation has been associated with a higher risk of disease later in life.[2] In the critical first weeks and months of life, perturbations to the infant gut microbiome have implications for growth development and health.[3]

### The Microbiome and Systemic Inflammation

It is evident that under certain conditions, disruption of the normal microbiota that colonise the intestinal tract can occur.[4] These conditions include systemic inflammatory processes, which can result in intestinal inflammation, where proinflammatory bacteria can flourish, interacting with the intestinal epithelium to cause cytokine release, activating key inflammatory pathways increasing morbidity and prolonging critical illness.[5] The pattern of cytokine release in patients undergoing CPB is described as comparable to those released in systemic inflammation such as trauma and sepsis.[6] However, the nature of gut

1  
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3 microbiota compositional changes in infants undergoing surgery with CPB remains  
4 understudied. This research aims to address this knowledge gap to enhance our  
5 understanding and inform care practices.  
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#### 10 CHD and Necrotising Enterocolitis (NEC)

11  
12 CHD affects approximately 1 in every 100 babies born throughout the US every year.[7] It is  
13 the most common congenital defect worldwide.[8] A diagnosis of 'Complex Congenital  
14 Heart Disease' can include conditions such as Hypoplastic Left Heart Syndrome (HLHS);  
15 Hypoplastic Right Heart Syndrome (HRHS), Transposition of the Great Arteries (TGA)  
16 requiring intervention in the first week of life, while CHD such as Atrioventricular Septal  
17 Defect (AVSD), Tetralogy of Fallot may require corrective surgery in the first few months of  
18 life. CHD requiring surgery involving cardiopulmonary bypass (CPB) present a greater risk to  
19 patients. This increased risk is not limited directly to the surgery, compromised ventricular  
20 function or low cardiac output state, but includes the risk of developing NEC.  
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31 There is a well-established connection between CHD and NEC, a potentially devastating  
32 intestinal condition of infancy. [9] NEC carries a reported incidence of between 3 and 9% in  
33 infants with CHD with all-cause mortality rates as high as 38% in children with 'cardiogenic'  
34 NEC.[10] While CHD remains one of the most common risk factors for NEC, the underlying  
35 pathophysiology of this association is complex. [11] Growing evidence suggests that  
36 perturbations in the early-life gut microbiota composition increase the risk for NEC. [3,12] A  
37 significant association between episodes of low cardiac output and shock in the  
38 development of NEC is recognised. [13, 14] It is reported that infants with certain types of  
39 CHD, mainly HLHS, may possess abnormal systemic vasculature contributing to the  
40 increased risk for NEC. [15] Whether it is those, or other causes of impaired perfusion to the  
41 gut, the resulting damage to the mucosal barrier can provide an entry point to bacteria  
42 provoking an inflammatory cascade, and the devastating consequences that can ensue.[16]  
43 The vulnerability of infants with CHD is enhanced during the course of surgical intervention  
44 involving CPB (11), and the role of the gut microbiome has received little research focus in  
45 this context.  
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#### Surgery involving CPB

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Infants diagnosed with CHD are at risk of alterations to their intestinal homeostasis, a further threat is presented in the context of surgery involving CPB. [11] There is evidence to suggest intestinal ischaemia reperfusion injury occurs after CPB and contributes to epithelial barrier dysfunction (EBD) potentially exposing the bloodstream to bacteria or bacterial products.[17] Although alterations in gut barrier integrity and resident microbiota have been demonstrated.[3] it is not fully understood what changes to the microbiome occur following CPB, and the nature and severity of EBD. While the gut microbiota in infants with CHD following CPB remains understudied, a small single centre case control study recently identified significant gut microbiota perturbations in patients with CHD.[18] This case-control study highlighted that children with CHD had a disrupted gut microbiome at baseline with an over-representation of pro-inflammatory bacteria, this was further exacerbated by CPB. Samples were collected pre-operatively and in a limited 24 and 48 hour time frame postoperatively. The significance of intraoperative variables including aortic cross clamp time and duration of CPB was not determined.

Our study proposes to address the knowledge gap and advance existing research by examining the gut microbiome of infants with CHD pre-operatively, and at defined time points up to 2 years of life/ postoperatively. This timeline will account for the recovery phase post cardiac surgery, including time to re-establish full feeds, wean from mechanical ventilation and circulatory support, and allow for surveillance of NEC postoperatively. Comparisons will be made with healthy age-matched infants recruited as part of the INFANTMET study.[19] As well as collecting intraoperative variables such as duration of CPB, and aortic cross clamp time, a novel aspect of this research will be to profile the metabolites in urine to assess potential metabolic biomarkers and pathway changes. Our research will recruit patients in a National Centre for Paediatric Cardiac Surgery, where 40 open cardiac surgeries are performed on infants annually. We therefore anticipate active recruitment will ensure the proposed target sample of 50 participants is achievable. No additional invasive procedures will be required for sample collection, enhancing the acceptability of the research for consenting parents or carers. This project will investigate the subdivisions of the gut microbiota of infants with CHD, and environmental factors such as the influence of mode of delivery, pre-operative fasting states and mode of feeding, and use of pre-

operative antibiotics. Understanding the status of the intestinal microbiome of infants with CHD and the effects of undergoing surgery with CPB is vital in informing best care practices to enhance patient outcomes.

## Objectives and Outcomes

The primary study objectives and outcomes are:

- To characterise the gut microbiota composition of infants with specified CHD undergoing surgery with CPB at specific time points perioperatively.
- To determine any differences in gut microbiota composition of infants who take part in this study pre and post-operatively compared with the microbiota of healthy babies from the INFANTMET study at matching time points.[19]
- To characterise the urine metabolite profile of infants with specified CHD undergoing surgery with CPB and compare with healthy infants from the INFANTMET study.[19]

Secondary objectives and outcomes:

To explore the influence of maternal and environmental factors on gut microbiome composition.

## Methods

### Study design

This study is a prospective cohort study of infants with CHD undergoing CPB at the National Centre for Paediatric Cardiac Surgery at Children's Health Ireland (CHI) at Crumlin, Dublin, Ireland. This single-site study will investigate the differences in the gut microbiome, metabolomics readouts, and stress levels between infants with CHD undergoing CPB and healthy age matched controls.

### Participant selection

This study will involve the collection of demographic and clinical maternal and infant data from infants diagnosed with CHD scheduled for surgery involving CPB. CHD is typically diagnosed antenatally with fetal echocardiography performed routinely at 20-24 weeks gestation. Any child presenting with a murmur or features of cardiac conditions, or symptoms of CHD will be diagnosed using clinical examination, including palpation, auscultation, electrocardiogram (ECG) and echocardiography. Cardiac diagnoses are classified according to cardiovascular physiology, i.e., left to right shunt, cyanosis with biventricular circulation, univentricular circulation and outflow tract obstruction, as presented in table 1.

Table 1. Classification of CHD Subtypes

Group	CHD Group	CHD Subtypes
1	Left to right shunt	AVSD, VSD, large ASD
2	Cyanotic CHD with biventricular circulation	D-TGA/IVS, D-TGA/VSD
3	Cyanotic CHD with univentricular circulation	HLHS, HRHS (Tricuspid Atresia) Pulmonary Atresia
4	RVOT Obstruction	Tetralogy of Fallot, dysplastic PS
5	LVOT Obstruction	Shones Syndrome, Coarctation of Aorta, Interrupted Aortic Arch
6	Others	Ebstein Anomaly, Truncus Arteriosus

To be eligible for the study, the participants must meet the terms of the inclusion and exclusion criteria as presented in Table 2.

Table 2. Inclusion and Exclusion Criteria.

Inclusion criteria	Exclusion criteria
Infants born full term ( $\geq 37$ weeks gestation)	Stillbirth or live birth where the baby is born alive but dies shortly after
Infants diagnosed with *CHD and scheduled for surgery involving CPB	Infants who are born healthy with no underlying illness, syndrome, or chronic disease
Infants born in Ireland to allow sample follow up	Participation in another study
Ability of the participant's parent/carers (in the investigator's opinion) to comprehend the full nature and purpose of the study	Infants not undergoing surgery involving cardiopulmonary bypass

Consent to participate in the study and willingness to comply with the protocol and study restrictions by the participant's parent/carers	Infants where parents/carers do not give consent to participate in the study
	Gastrointestinal pathology or intestinal surgery, excluding gastrostomy tube

\*CHD diagnoses and subtypes are presented in Table 1.

### Public and Patient Involvement (PPI) Statement

The mother of a child who had cardiac surgery as a baby was involved in reviewing the research questions, outcome measures and study literature at the study design phase. The PPI representative did not participate in the recruitment or the conduct of the study due to competing demands and availability. The PPI representative has offered to support dissemination of the study results through their involvement in charitable foundations and child and parent support fora.

### Recruitment

Participants meeting all inclusion criteria will be selected after admission to the hospital, outpatient clinic or cardiac day unit. Study-related information will be given in written form as well as explained by a member of the project team. No study-related activities will begin before the potentially eligible participants' parents/carers have signed the Informed Consent Form (ICF). Participants parents/carers will be asked to refer to the Privacy Notice on the hospital website or they can receive a hardcopy if they wish. Signed ICFs will be stored safely in a locked cabinet in the research office.

### Compensation

No compensation will be provided to the participants. There are no cost implications for the Health Service Executive (HSE) or to the participants. The management of patients and investigative tests will comply with current standards of care.

### Study timeline

After completing recruitment procedures, i.e., determining whether the patient meets the study inclusion criteria, discussing the study with the parents/caregivers and obtaining informed consent, clinical and demographic data will start to be collected. The study will be undertaken for a period of 2 years after the infant is initially recruited.

### Demographic Data

1  
2  
3 The infants' diagnosis including whether antenatal or postnatal diagnosis, co-morbidities,  
4 date of birth, gestational age, sex, mode of delivery, Appearance, Pulse, Activity, Grimace,  
5 Respiration (APGAR) scores, birth weight, head circumference, mode and type of feeding  
6 pre and post operatively, antibiotics administered post-delivery, complications and  
7 antenatal events will be recorded (case record form located in Appendix 1).  
8  
9

### 10 11 **Maternal Data**

12  
13  
14 Maternal history including age, antibiotics received, smoking status use of probiotics and  
15 significant antenatal events will be recorded.  
16

### 17 **Surgical course**

18  
19  
20 The type of surgery performed, duration of CPB and cross-clamp time will be recorded.  
21 Antibiotic use and any intraoperative events will be recorded as well as clinical information  
22 including arterial blood gas data.  
23  
24

### 25 **Post-operative Data**

26  
27  
28 Paediatric Index of Mortality (PIM) score, duration of mechanical ventilation, haematology  
29 variables including haemoglobin and haematocrit, renal data e.g. blood urea nitrogen (BUN)  
30 and creatinine, fluid balance, and cardiovascular support including vasoactive inotrope  
31 score, as well as duration of stay in paediatric intensive care unit will be recorded.  
32  
33

### 34 **Feeding Information Data**

35  
36  
37 Feeding information including the type of feed and duration of the feed prior to and after  
38 surgery will be recorded. The date the patient is established on full feeds will be recorded.  
39 Full feeding is defined as when the patient no longer requires parenteral nutrition or  
40 intravenous fluids.  
41  
42

### 43 **Discharge Information Data**

44  
45  
46 Discharge information data: This will include the patient's status on discharge from  
47 Paediatric Intensive Care Unit (PICU), as well as length of PICU and hospital stay.  
48  
49

### 50 **Complications**

51  
52  
53 The occurrence of complications will be recorded, for example the development of NEC. The  
54 timeline for recording NEC onset will be based on the initiation of triple antibiotic therapy,  
55 based on a full surgical review including clinical presentation, radiological and laboratory  
56 data. In addition, the occurrence of death, rehospitalisation for heart failure or cardiac  
57 problems will be included.  
58  
59  
60

## Subject withdrawal/exclusion

Under the Declaration of Helsinki, the research nurse will explain to the consenting adult that they have the right to withdraw from the study at any time and that this will in no way prejudice their future treatment. The reason for withdrawal will be recorded in the source documents and on the appropriate CRF. Consenting adults will be made aware that stored samples from individuals withdrawing from the study may have undergone processing and may be analysed in the study.

## Regulatory procedures

The study is conducted following the version Fortaleza, Brazil, October 2013 of the Declaration of Helsinki 1964. The Protocol and the ICF have been approved by the Clinical Research Ethics Committee of Children's Health Ireland GEN/826/20. As biological samples will be procured in one institution and sent to another, a data sharing agreement is in place between The Cardiology Department, CHI at Crumlin Hospital, and APC Microbiome Ireland, in Cork. This research is fully compliant with the guidelines as set out in The General Data Protection Regulation (GDPR), the Irish Data Protection Acts 1988 to 2018 including Protection Act 2018 (Section 36(2)) (Health Research) Regulations 2018.

## Data Statement

Once collected, the anonymised demographic, clinical and laboratory analysis data as well as statistical codes will be uploaded to the open access Research Repository University College Dublin.

## Sample collection and analysis

### Faecal samples

Stool samples will be collected at the following time points: within 24 hours after birth (timepoint (TP) 1), within 24 hrs pre surgery (TP 2), 1 week post-surgery (TP 3) 4 weeks post-surgery (TP 4), 24 weeks post-surgery (TP 5) at 52 weeks (TP 6) and 2 years of age (TP 7). Information about antibiotic therapy administered before or during the stool collection will be recorded.

As the study site is the National Centre for Paediatric Cardiology, all infants diagnosed with CHD antenatally are transferred to the study site from the Maternity Unit for management of CHD. The first study stool specimen is typically obtained after the infant is transferred to the study site and informed consent obtained, which is typically within 24 hours of delivery.

The sample will be collected by the bedside nurse or the parent/carers and transferred to the laboratory upon receipt of the sample during the weekdays or weekends. At night, the



1  
2  
3 sample will be kept in the dedicated fridge and transferred to the laboratory within 4-5  
4 hours for appropriate storage at -80°C until further analysis. A standard operating procedure  
5 (SOP) for sample collection when participant is no longer an in-patient is provided in  
6 Appendix 2.  
7  
8

## 9 10 Urine Samples

11  
12 Urine samples will be collected at 4-8 weeks post-surgery for metabolomic analysis using  
13 Sterisets Uricol Urine Collection Pack (MedGuard, Ireland). The urine sample will be  
14 collected from the urinary catheter if the participant is catheterised. Alternatively, a pad will  
15 be placed in the diaper and used to collect an unsoiled urine sample from the infant. The  
16 pad will then be placed in a biohazard bag and frozen immediately at -80 °C prior to  
17 processing. After all the sample collections are complete, they will be shipped to Teagasc  
18 Food Research, Moorepark, Ireland, using DHL overnight service for microbiome and  
19 metabolomics analyses. Styrofoam Saf-T-Pak STP-309 shipper box or equivalent will be used.  
20 DNA extraction will be performed on stool samples using the modification of the Repeated  
21 Bead Beating Plus Column (RBB+C) method.[20] LC-MS will be utilised for metabolomics  
22 analysis of urine.[19]  
23  
24  
25  
26  
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28

## 29 Sample collection for discharged participant

30  
31 Parents/carers will receive a sample collection discharge pack and a parent diary/instruction  
32 served as reminder to collect due samples at different time-points prior to discharge home.  
33 They will receive a follow up text message or phone call to remind them on the due sample.  
34 The sample collection discharge pack consists of urine/stool collection containers with study  
35 code, sterile pad, syringe, zip-lock bag, gloves, biohazard bag and cooler bag. Parents/carers  
36 are asked to keep the collected sample at the dedicated section of their home freezer. They  
37 will transport the collected sample in the cooler bag provided when attending out-patient  
38 department for appointments. They will ask a member of the project team to transfer the  
39 collected sample to the laboratory upon arrival at the hospital. The study researcher is  
40 available at the dedicated contact number for any queries.  
41  
42  
43  
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46

## 47 Adverse events and participant well-being

48  
49 There are no expected safety concerns related to the study. All study participants will be  
50 under the care of the cardiology team at the hospital with access to psychological support,  
51 as well as nursing and medical professionals, social workers and chaplaincy.  
52  
53  
54

## 55 Data collection and management

56  
57 The study diaries, study dataset, and paper/digital CRF systems will be used for recording  
58 data from each study participant. All the data collected in this study is pseudonymised, as  
59  
60

1  
2  
3 each of the participants will be assigned a specific study code and upon receipt, data will be  
4 referred to the study code. All study staff responsible for entering data into the CRF system  
5 received training in advance of the study commencement. This training included familiarity  
6 with the study diaries, study dataset, and paper/digital CRF system and have completed  
7 good clinical practice (GCP) in research training. They have individual access to the password  
8 protected study shared drive within the hospital. The study team will monitor the  
9 data/sample collection process. Any inconsistencies identified during the study will be  
10 presented as queries at the regular project team meeting.  
11  
12  
13  
14

### 15 **Comparison Data**

16  
17  
18 Data collected as part of the INFANTMET Study will serve as a healthy control comparison  
19 for this study. INFANTMET compared the gut microbiota development of breastfed infants  
20 born via C-section or vaginally at full-term or preterm at Cork University Maternity  
21 Hospital. Ethical approval for sample collection by Cork University Hospital Research Ethics  
22 Committee (reference number ECM(w) 07/02/2012). One hundred and ninety two infants  
23 were recruited to the INFANTMET study and stratified according to delivery mode and  
24 gestational age at birth. Faecal samples were collected from the infants at 1, 4, 8 and 24  
25 weeks of age and year 1, 2 and 4 of life and stored under controlled conditions. Urine  
26 samples were collected at 4 weeks of age for metabolomic analysis and stored in a freezer  
27 at -80°C prior to processing. Samples were analysed in accordance with the analysis  
28 proposed for the GuMIBear study. Although INFANTMET study participants did not have  
29 CHD, they nonetheless serve as a valuable comparison group. The stool and urine samples  
30 collected as part of the GuMIBear study will be as closely time matched as possible to the  
31 INFANTMET study samples to capture the major developmental period of the early life gut  
32 microbiota.  
33  
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### 40 **Bioinformatics and Statistical analysis**

#### 41 **Sample size justification**

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45  
46 Published research in this area is lacking. However one case control study by Salomon et al.  
47 (2021) included 17 cases and 12 control participants and was sufficiently powered to  
48 determine a statistically significant difference in beta diversity in cases versus controls  
49 ( $F=5.6$ ,  $p<0.001$ ). Our study proposes to include 50 patients with CHD undergoing surgery  
50 with CPB and age matched controls, almost three times the Solomon et al. (2021) study. We  
51 therefore anticipate that the proposed sample size is justified.  
52  
53  
54

#### 55 **Demographic and Clinical Data**

56  
57  
58 Demographic and clinical data and laboratory information will be tested for normality using  
59 the Shapiro-Wilk test. Descriptive statistics will be used to describe normally distributed  
60

1  
2  
3 data, and expressed as mean  $\pm$  standard deviation (SD). Continuous data not normally  
4 distributed will be reported as median and interquartile ranges. Categorical variables will be  
5 expressed as counts and percentages. Groups will be compared using chi square tests for  
6 categorical variables and independent-samples student's *t*- tests for normally distributed  
7 continuous variables. For variables not normally distributed, the Mann-Whitney *U* test will  
8 be used. Comparison will include subgroup analysis of participants who experienced post-  
9 operative complications including NEC with those that did not. Comparisons will also include  
10 cyanotic versus acyanotic heart disease subgroup analysis, as well as mode and type of  
11 feeding pre and post-operatively.  
12  
13  
14  
15

### 16 17 Microbiome analysis

18  
19 Metagenomic shotgun sequencing data will be analysed using bioBakery suite of tools  
20 ([https://huttenhower.sph.harvard.edu/biobakery\\_workflows/](https://huttenhower.sph.harvard.edu/biobakery_workflows/)). Trimmed and human reads  
21 filtered using KneadData (v0.7.2) with the default parameters. Quality controlled data will  
22 be taxonomically profiled at the species level with relative abundance by MetaPhlAn2.  
23 Functional profiling will be performed using HUMAnN3 and strain profiling using  
24 StrainPhlAn.  
25  
26  
27  
28

29 For alpha diversity analysis, samples will be rarefied to even depth and phyloseq::estimate  
30 richness will be used to calculate Chao1, Shannon and Simpson indices. Alpha diversity  
31 indices between groups will be univariately compared using the Wilcoxon rank sum test. A  
32 beta-diversity ordination will be generated using the Aitchison distance and visualised using  
33 Principal Component Analysis (PCA) plot. The Adonis function in the vegan package will be  
34 used to implement a permutational multivariate analysis of variance to test whether  
35 samples cluster beyond that expected by sampling variability. MaAsLin2 (Multivariate  
36 Associations with Linear Models) will be used to investigate multivariable associations  
37 between sequencing data and clinical metadata. MaAsLin2 performs boosted, additive,  
38 linear models to detect associations while adjusting for confounding factors. Sparse  
39 canonical correlation analysis (sCCA) will be used to calculate the overall correlation  
40 between metabolites and microbes, and to identify strongly associated biomarkers. Pairwise  
41 spearman rank correlation analysis will also be performed.  
42  
43  
44  
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### 49 Discussion

50  
51 Despite an increasing awareness of the early-life critical window of microbiome  
52 development on the health and wellness of infants, there remains much to learn about the  
53 interactions of the microbiome with the infant host with CHD undergoing surgery involving  
54 CPB. This study is designed to address this knowledge gap, and incorporates a sound  
55 methodology with particular strengths enhancing the value of its findings. The specimen  
56 collection strategy occurring at multiple time points over a 2 year period in the proposed  
57 study will deepen our understanding of the temporal dynamics of the colonising microbiota,  
58  
59  
60

1  
2  
3 and their interactions with host physiology. The study design will account for maternal and  
4 perioperative variables to determine changes to the microbiome. Access to existing  
5 microbiome data from healthy age matched infants provides a valuable opportunity to  
6 present high quality comparative information. While multi-centre trials capturing sufficient  
7 case numbers of NEC cases will offer robust conclusions, this study will offer valuable  
8 evidence to support the influence of CHD and CPB on the microbiome and intestinal  
9 epithelial barrier dysfunction (EBD). Future research can build on existing studies, and  
10 explore treatment strategies including recommendations for efficacious probiotic strain  
11 administration, including the supplements to promote a diverse gut microbiota to improve  
12 outcomes for this vulnerable population.  
13  
14  
15  
16  
17

### 18 Ethics and Dissemination

19  
20  
21 This research study is ethically approved (REC REF No: GEN/826/20). Study results will be  
22 available to patients with CHD and their families, carers, support networks, paediatric  
23 cardiology and microbiome societies and other researchers. Study findings will provide a  
24 deeper understanding of the gut microbiota of infants with CHD and inform perioperative  
25 management options including strategies to prioritise the integrity of the gut microbiota.  
26  
27  
28  
29  
30

### 31 Status of Study

32  
33 The trial is ongoing and as of November 10<sup>th</sup> 2022, 80 % of the participants have been  
34 recruited. Laboratory analysis has been carried out on 25% of study samples.  
35  
36

### 37 Funding Statement

38  
39  
40 This research received no specific grant from any funding agency in the public, commercial  
41 or not-for-profit sectors.  
42  
43

### 44 Competing interests

45  
46 The authors declare no association with commercial entities, either financial or non-  
47 financial.  
48  
49

### 50 Author contributions

51  
52  
53 CM, CJMcM, CS, KM devised the project, and the main conceptual ideas. CJMcM, CM, CS,  
54 RPR, FK, DJ, MHT were involved in the study design and writing of the manuscript. MOT, JB,  
55 NM, SD, CJMcM are involved in consenting participants, collecting samples and acquiring  
56 data. DJ, CS, FK and RPR are responsible for analysing study samples. All authors read  
57  
58  
59  
60

manuscript revisions, approved the final manuscript and accept accountability for the accuracy and integrity of the work.

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## Appendix 1: Case Report Form PDF Version

Appendix 2: SOP for Obtaining a Stool Sample when Participant is no longer Inpatient

## References

1. Skillington O MS, Gupta A, Mayer EA, Gill CIR, Del Rio D, O'Riordan KJ, Cryan JF, Ross RP and Stanton C The contrasting human gut microbiota in early and late life and implications for host health and disease. *Nutrition and Healthy Ageing*. 2012;6:157-78.
2. Cilieborg MS, Boye M, Sangild PT. Bacterial colonization and gut development in preterm neonates. *Early human development*. 2012;88 Suppl 1:S41-9.
3. Healy DB, Ryan CA, Ross RP, Stanton C, Dempsey EM. Clinical implications of preterm infant gut microbiome development. *Nature microbiology*. 2022;7(1):22-33.
4. Ohland CL, Jobin C. Microbial activities and intestinal homeostasis: A delicate balance between health and disease. *Cellular and molecular gastroenterology and hepatology*. 2015;1(1):28-40.
5. Cabrera-Perez J, Badovinac VP, Griffith TS. Enteric immunity, the gut microbiome, and sepsis: Rethinking the germ theory of disease. *Experimental biology and medicine*. 2017;242(2):127-39.
6. Halter J, Steinberg J, Fink G, Lutz C, Picone A, Maybury R, et al. Evidence of systemic cytokine release in patients undergoing cardiopulmonary bypass. *The journal of extra-corporeal technology*. 2005;37(3):272-7.
7. Centers for Disease Control and Prevention (CDC). Data and Statistics on Congenital Heart Defects. 2022 [updated January 24th 2022;26th July 2022]. Available from: <https://www.cdc.gov/ncbddd/heartdefects/data.html> Accessed 26<sup>th</sup> July 2022.
8. van der Linde D, Konings E, Slager M, Witsenburg M, Helbing W, Takkenburg J, Roos-Hesselink J. Birth Prevalence of Congenital Heart Disease Worldwide: A Systematic Review and Meta-Analysis. *Journal of the American College of Cardiology*. 2011;58(21):2241-2247.
9. Martin R, Makino H, Cetinyurek Yavuz A, Ben-Amor K, Roelofs M, Ishikawa E, et al. Early-Life Events, Including Mode of Delivery and Type of Feeding, Siblings and Gender, Shape the Developing Gut Microbiota. *PloS one*. 2016;11(6):e0158498.
10. Siano E, Lauriti G, Ceccanti S, Zani A. Cardiogenic Necrotizing Enterocolitis: A Clinically Distinct Entity from Classical Necrotizing Enterocolitis. *European journal of pediatric surgery : official journal of Austrian Association of Pediatric Surgery*. 2019;29(1):14-22.
11. Kelleher ST, McMahon CJ, James A. Necrotizing Enterocolitis in Children with Congenital Heart Disease: A Literature Review. *Pediatric cardiology*. 2021;42(8):1688-99.
12. Murphy K, O'Donovan AN, Caplice NM, Ross RP, Stanton C. Exploring the Gut Microbiota and Cardiovascular Disease. *Metabolites*. 2021;11(8).
13. Carlo WF, Kimball TR, Michelfelder EC, Border WL. Persistent diastolic flow reversal in abdominal aortic Doppler-flow profiles is associated with an increased risk of necrotizing enterocolitis in term infants with congenital heart disease. *Pediatrics*. 2007;119(2):330-5.

14. McElhinney DB, Hedrick HL, Bush DM, Pereira GR, Stafford PW, Gaynor JW, et al. Necrotizing enterocolitis in neonates with congenital heart disease: risk factors and outcomes. *Pediatrics*. 2000;106(5):1080-7.
15. Miller TA, Minich LL, Lambert LM, Joss-Moore L, Puchalski MD. Abnormal abdominal aorta hemodynamics are associated with necrotizing enterocolitis in infants with hypoplastic left heart syndrome. *Pediatric cardiology*. 2014;35(4):616-21.
16. Nino DF, Sodhi CP, Hackam DJ. Necrotizing enterocolitis: new insights into pathogenesis and mechanisms. *Nature reviews Gastroenterology & hepatology*. 2016;13(10):590-600.
17. Typpo KV, Larmonier CB, Deschenes J, Redford D, Kiela PR, Ghishan FK. Clinical characteristics associated with postoperative intestinal epithelial barrier dysfunction in children with congenital heart disease. *Pediatric critical care medicine : a journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies*. 2015;16(1):37-44.
18. Salomon J, Ericsson A, Price A, Manithody C, Murry DJ, Chhonker YS, et al. Dysbiosis and Intestinal Barrier Dysfunction in Pediatric Congenital Heart Disease Is Exacerbated Following Cardiopulmonary Bypass. *JACC Basic to translational science*. 2021;6(4):311-27.
19. Hill CJ, Lynch DB, Murphy K, Ulaszewska M, Jeffery IB, O'Shea CA, et al. Evolution of gut microbiota composition from birth to 24 weeks in the INFANTMET Cohort. *Microbiome*. 2017;5(1):4.
20. Yu Z, Morrisson M. Improved extraction of PCR-quality community DNA from digesta and fecal samples. *Biotechniques*. 2004;36(5):808-12.



^999 – missing data; 0 – no data in medical notes

**CASE RECORD FORM**

**Stool Sample Collections**

Period/ Time Frame	Projected Date	Sample Date	Sample Collected	Comments
1. Within 24h of Birth			<input type="checkbox"/> YES <input type="checkbox"/> NO	
2. Pre-operatively			<input type="checkbox"/> YES <input type="checkbox"/> NO	
3. Week 1 of life/Post-Op			<input type="checkbox"/> YES <input type="checkbox"/> NO	
4a. Week 4 to 8 life/Post-op			<input type="checkbox"/> YES <input type="checkbox"/> NO	
4b. <b>Urine</b> Sample Week 4 to 8			<input type="checkbox"/> YES <input type="checkbox"/> NO	
5. Week 24 of life/Post-Op			<input type="checkbox"/> YES <input type="checkbox"/> NO	
6. Week 52 of life/Post-Op			<input type="checkbox"/> YES <input type="checkbox"/> NO	
7. Year 2 of life/Post-Op			<input type="checkbox"/> YES <input type="checkbox"/> NO	

**PATIENT DEMOGRAPHIC**

Date of Enrolment:	Date of Birth:	Cardiac Classification Group No:
Gender: <input type="checkbox"/> Male <input type="checkbox"/> Female <input type="checkbox"/> Other/Ambiguous	Gestational Age: APGAR at ① ⑤ min [ ] [ ]	Multiple <input type="checkbox"/> Yes <input type="checkbox"/> No Birth Order:
Reason for admission:		
Comorbidities:		
Mode of Delivery: SVD: <input type="checkbox"/> Yes <input type="checkbox"/> No LSCS: <input type="checkbox"/> Yes <input type="checkbox"/> No, if Yes: <input type="checkbox"/> Elective/ <input type="checkbox"/> Emergency	Weight at Birth: _____ . _____ KGs Head Circumference: _____ cms	
Antibiotics to Infant post-delivery; <input type="checkbox"/> Yes <input type="checkbox"/> No If Yes, list:	Timing of cardiac diagnosis: Postnatal <input type="checkbox"/> Antenatal <input type="checkbox"/>	
Significant Antenatal Events:		

**MATERNAL INFORMATION**

Maternal Age (years) at Birth:	Gestational Age at Booking Appt:
Antibiotics given Pre-Delivery: <input type="checkbox"/> Yes <input type="checkbox"/> No List:	Maternal Probiotics taken during pregnancy: <input type="checkbox"/> Yes <input type="checkbox"/> No List:
Maternal Smoking during Pregnancy: <input type="checkbox"/> Yes <input type="checkbox"/> No	Other Household Members Smoking during Pregnancy: <input type="checkbox"/> Yes <input type="checkbox"/> No





^999 – missing data; 0 – no data in medical notes

### SURGERY INFORMATION

Surgery Date:			
Surgery Performed:			
Pre-Op Antibiotics <sup>1?</sup> List:		Post-Op Antibiotics <sup>1?</sup> List:	
1. Abs <48hrs pre sampling		1. Abs <48hrs pre sampling	
2. Abx <72hrs pre sampling		2. Abx <72hrs pre sampling	
3. Abx in last 7 days/during sample		3. Abx in last 7 days/during sample	
ABG	pH	PO <sup>2</sup>	
First Pre-Op ABG:		Cardiopulmonary Bypass Duration:	
First Post-Op ABG:		Aortic Cross Clamp Duration:	
Significant Intraoperative Events:			

<sup>1</sup>Antibiotic treatment at time of stool sampling as below. Important not to include antibiotics which were started post the stool sampling

- 1) Abs < 48hrs pre sampling
- 2) Abx < 72 hrs pre sampling
- 3) Abx in previous 7 days/ during sample collection

### POST-OPERATIVE INFORMATION

PIM3 Score:		No. of days in ICU (1 <sup>st</sup> adm):	
Mechanical Vent:	<input type="checkbox"/> Yes <input type="checkbox"/> No	No of Days on ECLS:	
No of Days Vent:		No of Days on RRT:	
Agent	Day 1	Day 2	Day 3
Milrinone			
Epinephrine			
Norepinephrine			
Vasopressin			
Midazolam			
Morphine			
Clonidine			
Others			
Fluid Balance:			
BUN:			
Creatinine:			
HCT:			
Hgb:			

### FEEDING INFORMATION

Mode of Feeding (note <b>date initiated</b> and <b>date d/c</b> ):		
Breastmilk:	Infant Formula:	Other:
Prebiotics given to Infant: <input type="checkbox"/> Yes <input type="checkbox"/> No	Type and Date Given:	
Excessive Infantile Crying (cried for $\geq 3$ Hrs for 3 Days in one week): <input type="checkbox"/> Yes <input type="checkbox"/> No		



# GuMIBear Study



Study ID: \_\_\_\_\_ GMB: \_\_\_\_\_

1 ^999 – missing data; 0 – no data in medical notes

Date Trophic Feeds Commenced:		Type of Feed Used:	
Time to Establishment of full feed <sup>2</sup> :			
Development of NEC <sup>3</sup> :			
Days post-op when developed NEC?			
Gut stasis:	Not applicable		
Management Strategy:			

2 Full feed – No longer requires parenteral nutrition or intravenous fluids supplement regardless the TFI.

3 NEC – Initiation of triple IV antibiotic therapy and nil by mouth for at least 5 days, based on a full surgical review including clinical presentation, radiological and laboratory data.

## DISCHARGE INFORMATION

Date of Discharge			
Ward:	Home:	RIP:	

## READMISSION TO ICU

Total ICU Readmission days:				
	Date of Admission	Date of Discharge	Total ICU stays	Reason for admission
1.				
2.				
3.				
4.				
5.				

## DATA ENTRY BY (NAME)

Admission	Date	Paper	Date	G-Drive
First				
Second				
Third				

Participant Withdrawal from Study: <input type="checkbox"/> Yes <input type="checkbox"/> No
GCP Procedure Followed: <input type="checkbox"/> Yes <input type="checkbox"/> No
See Study Folder Appendix 4. Signed: _____ Date: _____



## The Gut Microbiota of Infants with Complex Congenital Heart Disease Undergoing Cardiopulmonary Bypass



( GuMiBear)

### STANDARD OPERATING PROCEDURE FOR STOOL SAMPLE COLLECTION AS AN OUTPATIENT

**Purpose:** To collect infant stool samples while the infant is an out-patient from CHI at Crumlin

**Objective:** To collect infant stool samples for the study in a uniform manner and under a set of conditions, so that they can be processed by the laboratories to achieve optimal results.

**Procedure:**

1. Ensure that at least one legal guardian has provided written informed consent for their infant to participate in the study and that they are happy for their infant to remain in the study.
2. Ensure that in the hospital chart of the infant that it is noted that he/she is participating in the study and contact details of the study team.
3. Ensure the parent/guardian has been supplied with a study pack containing the requisites for the collection of the stool sample
  - a. Completed labels
  - b. Disposable Gloves
  - c. Stool collection containers
  - d. Bio-hazard bags
4. Explain to the legal guardian that a minimum of a teaspoon of stool has to be collected. Explain to the legal guardian when the samples have to be collected as close as possible to the next out-patient appointment.
5. Explain to parent/guardian how to collect the sample as follows:
  - a. Have requisites for collection at the ready.(Gloves, sample container, red biohazard bag & labels)
  - b. Place appropriate label on the sample bottle.
  - c. Wear disposable gloves
  - d. Unscrew cap of sample bottle



## The Gut Microbiota of Infants with Complex Congenital Heart Disease Undergoing Cardiopulmonary Bypass



( GuMiBear)

- e. Spoon in stool sample (1 teaspoon in volume)
  - f. Screw cap on tightly
  - g. Place in red hazard bag
  - h. Remove disposable gloves
  - i. Dispose with soiled nappy
  - j. Wash hands
  - k. Place stool sample in fridge
  - l. Text research nurse that sample is ready for collection.
6. Label the sample bottles with Subject Number, Date of Birth, Initials, Sample Number, Date of Collection
  7. Ask the parent/guardian to attach the appropriate label to the sample container and immediately place container in freezer.
  8. Text the parent/guardian the night before the sample is to be collected. Meet the parent/guardian in out-patients to collect the stool sample and store the sample in the study container in the designated study freezer.
  9. Update the study spreadsheet to indicate sample has been obtained.

# BMJ Open

## A Protocol for a Prospective Cohort Study Exploring the Gut Microbiota of Infants with Congenital Heart Disease Undergoing Cardiopulmonary Bypass (the GuMiBear study)

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Manuscripts

**Title:** A Protocol for a Prospective Cohort Study Exploring the Gut Microbiota of Infants with Congenital Heart Disease Undergoing Cardiopulmonary Bypass (the GuMiBear study)

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## Abstract

### Introduction

The gut microbiota develops from birth and matures significantly during the first 24 months of life, playing a major role in infant health and development. The composition of the gut microbiota is influenced by several factors including mode of delivery, gestational age, feed type and treatment with antibiotics. Alterations in the pattern of gut microbiota development and composition can be associated with illness and compromised health outcomes.

Infants diagnosed with 'Congenital Heart Disease' (CHD) often require surgery involving cardiopulmonary bypass (CPB) early in life. The impact of this type of surgery on the integrity of the gut microbiome is poorly understood. In addition, these infants are at significant risk of developing the potentially devastating intestinal condition Necrotising Enterocolitis (NEC).

### Methods and Analysis

This study will employ a prospective cohort study methodology to investigate the gut microbiota and urine metabolome of infants with CHD undergoing surgery involving CPB. Stool and urine samples, demographic and clinical data will be collected from eligible infants based at the National Centre for Paediatric Cardiac Surgery in Ireland. Shotgun metagenome

1  
2  
3 sequencing will be performed on stool samples and urine metabolomic analysis will identify  
4 metabolic biomarkers. The impact of the underlying diagnosis, surgery involving CPB, and  
5 the influence of environmental factors will be explored. Data from healthy age matched  
6 infants from the INFANTMET study will serve as a control for this study.  
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## 12 **Abbreviations:**

13  
14 APGAR Score Newborn assessment score rating: Appearance, Pulse, Activity, Grimace,  
15 Respiration  
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18 ASD Atrial Septal Defect  
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21 AVSD Atrioventricular Septal Defect  
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23  
24 BUN Blood Urea Nitrogen  
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27 CHC Children's Heart Centre  
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30 CHD Congenital Heart Disease  
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32  
33 CHI Children's Health Ireland  
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35  
36 CPB Cardiopulmonary Bypass  
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38  
39 CRF Case Report Form  
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41  
42 D-TGA Dextro-Transposition of the Great Arteries  
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44  
45 EBD Epithelial Barrier Dysfunction  
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47  
48 ECG Electrocardiogram  
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50  
51 GCP Good Clinical Practice  
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54 GDPR General Data Protection Regulation  
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56  
57 HLHS Hypoplastic Left Heart Syndrome  
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60 HRHS Hypoplastic Right Heart Syndrome

HSE Health Service Executive

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3	ICF	Informed Consent Form
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5		
6	IV	Intravenous
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8	IVS	Intra Ventricular Septum
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11	L-TGA	Levo-Transposition of the Great Arteries
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13		
14	LVOT	Left Ventricular Outflow Tract
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16	MaAsLin2	Multivariate Associations with Linear Models
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18		
19	NEC	Necrotising Enterocolitis
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21		
22	PCA	Principal Component Analysis
23		
24	PICU	Paediatric Intensive Care Unit
25		
26		
27	PIM3	Paediatric Index of Mortality
28		
29		
30	PPI	Public and Patient Involvement
31		
32	PS	Pulmonary Valve Stenosis
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34		
35	RBB+C	Repeated Beat Beating Plus Column (RBB+C) method
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37	RVOT	Right Ventricular Outflow Tract
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39		
40	sCCA	Sparse canonical correlation analysis
41		
42		
43	SD	Standard Deviation
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45		
46	SOP	Standard Operating Procedure
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48	TGA	Transposition of the Great Arteries
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51	TP	Time Point
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53	VSD	Ventricular Septal Defect
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**Keywords:** Congenital Heart disease, Microbiota, Infant, Antibiotic, Development, Cardiac Surgery, Cardiopulmonary Bypass

1  
2  
3 Word Count: 3,887  
4  
5

## 6 **Article Summary**

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### 8 **Strengths and Limitations of this Study**

9

- 10 ● GuMIBear is the first study to investigate the gut microbiome of infants with CHD  
11 undergoing surgery with CPB over a 2 year timeframe
  - 12 ● Comparison with age matched healthy controls allow insights into clinically relevant  
13 microbiome alterations throughout and beyond the surgical course
  - 14 ● The study involves urine metabolomic analysis providing novel insight into the  
15 metabolite profile of study participants compared with healthy age matched infants
  - 16 ● The primary limitation of GuMIBear is that it is a single centre study limiting the  
17 generalisability of the findings
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## 26 **Introduction**

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### 28 **What is currently known**

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30  
31 The establishment of gut microbiota begins at birth and continues over the first years of life.  
32 Continued evolution of the gut microbiome after birth is governed by host factors such as  
33 both the adaptive and innate immune system, as well as external factors such as diet,  
34 medication and toxin exposure, and illness.[1] Understanding the role of the gut  
35 microbiome in metabolism, immune function and nutrition is gaining increasing recognition,  
36 as it is accepted that an altered colonisation has been associated with a higher risk of  
37 disease later in life.[2] In the critical first weeks and months of life, perturbations to the  
38 infant gut microbiome have implications for growth development and health.[3]  
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### 48 **The Microbiome and Systemic Inflammation**

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51 It is evident that under certain conditions, disruption of the normal microbiota that colonise  
52 the intestinal tract can occur.[4] These conditions include systemic inflammatory processes,  
53 which can result in intestinal inflammation, where proinflammatory bacteria can flourish,  
54 interacting with the intestinal epithelium to cause cytokine release, activating key  
55 inflammatory pathways increasing morbidity and prolonging critical illness.[5] The pattern  
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3 of cytokine release in patients undergoing CPB is described as comparable to those released  
4 in systemic inflammation such as trauma and sepsis.[6] However, the nature of gut  
5 microbiota compositional changes in infants undergoing surgery with CPB remains  
6 understudied. This research aims to address this knowledge gap to enhance our  
7 understanding and inform care practices.  
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### 13 14 CHD and Necrotising Enterocolitis (NEC)

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16 CHD affects approximately 1 in every 100 babies born throughout the US every year.[7] It is  
17 the most common congenital defect worldwide.[8] A diagnosis of 'Complex Congenital  
18 Heart Disease' can include conditions such as Hypoplastic Left Heart Syndrome (HLHS);  
19 Hypoplastic Right Heart Syndrome (HRHS), Transposition of the Great Arteries (TGA)  
20 requiring intervention in the first week of life, while CHD such as Atrioventricular Septal  
21 Defect (AVSD), Tetralogy of Fallot may require corrective surgery in the first few months of  
22 life. CHD requiring surgery involving cardiopulmonary bypass (CPB) present a greater risk to  
23 patients. This increased risk is not limited directly to the surgery, compromised ventricular  
24 function or low cardiac output state, but includes the risk of developing NEC.  
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34 There is a well-established connection between CHD and NEC, a potentially devastating  
35 intestinal condition of infancy. [9] NEC carries a reported incidence of between 3 and 9% in  
36 infants with CHD with all-cause mortality rates as high as 38% in children with 'cardiogenic'  
37 NEC.[10] While CHD remains one of the most common risk factors for NEC, the underlying  
38 pathophysiology of this association is complex. [11] Growing evidence suggests that  
39 perturbations in the early-life gut microbiota composition increase the risk for NEC. [3,12] A  
40 significant association between episodes of low cardiac output and shock in the  
41 development of NEC is recognised. [13, 14] It is reported that infants with certain types of  
42 CHD, mainly HLHS, may possess abnormal systemic vasculature contributing to the  
43 increased risk for NEC. [15] Whether it is those, or other causes of impaired perfusion to the  
44 gut, the resulting damage to the mucosal barrier can provide an entry point to bacteria  
45 provoking an inflammatory cascade, and the devastating consequences that can ensue.[16]  
46 The vulnerability of infants with CHD is enhanced during the course of surgical intervention  
47 involving CPB (11), and the role of the gut microbiome has received little research focus in  
48 this context.  
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## Surgery involving CPB

Infants diagnosed with CHD are at risk of alterations to their intestinal homeostasis, a further threat is presented in the context of surgery involving CPB. [11] There is evidence to suggest intestinal ischaemia reperfusion injury occurs after CPB and contributes to epithelial barrier dysfunction (EBD) potentially exposing the bloodstream to bacteria or bacterial products.[17] Although alterations in gut barrier integrity and resident microbiota have been demonstrated.[3] it is not fully understood what changes to the microbiome occur following CPB, and the nature and severity of EBD. While the gut microbiota in infants with CHD following CPB remains understudied, a small single centre case control study recently identified significant gut microbiota perturbations in patients with CHD.[18] This case-control study highlighted that children with CHD had a disrupted gut microbiome at baseline with an over-representation of pro-inflammatory bacteria, this was further exacerbated by CPB. Samples were collected pre-operatively and in a limited 24 and 48 hour time frame postoperatively. The significance of intraoperative variables including aortic cross clamp time and duration of CPB was not determined.

Our study proposes to address the knowledge gap and advance existing research by examining the gut microbiome of infants with CHD pre-operatively, and at defined time points up to 2 years of life/ postoperatively. This timeline will account for the recovery phase post cardiac surgery, including time to re-establish full feeds, wean from mechanical ventilation and circulatory support, and allow for surveillance of outcome measures including NEC, repeat surgery, and mortality postoperatively. Comparisons will be made with healthy age-matched infants recruited as part of the INFANTMET study.[19] As well as collecting intraoperative variables such as duration of CPB and aortic cross clamp time, a novel aspect of this research will be to profile the metabolites in urine to assess potential metabolic biomarkers and pathway changes. Our research will recruit patients in a National Centre for Paediatric Cardiac Surgery, where 40 open cardiac surgeries are performed on infants annually. We therefore anticipate active recruitment will ensure the proposed target sample of 50 participants is achievable. No additional invasive procedures will be required for sample collection, enhancing the acceptability of the research for consenting parents or

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2  
3 carers. This project will investigate the subdivisions of the gut microbiota of infants with  
4 CHD, and environmental factors such as the influence of mode of delivery, pre-operative  
5 fasting states and mode of feeding, and use of pre-operative antibiotics. Understanding the  
6 status of the intestinal microbiome of infants with CHD and the effects of undergoing  
7 surgery with CPB is vital in informing best care practices to enhance patient outcomes.  
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## 13 **Objectives and Outcomes**

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17 The primary study objectives and outcomes are:

- 18 ● To characterise the gut microbiota composition of infants with specified CHD  
19 undergoing surgery with CPB at specific time points perioperatively.
- 20 ● To compare any differences in gut microbiota composition of infants who take part in  
21 this study at defined time points pre and post-operatively and compare with the  
22 microbiota of healthy babies from the INFANTMET study at matching time points.[19]
- 23 ● To characterise the urine metabolite profile of infants with specified CHD undergoing  
24 surgery with CPB and compare with healthy infants from the INFANTMET study.[19]

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31 Secondary objectives and outcomes:

32 To explore the influence of maternal and environmental factors on gut microbiome  
33 composition.  
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## 35 **Methods**

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39 Study design

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41 This study is a prospective cohort study of infants with CHD undergoing CPB at the National  
42 Centre for Paediatric Cardiac Surgery at Children's Health Ireland (CHI) at Crumlin, Dublin,  
43 Ireland. This single-site study will investigate the differences in the gut microbiome,  
44 metabolomics readouts, and stress levels between infants with CHD undergoing CPB and  
45 healthy age matched controls.  
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49 Participant selection

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51 This study will involve the collection of demographic and clinical maternal and infant data  
52 from infants diagnosed with CHD scheduled for surgery involving CPB. CHD is typically  
53 diagnosed antenatally with fetal echocardiography performed routinely at 20-24 weeks  
54 gestation. Any child presenting with a murmur or features of cardiac conditions, or  
55 symptoms of CHD will be diagnosed using clinical examination, including palpation,  
56 auscultation, electrocardiogram (ECG) and echocardiography. Cardiac diagnoses are  
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classified according to cardiovascular physiology, i.e., left to right shunt, cyanosis with biventricular circulation, univentricular circulation and outflow tract obstruction, as presented in table 1.

Table 1. Classification of CHD Subtypes

Group	CHD Group	CHD Subtypes
1	Left to right shunt	AVSD, VSD, large ASD
2	Cyanotic CHD with biventricular circulation	D-TGA/IVS, D-TGA/VSD
3	Cyanotic CHD with univentricular circulation	HLHS, HRHS (Tricuspid Atresia) Pulmonary Atresia
4	RVOT Obstruction	Tetralogy of Fallot, dysplastic PS
5	LVOT Obstruction	Shones Syndrome, Coarctation of Aorta, Interrupted Aortic Arch
6	Others	Ebstein Anomaly, Truncus Arteriosus

To be eligible for the study, the participants must meet the terms of the inclusion and exclusion criteria as presented in Table 2.

Table 2. Inclusion and Exclusion Criteria.

Inclusion criteria	Exclusion criteria
Infants born full term ( $\geq 37$ weeks gestation)	Stillbirth or live birth where the baby is born alive but dies shortly after
Infants diagnosed with *CHD and scheduled for surgery involving CPB	Infants who are born healthy with no underlying illness, syndrome, or chronic disease
Infants born in Ireland to allow sample follow up	Participation in another study

Ability of the participant's parent/carers (in the investigator's opinion) to comprehend the full nature and purpose of the study	Infants not undergoing surgery involving cardiopulmonary bypass
Consent to participate in the study and willingness to comply with the protocol and study restrictions by the participant's parent/carers	Infants where parents/carers do not give consent to participate in the study
	Gastrointestinal pathology or intestinal surgery, excluding gastrostomy tube

\*CHD diagnoses and subtypes are presented in Table 1.

### Public and Patient Involvement (PPI) Statement

The mother of a child who had cardiac surgery as a baby was involved in reviewing the research questions, outcome measures and study literature at the study design phase. The PPI representative did not participate in the recruitment or the conduct of the study due to competing demands and availability. The PPI representative has offered to support dissemination of the study results through their involvement in charitable foundations and child and parent support fora.

### Recruitment

Participants meeting all inclusion criteria will be selected after admission to the hospital, outpatient clinic or cardiac day unit. Study-related information will be given in written form as well as explained by a member of the project team. No study-related activities will begin before the potentially eligible participants' parents/carers have signed the Informed Consent Form (ICF). Participants parents/carers will be asked to refer to the Privacy Notice on the hospital website or they can receive a hardcopy if they wish. Signed ICFs will be stored safely in a locked cabinet in the research office.

### Compensation

No compensation will be provided to the participants. There are no cost implications for the Health Service Executive (HSE) or to the participants. The management of patients and investigative tests will comply with current standards of care.

### Study timeline

After completing recruitment procedures, i.e., determining whether the patient meets the study inclusion criteria, discussing the study with the parents/caregivers and obtaining

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2  
3 informed consent, clinical and demographic data will start to be collected. The study will be  
4 undertaken for a period of 2 years after the infant is initially recruited.  
5  
6

### 7 **Demographic Data**

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9  
10 The infants' diagnosis including whether antenatal or postnatal diagnosis, co-morbidities,  
11 date of birth, gestational age, sex, mode of delivery, Appearance, Pulse, Activity, Grimace,  
12 Respiration (APGAR) scores, birth weight, head circumference, mode and type of feeding  
13 pre and post operatively, antibiotics administered post-delivery, complications and  
14 antenatal events will be recorded (case record form located in Appendix 1).  
15  
16

### 17 **Maternal Data**

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20 Maternal history including age, antibiotics received, smoking status use of probiotics and  
21 significant antenatal events will be recorded.  
22  
23

### 24 **Surgical course**

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26  
27 The type of surgery performed, duration of CPB and cross-clamp time will be recorded.  
28 Antibiotic use and any intraoperative events will be recorded as well as clinical information  
29 including arterial blood gas data.  
30  
31

### 32 **Post-operative Data**

33  
34  
35 Paediatric Index of Mortality (PIM) score, duration of mechanical ventilation, haematology  
36 variables including haemoglobin and haematocrit, renal data e.g. blood urea nitrogen (BUN)  
37 and creatinine, fluid balance, and cardiovascular support including vasoactive inotrope  
38 score, as well as duration of stay in paediatric intensive care unit will be recorded.  
39  
40

### 41 **Feeding Information Data**

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43  
44 Feeding information including the type of feed and duration of the feed prior to and after  
45 surgery will be recorded. The date the patient is established on full feeds will be recorded.  
46 Full feeding is defined as when the patient no longer requires parenteral nutrition or  
47 intravenous fluids.  
48  
49

### 50 **Discharge Information Data**

51  
52  
53 Discharge information data: This will include the patient's status on discharge from  
54 Paediatric Intensive Care Unit (PICU), as well as length of PICU and hospital stay.  
55  
56

### 57 **Complications**

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2  
3 The occurrence of complications will be recorded, for example the development of NEC. The  
4 timeline for recording NEC onset will be based on the initiation of triple antibiotic therapy,  
5 based on a full surgical review including clinical presentation, radiological and laboratory  
6 data. In addition, the occurrence of death, rehospitalisation for heart failure or cardiac  
7 problems will be included.  
8  
9

### 10 11 **Subject withdrawal/exclusion** 12

13  
14 Under the Declaration of Helsinki, the research nurse will explain to the consenting adult  
15 that they have the right to withdraw from the study at any time and that this will in no way  
16 prejudice their future treatment. The reason for withdrawal will be recorded in the source  
17 documents and on the appropriate CRF. Consenting adults will be made aware that stored  
18 samples from individuals withdrawing from the study may have undergone processing and  
19 may be analysed in the study.  
20  
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22

### 23 24 **Regulatory procedures** 25

26 The study is conducted following the version Fortaleza, Brazil, October 2013 of the  
27 Declaration of Helsinki 1964. The Protocol and the ICF have been approved by the Clinical  
28 Research Ethics Committee of Children's Health Ireland GEN/826/20. As biological samples  
29 will be procured in one institution and sent to another, a data sharing agreement is in place  
30 between The Cardiology Department, CHI at Crumlin Hospital, and APC Microbiome Ireland,  
31 in Cork. This research is fully compliant with the guidelines as set out in The General Data  
32 Protection Regulation (GDPR), the Irish Data Protection Acts 1988 to 2018 including  
33 Protection Act 2018 (Section 36(2)) (Health Research) Regulations 2018.  
34  
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37

### 38 39 **Data Statement** 40

41 Once collected, the anonymised demographic, clinical and laboratory analysis data as well  
42 as statistical codes will be uploaded to the open access Research Repository University  
43 College Dublin.  
44  
45  
46

### 47 48 **Sample collection and analysis** 49

#### 50 51 **Faecal samples**

52 Stool samples will be collected at the following time points: within 24 hours after birth  
53 (timepoint (TP) 1), within 24 hrs pre surgery (TP 2), 1 week post-surgery (TP 3) 4 weeks post-  
54 surgery (TP 4), 24 weeks post-surgery (TP 5) at 52 weeks (TP 6) and 2 years of age (TP 7).  
55 Information about antibiotic therapy administered before or during the stool collection will  
56 be recorded.  
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3 As the study site is the National Centre for Paediatric Cardiology, all infants diagnosed with  
4 CHD antenatally are transferred to the study site from the Maternity Unit for management  
5 of CHD. The first study stool specimen is typically obtained after the infant is transferred to  
6 the study site and informed consent obtained, which is typically within 24 hours of delivery.  
7  
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10 The sample will be collected by the bedside nurse or the parent/carers and transferred to  
11 the laboratory upon receipt of the sample during the weekdays or weekends. At night, the  
12 sample will be kept in the dedicated fridge and transferred to the laboratory within 4-5  
13 hours for appropriate storage at -80°C until further analysis. A standard operating procedure  
14 (SOP) for sample collection when participant is no longer an in-patient is provided in  
15 Appendix 2.  
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### 19 Urine Samples

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22 Testing the urinary metabolomics of study participants will allow the potential identification of  
23 altered metabolomic profiles, and explore the role of microbiota in such alterations. Mirroring  
24 the INFANTMET study, urine samples will be collected at 4-8 weeks post-surgery for  
25 metabolomic analysis using Sterisets Uricol Urine Collection Pack (MedGuard, Ireland). The  
26 urine sample will be collected from the urinary catheter if the participant is catheterised.  
27 Alternatively, a pad will be placed in the diaper and used to collect an unsoiled urine sample  
28 from the infant. The pad will then be placed in a biohazard bag and frozen immediately at -  
29 80 °C prior to processing. After all the sample collections are complete, they will be shipped  
30 to Teagasc Food Research, Moorepark, Ireland, using DHL overnight service for microbiome  
31 and metabolomics analyses. Styrofoam Saf-T-Pak STP-309 shipper box or equivalent will be  
32 used. DNA extraction will be performed on stool samples using the modification of the  
33 Repeated Bead Beating Plus Column (RBB+C) method.[20] LC-MS will be utilised for  
34 metabolomics analysis of urine.[19]  
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### 41 Sample collection for discharged participant

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44 Parents/carers will receive a sample collection discharge pack and a parent diary/instruction  
45 served as reminder to collect due samples at different time-points prior to discharge home.  
46 They will receive a follow up text message or phone call to remind them on the due sample.  
47 The sample collection discharge pack consists of urine/stool collection containers with study  
48 code, sterile pad, syringe, zip-lock bag, gloves, biohazard bag and cooler bag. Parents/carers  
49 are asked to keep the collected sample at the dedicated section of their home freezer. They  
50 will transport the collected sample in the cooler bag provided when attending out-patient  
51 department for appointments. They will ask a member of the project team to transfer the  
52 collected sample to the laboratory upon arrival at the hospital. The study researcher is  
53 available at the dedicated contact number for any queries.  
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### 59 Adverse events and participant well-being



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3 There are no expected safety concerns related to the study. All study participants will be  
4 under the care of the cardiology team at the hospital with access to psychological support,  
5 as well as nursing and medical professionals, social workers and chaplaincy.  
6  
7

### 8 **Data collection and management**

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11 The study diaries, study dataset, and paper/digital CRF systems will be used for recording  
12 data from each study participant. All the data collected in this study is pseudonymised, as  
13 each of the participants will be assigned a specific study code and upon receipt, data will be  
14 referred to the study code. All study staff responsible for entering data into the CRF system  
15 received training in advance of the study commencement. This training included familiarity  
16 with the study diaries, study dataset, and paper/digital CRF system and have completed  
17 good clinical practice (GCP) in research training. They have individual access to the password  
18 protected study shared drive within the hospital. The study team will monitor the  
19 data/sample collection process. Any inconsistencies identified during the study will be  
20 presented as queries at the regular project team meeting.  
21  
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### 26 **Comparison Data**

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29 Data collected as part of the INFANTMET Study will serve as a healthy control comparison  
30 for this study. INFANTMET compared the gut microbiota development of breastfed infants  
31 born via C-section or vaginally at full-term or preterm at Cork University Maternity  
32 Hospital. Ethical approval for sample collection by Cork University Hospital Research Ethics  
33 Committee (reference number ECM(w) 07/02/2012). One hundred and ninety two infants  
34 were recruited to the INFANTMET study and stratified according to delivery mode and  
35 gestational age at birth. Faecal samples were collected from the infants at 1, 4, 8 and 24  
36 weeks of age and year 1, 2 and 4 of life and stored under controlled conditions. Urine  
37 samples were collected at 4 weeks of age for metabolomic analysis and stored in a freezer  
38 at -80°C prior to processing. Samples were analysed in accordance with the analysis  
39 proposed for the GuMIBear study. Although INFANTMET study participants did not have  
40 CHD, they nonetheless serve as a valuable comparison group. The stool and urine samples  
41 collected as part of the GuMIBear study will be as closely time matched as possible to the  
42 INFANTMET study samples to capture the major developmental period of the early life gut  
43 microbiota.  
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### 51 **Bioinformatics and Statistical analysis**

#### 52 **Sample size justification**

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56 Published research in this area is lacking. However one case control study by Salomon et al.  
57 (2021) included 17 cases and 12 control participants and was sufficiently powered to  
58 determine a statistically significant difference in beta diversity in cases versus controls  
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3 (F=5.6, p<0.001). Our study proposes to include 50 patients with CHD undergoing surgery  
4 with CPB and age matched controls, almost three times the Solomon et al. (2021) study. We  
5 therefore anticipate that the proposed sample size is justified.  
6  
7

## 8 Demographic and Clinical Data 9

10  
11 Demographic and clinical data and laboratory information will be tested for normality using  
12 the Shapiro-Wilk test. Descriptive statistics will be used to describe normally distributed  
13 data, and expressed as mean  $\pm$  standard deviation (SD). Continuous data not normally  
14 distributed will be reported as median and interquartile ranges. Categorical variables will be  
15 expressed as counts and percentages. Groups will be compared using chi square tests for  
16 categorical variables and independent-samples student's *t*- tests for normally distributed  
17 continuous variables. For variables not normally distributed, the Mann-Whitney *U* test will  
18 be used. Comparison will include subgroup analysis of participants who experienced post-  
19 operative complications including NEC with those that did not. Comparisons will also include  
20 cyanotic versus acyanotic heart disease subgroup analysis, as well as mode and type of  
21 feeding pre and post-operatively.  
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## 28 Microbiome analysis 29

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31 Metagenomic shotgun sequencing data will be analysed using bioBakery suite of tools  
32 ([https://huttenhower.sph.harvard.edu/biobakery\\_workflows/](https://huttenhower.sph.harvard.edu/biobakery_workflows/)). Trimmed and human reads  
33 filtered using KneadData (v0.7.2) with the default parameters. Quality controlled data will  
34 be taxonomically profiled at the species level with relative abundance by MetaPhlAn2.  
35 Functional profiling will be performed using HUMAnN3 and strain profiling using  
36 StrainPhlAn.  
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41 For alpha diversity analysis, samples will be rarefied to even depth and phyloseq::estimate  
42 richness will be used to calculate Chao1, Shannon and Simpson indices. Alpha diversity  
43 indices between groups will be univariately compared using the Wilcoxon rank sum test. A  
44 beta-diversity ordination will be generated using the Aitchison distance and visualised using  
45 Principal Component Analysis (PCA) plot. The Adonis function in the vegan package will be  
46 used to implement a permutational multivariate analysis of variance to test whether  
47 samples cluster beyond that expected by sampling variability. MaAsLin2 (Multivariate  
48 Associations with Linear Models) will be used to investigate multivariable associations  
49 between sequencing data and clinical metadata. MaAsLin2 performs boosted, additive,  
50 linear models to detect associations while adjusting for confounding factors. Sparse  
51 canonical correlation analysis (sCCA) will be used to calculate the overall correlation  
52 between metabolites and microbes, and to identify strongly associated biomarkers. Pairwise  
53 spearman rank correlation analysis will also be performed.  
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## 60 Discussion

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3 Despite an increasing awareness of the early-life critical window of microbiome  
4 development on the health and wellness of infants, there remains much to learn about the  
5 interactions of the microbiome with the infant host with CHD undergoing surgery involving  
6 CPB. This study is designed to address this knowledge gap, and incorporates a sound  
7 methodology with particular strengths enhancing the value of its findings. The specimen  
8 collection strategy occurring at multiple time points over a 2 year period in the proposed  
9 study will deepen our understanding of the temporal dynamics of the colonising microbiota,  
10 and their interactions with host physiology. The study design will account for maternal and  
11 perioperative variables to determine changes to the microbiome. Access to existing  
12 microbiome data from healthy age matched infants provides a valuable opportunity to  
13 present high quality comparative information. A limitation of this study may include the  
14 failure to recruit infants with CHD not identified antenatally, despite active fetal screening  
15 services. While multi-centre trials capturing sufficient case numbers of NEC cases will offer  
16 robust conclusions, this study will offer valuable evidence to support the influence of CHD  
17 and CPB on the microbiome and intestinal epithelial barrier dysfunction (EBD). Future  
18 research can build on existing studies, and explore treatment strategies including  
19 recommendations for efficacious probiotic strain administration, including the supplements  
20 to promote a diverse gut microbiota to improve outcomes for this vulnerable population.  
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### 30 Ethics and Dissemination

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32 This research study is ethically approved (REC REF No: GEN/826/20). Study results will be  
33 available to patients with CHD and their families, carers, support networks, paediatric  
34 cardiology and microbiome societies and other researchers. Study findings will provide a  
35 deeper understanding of the gut microbiota of infants with CHD and inform perioperative  
36 management options including strategies to prioritise the integrity of the gut microbiota.  
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### 42 Status of Study

43  
44 The trial is ongoing and as of 5<sup>th</sup> February 2023 , 84 % of the participants have been  
45 recruited. Laboratory analysis has been carried out on 25% of study samples.  
46  
47

### 48 Funding Statement

49  
50 This research received no specific grant from any funding agency in the public, commercial  
51 or not-for-profit sectors.  
52  
53

### 54 Competing interests

55  
56 The authors declare no association with commercial entities, either financial or non-  
57 financial.  
58  
59  
60

## Author contributions

CM, CJMcM, CS, KM devised the project, and the main conceptual ideas. CJMcM, CM, CS, RPR, FK, DJ, MHT were involved in the study design and writing of the manuscript. MOT, JB, NM, SD, CJMcM are involved in consenting participants, collecting samples and acquiring data. DJ, CS, FK and RPR are responsible for analysing study samples. All authors read manuscript revisions, approved the final manuscript and accept accountability for the accuracy and integrity of the work.

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## Appendix 1: Case Report Form PDF Version

Appendix 2: SOP for Obtaining a Stool Sample when Participant is no longer Inpatient

## References

1. Skillington O MS, Gupta A, Mayer EA, Gill CIR, Del Rio D, O'Riordan KJ, Cryan JF, Ross RP and Stanton C The contrasting human gut microbiota in early and late life and implications for host health and disease. *Nutrition and Healthy Ageing*. 2012;6:157-78.
2. Cilieborg MS, Boye M, Sangild PT. Bacterial colonization and gut development in preterm neonates. *Early human development*. 2012;88 Suppl 1:S41-9.
3. Healy DB, Ryan CA, Ross RP, Stanton C, Dempsey EM. Clinical implications of preterm infant gut microbiome development. *Nature microbiology*. 2022;7(1):22-33.
4. Ohland CL, Jobin C. Microbial activities and intestinal homeostasis: A delicate balance between health and disease. *Cellular and molecular gastroenterology and hepatology*. 2015;1(1):28-40.
5. Cabrera-Perez J, Badovinac VP, Griffith TS. Enteric immunity, the gut microbiome, and sepsis: Rethinking the germ theory of disease. *Experimental biology and medicine*. 2017;242(2):127-39.
6. Halter J, Steinberg J, Fink G, Lutz C, Picone A, Maybury R, et al. Evidence of systemic cytokine release in patients undergoing cardiopulmonary bypass. *The journal of extra-corporeal technology*. 2005;37(3):272-7.
7. Centers for Disease Control and Prevention (CDC). Data and Statistics on Congenital Heart Defects. 2022 [updated January 24th 2022;26th July 2022]. Available from: <https://www.cdc.gov/ncbddd/heartdefects/data.html> Accessed 26<sup>th</sup> July 2022.
8. van der Linde D, Konings E, Slager M, Witsenburg M, Helbing W, Takkenburg J, Roos-Hesselink J. Birth Prevalence of Congenital Heart Disease Worldwide: A Systematic Review and Meta-Analysis. *Journal of the American College of Cardiology*. 2011;58(21):2241-2247.
9. Martin R, Makino H, Cetinyurek Yavuz A, Ben-Amor K, Roelofs M, Ishikawa E, et al. Early-Life Events, Including Mode of Delivery and Type of Feeding, Siblings and Gender, Shape the Developing Gut Microbiota. *PloS one*. 2016;11(6):e0158498.
10. Siano E, Lauriti G, Ceccanti S, Zani A. Cardiogenic Necrotizing Enterocolitis: A Clinically Distinct Entity from Classical Necrotizing Enterocolitis. *European journal of pediatric surgery : official*

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3 journal of Austrian Association of Pediatric Surgery. 2019;29(1):14-22.

4 11. Kelleher ST, McMahon CJ, James A. Necrotizing Enterocolitis in Children with Congenital  
5 Heart Disease: A Literature Review. *Pediatric cardiology*. 2021;42(8):1688-99.

6 12. Murphy K, O'Donovan AN, Caplice NM, Ross RP, Stanton C. Exploring the Gut Microbiota and  
7 Cardiovascular Disease. *Metabolites*. 2021;11(8).

8 13. Carlo WF, Kimball TR, Michelfelder EC, Border WL. Persistent diastolic flow reversal in  
9 abdominal aortic Doppler-flow profiles is associated with an increased risk of necrotizing  
10 enterocolitis in term infants with congenital heart disease. *Pediatrics*. 2007;119(2):330-5.

11 14. McElhinney DB, Hedrick HL, Bush DM, Pereira GR, Stafford PW, Gaynor JW, et al. Necrotizing  
12 enterocolitis in neonates with congenital heart disease: risk factors and outcomes. *Pediatrics*.  
13 2000;106(5):1080-7.

14 15. Miller TA, Minich LL, Lambert LM, Joss-Moore L, Puchalski MD. Abnormal abdominal aorta  
15 hemodynamics are associated with necrotizing enterocolitis in infants with hypoplastic left heart  
16 syndrome. *Pediatric cardiology*. 2014;35(4):616-21.

17 16. Nino DF, Sodhi CP, Hackam DJ. Necrotizing enterocolitis: new insights into pathogenesis and  
18 mechanisms. *Nature reviews Gastroenterology & hepatology*. 2016;13(10):590-600.

19 17. Typpo KV, Larmonier CB, Deschenes J, Redford D, Kiela PR, Ghishan FK. Clinical  
20 characteristics associated with postoperative intestinal epithelial barrier dysfunction in children with  
21 congenital heart disease. *Pediatric critical care medicine : a journal of the Society of Critical Care  
22 Medicine and the World Federation of Pediatric Intensive and Critical Care Societies*. 2015;16(1):37-  
23 44.

24 18. Salomon J, Ericsson A, Price A, Manithody C, Murry DJ, Chhonker YS, et al. Dysbiosis and  
25 Intestinal Barrier Dysfunction in Pediatric Congenital Heart Disease Is Exacerbated Following  
26 Cardiopulmonary Bypass. *JACC Basic to translational science*. 2021;6(4):311-27.

27 19. Hill CJ, Lynch DB, Murphy K, Ulaszewska M, Jeffery IB, O'Shea CA, et al. Evolution of gut  
28 microbiota composition from birth to 24 weeks in the INFANTMET Cohort. *Microbiome*. 2017;5(1):4.

29 20. Yu Z, Morrisson M. Improved extraction of PCR-quality community DNA from digesta and  
30 fecal samples. *Biotechniques*. 2004;36(5):808-12.



^999 – missing data; 0 – no data in medical notes

**CASE RECORD FORM**

**Stool Sample Collections**

Period/ Time Frame	Projected Date	Sample Date	Sample Collected	Comments
1. Within 24h of Birth			<input type="checkbox"/> YES <input type="checkbox"/> NO	
2. Pre-operatively			<input type="checkbox"/> YES <input type="checkbox"/> NO	
3. Week 1 of life/Post-Op			<input type="checkbox"/> YES <input type="checkbox"/> NO	
4a. Week 4 to 8 life/Post-op			<input type="checkbox"/> YES <input type="checkbox"/> NO	
4b. <b>Urine</b> Sample Week 4 to 8			<input type="checkbox"/> YES <input type="checkbox"/> NO	
5. Week 24 of life/Post-Op			<input type="checkbox"/> YES <input type="checkbox"/> NO	
6. Week 52 of life/Post-Op			<input type="checkbox"/> YES <input type="checkbox"/> NO	
7. Year 2 of life/Post-Op			<input type="checkbox"/> YES <input type="checkbox"/> NO	

**PATIENT DEMOGRAPHIC**

Date of Enrolment:		Date of Birth:		Cardiac Classification Group No:
Gender:	<input type="checkbox"/> Male <input type="checkbox"/> Female <input type="checkbox"/> Other/Ambiguous	Gestational Age:		Multiple <input type="checkbox"/> Yes <input type="checkbox"/> No
		APGAR at ① ⑤ min	<input type="text"/> <input type="text"/>	Birth Order:
Reason for admission:				
Comorbidities:				
Mode of Delivery:		Weight at Birth: _____ . _____ KGs		
SVD: <input type="checkbox"/> Yes <input type="checkbox"/> No		Head Circumference: _____ cms		
LSCS: <input type="checkbox"/> Yes <input type="checkbox"/> No, if Yes: <input type="checkbox"/> Elective/ <input type="checkbox"/> Emergency		Timing of cardiac diagnosis:		
Antibiotics to Infant post-delivery; <input type="checkbox"/> Yes <input type="checkbox"/> No		Postnatal <input type="checkbox"/> Antenatal <input type="checkbox"/>		
If Yes, list:				
Significant Antenatal Events:				

**MATERNAL INFORMATION**

Maternal Age (years) at Birth:	Gestational Age at Booking Appt:
Antibiotics given Pre-Delivery: <input type="checkbox"/> Yes <input type="checkbox"/> No	Maternal Probiotics taken during pregnancy:
List:	<input type="checkbox"/> Yes <input type="checkbox"/> No List:
Maternal Smoking during Pregnancy: <input type="checkbox"/> Yes <input type="checkbox"/> No	Other Household Members Smoking during Pregnancy: <input type="checkbox"/> Yes <input type="checkbox"/> No



^999 – missing data; 0 – no data in medical notes

### SURGERY INFORMATION

Surgery Date:			
Surgery Performed:			
Pre-Op Antibiotics <sup>1?</sup> List:		Post-Op Antibiotics <sup>1?</sup> List:	
1. Abs <48hrs pre sampling		1. Abs <48hrs pre sampling	
2. Abx <72hrs pre sampling		2. Abx <72hrs pre sampling	
3. Abx in last 7 days/during sample		3. Abx in last 7 days/during sample	
ABG	pH	PO <sup>2</sup>	
First Pre-Op ABG:		Cardiopulmonary Bypass Duration:	
First Post-Op ABG:		Aortic Cross Clamp Duration:	
Significant Intraoperative Events:			

<sup>1</sup>Antibiotic treatment at time of stool sampling as below. Important not to include antibiotics which were started post the stool sampling

- 1) Abs < 48hrs pre sampling
- 2) Abx < 72 hrs pre sampling
- 3) Abx in previous 7 days/ during sample collection

### POST-OPERATIVE INFORMATION

PIM3 Score:		No. of days in ICU (1 <sup>st</sup> adm):	
Mechanical Vent:	<input type="checkbox"/> Yes <input type="checkbox"/> No	No of Days on ECLS:	
No of Days Vent:		No of Days on RRT:	
Agent	Day 1	Day 2	Day 3
Milrinone			
Epinephrine			
Norepinephrine			
Vasopressin			
Midazolam			
Morphine			
Clonidine			
Others			
Fluid Balance:			
BUN:			
Creatinine:			
HCT:			
Hgb:			

### FEEDING INFORMATION

Mode of Feeding (note <b>date initiated</b> and <b>date d/c</b> ):		
Breastmilk:	Infant Formula:	Other:
Prebiotics given to Infant: <input type="checkbox"/> Yes <input type="checkbox"/> No	Type and Date Given:	
Excessive Infantile Crying (cried for $\geq 3$ Hrs for 3 Days in one week): <input type="checkbox"/> Yes <input type="checkbox"/> No		



# GuMIBear Study



Study ID: \_\_\_\_\_ GMB: \_\_\_\_\_

1 ^999 – missing data; 0 – no data in medical notes

Date Trophic Feeds Commenced:		Type of Feed Used:	
Time to Establishment of full feed <sup>2</sup> :			
Development of NEC <sup>3</sup> :			
Days post-op when developed NEC?			
Gut stasis:	Not applicable		
Management Strategy:			

2 Full feed – No longer requires parenteral nutrition or intravenous fluids supplement regardless the TFI.

3 NEC – Initiation of triple IV antibiotic therapy and nil by mouth for at least 5 days, based on a full surgical review including clinical presentation, radiological and laboratory data.

## DISCHARGE INFORMATION

Date of Discharge			
Ward:	Home:	RIP:	

## READMISSION TO ICU

Total ICU Readmission days:				
	Date of Admission	Date of Discharge	Total ICU stays	Reason for admission
1.				
2.				
3.				
4.				
5.				

## DATA ENTRY BY (NAME)

Admission	Date	Paper	Date	G-Drive
First				
Second				
Third				

Participant Withdrawal from Study: <input type="checkbox"/> Yes <input type="checkbox"/> No
GCP Procedure Followed: <input type="checkbox"/> Yes <input type="checkbox"/> No
See Study Folder Appendix 4. Signed: _____ Date: _____





## The Gut Microbiota of Infants with Complex Congenital Heart Disease Undergoing Cardiopulmonary Bypass



( GuMiBear)

### STANDARD OPERATING PROCEDURE FOR STOOL SAMPLE COLLECTION AS AN OUTPATIENT

**Purpose:** To collect infant stool samples while the infant is an out-patient from CHI at Crumlin

**Objective:** To collect infant stool samples for the study in a uniform manner and under a set of conditions, so that they can be processed by the laboratories to achieve optimal results.

**Procedure:**

1. Ensure that at least one legal guardian has provided written informed consent for their infant to participate in the study and that they are happy for their infant to remain in the study.
2. Ensure that in the hospital chart of the infant that it is noted that he/she is participating in the study and contact details of the study team.
3. Ensure the parent/guardian has been supplied with a study pack containing the requisites for the collection of the stool sample
  - a. Completed labels
  - b. Disposable Gloves
  - c. Stool collection containers
  - d. Bio-hazard bags
4. Explain to the legal guardian that a minimum of a teaspoon of stool has to be collected. Explain to the legal guardian when the samples have to be collected as close as possible to the next out-patient appointment.
5. Explain to parent/guardian how to collect the sample as follows:
  - a. Have requisites for collection at the ready.(Gloves, sample container, red biohazard bag & labels)
  - b. Place appropriate label on the sample bottle.
  - c. Wear disposable gloves
  - d. Unscrew cap of sample bottle



## The Gut Microbiota of Infants with Complex Congenital Heart Disease Undergoing Cardiopulmonary Bypass



( GuMiBear)

- e. Spoon in stool sample (1 teaspoon in volume)
  - f. Screw cap on tightly
  - g. Place in red hazard bag
  - h. Remove disposable gloves
  - i. Dispose with soiled nappy
  - j. Wash hands
  - k. Place stool sample in fridge
  - l. Text research nurse that sample is ready for collection.
6. Label the sample bottles with Subject Number, Date of Birth, Initials, Sample Number, Date of Collection
  7. Ask the parent/guardian to attach the appropriate label to the sample container and immediately place container in freezer.
  8. Text the parent/guardian the night before the sample is to be collected. Meet the parent/guardian in out-patients to collect the stool sample and store the sample in the study container in the designated study freezer.
  9. Update the study spreadsheet to indicate sample has been obtained.

# BMJ Open

## A Protocol for a Prospective Cohort Study Exploring the Gut Microbiota of Infants with Congenital Heart Disease Undergoing Cardiopulmonary Bypass (the GuMiBear study)

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-067016.R3
Article Type:	Protocol
Date Submitted by the Author:	01-Mar-2023
Complete List of Authors:	<p>Magner, Claire; University College Dublin, School of Nursing, Midwifery and Health Systems          Jenkins, Dominic; Children's Health Ireland at Crumlin          Koc, Fatma; University College Cork, School of Microbiology; Teagasc Food Research Centre, Food Biosciences          Tan, Mong Hoi; Children's Health Ireland at Crumlin          O'Toole, Molly; Children's Health Ireland at Crumlin          Boyle, Jordan; Children's Health Ireland at Crumlin          Maguire, Niamh; Children's Health Ireland at Crumlin          Duignan, Sophie; Children's Health Ireland at Crumlin          Murphy, Kiera; University College Cork APC Microbiome Institute; Teagasc Food Research Centre, Food Biosciences          Ross, Paul; University College Cork College of Science Engineering and Food Science          Stanton, Catherine; University College Cork APC Microbiome Institute; Teagasc Food Research Centre          McMahon, Colin J.; Children's Health Ireland at Crumlin, Department of Paediatric Cardiology; University College Dublin School of Medicine</p>
<b>Primary Subject Heading</b>:	Cardiovascular medicine
Secondary Subject Heading:	Paediatrics
Keywords:	Congenital heart disease < CARDIOLOGY, Cardiac surgery < SURGERY, Paediatric cardiology < CARDIOLOGY, Quality in health care < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, Biochemistry < NATURAL SCIENCE DISCIPLINES

SCHOLARONE™  
Manuscripts

**Title:** A Protocol for a Prospective Cohort Study Exploring the Gut Microbiota of Infants with Congenital Heart Disease Undergoing Cardiopulmonary Bypass (the GuMiBear study)

**Authors:** Claire Magner<sup>1</sup>, Dominic Jenkins<sup>2</sup>, Fatma Koc<sup>3,4,5</sup>, Mong Hoi Tan<sup>2</sup>, Molly O'Toole<sup>2</sup>, Jordan Boyle<sup>2</sup>, Niamh Maguire<sup>2</sup>, Sophie Duignan<sup>2</sup>, Kiera Murphy<sup>4,5</sup>, R. Paul Ross<sup>4</sup>, Catherine Stanton<sup>4,5</sup>, Colin J. McMahon<sup>6</sup>

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## Abstract

### Introduction

The gut microbiota develops from birth and matures significantly during the first 24 months of life, playing a major role in infant health and development. The composition of the gut microbiota is influenced by several factors including mode of delivery, gestational age, feed type and treatment with antibiotics. Alterations in the pattern of gut microbiota development and composition can be associated with illness and compromised health outcomes.

Infants diagnosed with 'Congenital Heart Disease' (CHD) often require surgery involving cardiopulmonary bypass (CPB) early in life. The impact of this type of surgery on the integrity of the gut microbiome is poorly understood. In addition, these infants are at significant risk of developing the potentially devastating intestinal condition Necrotising Enterocolitis (NEC).

### Methods and Analysis

This study will employ a prospective cohort study methodology to investigate the gut microbiota and urine metabolome of infants with CHD undergoing surgery involving CPB. Stool and urine samples, demographic and clinical data will be collected from eligible infants based at the National Centre for Paediatric Cardiac Surgery in Ireland. Shotgun metagenome

sequencing will be performed on stool samples and urine metabolomic analysis will identify metabolic biomarkers. The impact of the underlying diagnosis, surgery involving CPB, and the influence of environmental factors will be explored. Data from healthy age matched infants from the INFANTMET study will serve as a control for this study.

#### Ethics and Dissemination

This study has received full ethical approval from the Clinical Research Ethics Committee of Children's Health Ireland GEN/826/20.

#### Abbreviations:

APGAR Score	Newborn assessment score rating: Appearance, Pulse, Activity, Grimace, Respiration
ASD	Atrial Septal Defect
AVSD	Atrioventricular Septal Defect
BUN	Blood Urea Nitrogen
CHC	Children's Heart Centre
CHD	Congenital Heart Disease
CHI	Children's Health Ireland
CPB	Cardiopulmonary Bypass
CRF	Case Report Form
D-TGA	Dextro-Transposition of the Great Arteries
EBD	Epithelial Barrier Dysfunction
ECG	Electrocardiogram
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
HLHS	Hypoplastic Left Heart Syndrome

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2		
3	HRHS	Hypoplastic Right Heart Syndrome
4		
5		
6	HSE	Health Service Executive
7		
8	ICF	Informed Consent Form
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10		
11	IV	Intravenous
12		
13		
14	IVS	Intra Ventricular Septum
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16	L-TGA	Levo-Transposition of the Great Arteries
17		
18		
19	LVOT	Left Ventricular Outflow Tract
20		
21		
22	MaAsLin2	Multivariate Associations with Linear Models
23		
24	NEC	Necrotising Enterocolitis
25		
26		
27	PCA	Principal Component Analysis
28		
29	PICU	Paediatric Intensive Care Unit
30		
31		
32	PIM3	Paediatric Index of Mortality
33		
34		
35	PPI	Public and Patient Involvement
36		
37	PS	Pulmonary Valve Stenosis
38		
39		
40	RBB+C	Repeated Beat Beating Plus Column (RBB+C) method
41		
42	RVOT	Right Ventricular Outflow Tract
43		
44		
45	sCCA	Sparse canonical correlation analysis
46		
47		
48	SD	Standard Deviation
49		
50	SOP	Standard Operating Procedure
51		
52		
53	TGA	Transposition of the Great Arteries
54		
55		
56	TP	Time Point
57		
58	VSD	Ventricular Septal Defect
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60		

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6 **Keywords:** Congenital Heart disease, Microbiota, Infant, Antibiotic, Development, Cardiac  
7 Surgery, Cardiopulmonary Bypass  
8

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10 Word Count: 3,887  
11

## 12 **Article Summary**

### 13 **Strengths and Limitations of this Study**

- 14 ● The GuMIBear study will collect microbiome data from children with CHD at 7 time  
15 points over a 2 year timeframe
  - 16 ● The study design incorporates comparison with age matched healthy controls,  
17 allowing insights into clinically relevant microbiome alterations throughout and  
18 beyond the surgical course
  - 19 ● The study involves urine metabolomic analysis providing novel insights into the  
20 metabolite profile of study participants compared with healthy age matched infants
  - 21 ● The primary limitation of GuMIBear is that it is a single centre study limiting the  
22 generalisability of the findings
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## 34 **Introduction**

### 35 **What is currently known**

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39 The establishment of gut microbiota begins at birth and continues over the first years of life.  
40 Continued evolution of the gut microbiome after birth is governed by host factors such as  
41 both the adaptive and innate immune system, as well as external factors such as diet,  
42 medication and toxin exposure, and illness.[1] Understanding the role of the gut  
43 microbiome in metabolism, immune function and nutrition is gaining increasing recognition,  
44 as it is accepted that an altered colonisation has been associated with a higher risk of  
45 disease later in life.[2] In the critical first weeks and months of life, perturbations to the  
46 infant gut microbiome have implications for growth development and health.[3]  
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### 55 **The Microbiome and Systemic Inflammation**

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3 It is evident that under certain conditions, disruption of the normal microbiota that colonise  
4 the intestinal tract can occur.[4] These conditions include systemic inflammatory processes,  
5 which can result in intestinal inflammation, where proinflammatory bacteria can flourish,  
6 interacting with the intestinal epithelium to cause cytokine release, activating key  
7 inflammatory pathways increasing morbidity and prolonging critical illness.[5] The pattern  
8 of cytokine release in patients undergoing CPB is described as comparable to those released  
9 in systemic inflammation such as trauma and sepsis.[6] However, the nature of gut  
10 microbiota compositional changes in infants undergoing surgery with CPB remains  
11 understudied. This research aims to address this knowledge gap to enhance our  
12 understanding and inform care practices.  
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### 23 CHD and Necrotising Enterocolitis (NEC)

24 CHD affects approximately 1 in every 100 babies born throughout the US every year.[7] It is  
25 the most common congenital defect worldwide.[8] A diagnosis of 'Complex Congenital  
26 Heart Disease' can include conditions such as Hypoplastic Left Heart Syndrome (HLHS);  
27 Hypoplastic Right Heart Syndrome (HRHS), Transposition of the Great Arteries (TGA)  
28 requiring intervention in the first week of life, while CHD such as Atrioventricular Septal  
29 Defect (AVSD), Tetralogy of Fallot may require corrective surgery in the first few months of  
30 life. CHD requiring surgery involving cardiopulmonary bypass (CPB) present a greater risk to  
31 patients. This increased risk is not limited directly to the surgery, compromised ventricular  
32 function or low cardiac output state, but includes the risk of developing NEC.  
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44 There is a well-established connection between CHD and NEC, a potentially devastating  
45 intestinal condition of infancy. [9] NEC carries a reported incidence of between 3 and 9% in  
46 infants with CHD with all-cause mortality rates as high as 38% in children with 'cardiogenic'  
47 NEC.[10] While CHD remains one of the most common risk factors for NEC, the underlying  
48 pathophysiology of this association is complex. [11] Growing evidence suggests that  
49 perturbations in the early-life gut microbiota composition increase the risk for NEC. [3,12] A  
50 significant association between episodes of low cardiac output and shock in the  
51 development of NEC is recognised. [13, 14] It is reported that infants with certain types of  
52 CHD, mainly HLHS, may possess abnormal systemic vasculature contributing to the  
53 increased risk for NEC. [15] Whether it is those, or other causes of impaired perfusion to the  
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3 gut, the resulting damage to the mucosal barrier can provide an entry point to bacteria  
4 provoking an inflammatory cascade, and the devastating consequences that can ensue.[16]  
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6 The vulnerability of infants with CHD is enhanced during the course of surgical intervention  
7 involving CPB (11), and the role of the gut microbiome has received little research focus in  
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9 this context.  
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#### 14 Surgery involving CPB

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18 Infants diagnosed with CHD are at risk of alterations to their intestinal homeostasis, a  
19 further threat is presented in the context of surgery involving CPB. [11] There is evidence to  
20 suggest intestinal ischaemia reperfusion injury occurs after CPB and contributes to epithelial  
21 barrier dysfunction (EBD) potentially exposing the bloodstream to bacteria or bacterial  
22 products.[17] Although alterations in gut barrier integrity and resident microbiota have  
23 been demonstrated.[3] it is not fully understood what changes to the microbiome occur  
24 following CPB, and the nature and severity of EBD. While the gut microbiota in infants with  
25 CHD following CPB remains understudied, a small single centre case control study recently  
26 identified significant gut microbiota perturbations in patients with CHD.[18] This case-  
27 control study highlighted that children with CHD had a disrupted gut microbiome at baseline  
28 with an over-representation of pro-inflammatory bacteria, this was further exacerbated by  
29 CPB. Samples were collected pre-operatively and in a limited 24 and 48 hour time frame  
30 postoperatively. The significance of intraoperative variables including aortic cross clamp  
31 time and duration of CPB was not determined.  
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45 Our study proposes to address the knowledge gap and advance existing research by  
46 examining the gut microbiome of infants with CHD pre-operatively, and at defined time  
47 points up to 2 years of life/ postoperatively. This timeline will account for the recovery  
48 phase post cardiac surgery, including time to re-establish full feeds, wean from mechanical  
49 ventilation and circulatory support, and allow for surveillance of outcome measures  
50 including NEC, repeat surgery, and mortality postoperatively. Comparisons will be made  
51 with healthy age-matched infants recruited as part of the INFANTMET study.[19] As well as  
52 collecting intraoperative variables such as duration of CPB and aortic cross clamp time, a  
53 novel aspect of this research will be to profile the metabolites in urine to assess potential  
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3 metabolic biomarkers and pathway changes. Our research will recruit patients in a National  
4 Centre for Paediatric Cardiac Surgery, where 40 open cardiac surgeries are performed on  
5 infants annually. We therefore anticipate active recruitment will ensure the proposed target  
6 sample of 50 participants is achievable. No additional invasive procedures will be required  
7 for sample collection, enhancing the acceptability of the research for consenting parents or  
8 carers. This project will investigate the subdivisions of the gut microbiota of infants with  
9 CHD, and environmental factors such as the influence of mode of delivery, pre-operative  
10 fasting states and mode of feeding, and use of pre-operative antibiotics. Understanding the  
11 status of the intestinal microbiome of infants with CHD and the effects of undergoing  
12 surgery with CPB is vital in informing best care practices to enhance patient outcomes.  
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### 23 **Objectives and Outcomes**

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26 The primary study objectives and outcomes are:

- 27 ● To characterise the gut microbiota composition of infants with specified CHD  
28 undergoing surgery with CPB at specific time points perioperatively.
  - 29 ● To compare any differences in gut microbiota composition of infants who take part in  
30 this study at defined time points pre and post-operatively and compare with the  
31 microbiota of healthy babies from the INFANTMET study at matching time points.[19]
  - 32 ● To characterise the urine metabolite profile of infants with specified CHD undergoing  
33 surgery with CPB and compare with healthy infants from the INFANTMET study.[19]
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40 Secondary objectives and outcomes:

41 To explore the influence of maternal and environmental factors on gut microbiome  
42 composition.  
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### 45 **Methods**

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48 Study design

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50 This study is a prospective cohort study of infants with CHD undergoing CPB at the National  
51 Centre for Paediatric Cardiac Surgery at Children's Health Ireland (CHI) at Crumlin, Dublin,  
52 Ireland. This single-site study will investigate the differences in the gut microbiome,  
53 metabolomics readouts, and stress levels between infants with CHD undergoing CPB and  
54 healthy age matched controls.  
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58 Participant selection  
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This study will involve the collection of demographic and clinical maternal and infant data from infants diagnosed with CHD scheduled for surgery involving CPB. CHD is typically diagnosed antenatally with fetal echocardiography performed routinely at 20-24 weeks gestation. Any child presenting with a murmur or features of cardiac conditions, or symptoms of CHD will be diagnosed using clinical examination, including palpation, auscultation, electrocardiogram (ECG) and echocardiography. Cardiac diagnoses are classified according to cardiovascular physiology, i.e., left to right shunt, cyanosis with biventricular circulation, univentricular circulation and outflow tract obstruction, as presented in table 1.

Table 1. Classification of CHD Subtypes

Group	CHD Group	CHD Subtypes
1	Left to right shunt	AVSD, VSD, large ASD
2	Cyanotic CHD with biventricular circulation	D-TGA/IVS, D-TGA/VSD
3	Cyanotic CHD with univentricular circulation	HLHS, HRHS (Tricuspid Atresia) Pulmonary Atresia
4	RVOT Obstruction	Tetralogy of Fallot, dysplastic PS
5	LVOT Obstruction	Shones Syndrome, Coarctation of Aorta, Interrupted Aortic Arch
6	Others	Ebstein Anomaly, Truncus Arteriosus

To be eligible for the study, the participants must meet the terms of the inclusion and exclusion criteria as presented in Table 2.

Table 2. Inclusion and Exclusion Criteria.

Inclusion criteria	Exclusion criteria
Infants born full term ( $\geq 37$ weeks gestation)	Stillbirth or live birth where the baby is born alive but dies shortly after

Infants diagnosed with *CHD and scheduled for surgery involving CPB	Infants who are born healthy with no underlying illness, syndrome, or chronic disease
Infants born in Ireland to allow sample follow up	Participation in another study
Ability of the participant's parent/carers (in the investigator's opinion) to comprehend the full nature and purpose of the study	Infants not undergoing surgery involving cardiopulmonary bypass
Consent to participate in the study and willingness to comply with the protocol and study restrictions by the participant's parent/carers	Infants where parents/carers do not give consent to participate in the study
	Gastrointestinal pathology or intestinal surgery, excluding gastrostomy tube

\*CHD diagnoses and subtypes are presented in Table 1.

### Public and Patient Involvement (PPI) Statement

The mother of a child who had cardiac surgery as a baby was involved in reviewing the research questions, outcome measures and study literature at the study design phase. The PPI representative did not participate in the recruitment or the conduct of the study due to competing demands and availability. The PPI representative has offered to support dissemination of the study results through their involvement in charitable foundations and child and parent support fora.

### Recruitment

Participants meeting all inclusion criteria will be selected after admission to the hospital, outpatient clinic or cardiac day unit. Study-related information will be given in written form as well as explained by a member of the project team. No study-related activities will begin before the potentially eligible participants' parents/carers have signed the Informed Consent Form (ICF). Participants parents/carers will be asked to refer to the Privacy Notice on the hospital website or they can receive a hardcopy if they wish. Signed ICFs will be stored safely in a locked cabinet in the research office.

### Compensation

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3 No compensation will be provided to the participants. There are no cost implications for the  
4 Health Service Executive (HSE) or to the participants. The management of patients and  
5 investigative tests will comply with current standards of care.  
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### 8 **Study timeline**

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11 After completing recruitment procedures, i.e., determining whether the patient meets the  
12 study inclusion criteria, discussing the study with the parents/caregivers and obtaining  
13 informed consent, clinical and demographic data will start to be collected. The study will be  
14 undertaken for a period of 2 years after the infant is initially recruited.  
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### 17 **Demographic Data**

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20 The infants' diagnosis including whether antenatal or postnatal diagnosis, co-morbidities,  
21 date of birth, gestational age, sex, mode of delivery, Appearance, Pulse, Activity, Grimace,  
22 Respiration (APGAR) scores, birth weight, head circumference, mode and type of feeding  
23 pre and post operatively, antibiotics administered post-delivery, complications and  
24 antenatal events will be recorded (case record form located in Appendix 1).  
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### 28 **Maternal Data**

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31 Maternal history including age, antibiotics received, smoking status use of probiotics and  
32 significant antenatal events will be recorded.  
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### 35 **Surgical course**

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38 The type of surgery performed, duration of CPB and cross-clamp time will be recorded.  
39 Antibiotic use and any intraoperative events will be recorded as well as clinical information  
40 including arterial blood gas data.  
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### 43 **Post-operative Data**

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46 Paediatric Index of Mortality (PIM) score, duration of mechanical ventilation, haematology  
47 variables including haemoglobin and haematocrit, renal data e.g. blood urea nitrogen (BUN)  
48 and creatinine, fluid balance, and cardiovascular support including vasoactive inotrope  
49 score, as well as duration of stay in paediatric intensive care unit will be recorded.  
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### 52 **Feeding Information Data**

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55 Feeding information including the type of feed and duration of the feed prior to and after  
56 surgery will be recorded. The date the patient is established on full feeds will be recorded.  
57 Full feeding is defined as when the patient no longer requires parenteral nutrition or  
58 intravenous fluids.  
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### **Discharge Information Data**

Discharge information data: This will include the patient's status on discharge from Paediatric Intensive Care Unit (PICU), as well as length of PICU and hospital stay.

### **Complications**

The occurrence of complications will be recorded, for example the development of NEC. The timeline for recording NEC onset will be based on the initiation of triple antibiotic therapy, based on a full surgical review including clinical presentation, radiological and laboratory data. In addition, the occurrence of death, rehospitalisation for heart failure or cardiac problems will be included.

### **Subject withdrawal/exclusion**

Under the Declaration of Helsinki, the research nurse will explain to the consenting adult that they have the right to withdraw from the study at any time and that this will in no way prejudice their future treatment. The reason for withdrawal will be recorded in the source documents and on the appropriate CRF. Consenting adults will be made aware that stored samples from individuals withdrawing from the study may have undergone processing and may be analysed in the study.

### **Regulatory procedures**

The study is conducted following the version Fortaleza, Brazil, October 2013 of the Declaration of Helsinki 1964. The Protocol and the ICF have been approved by the Clinical Research Ethics Committee of Children's Health Ireland GEN/826/20. As biological samples will be procured in one institution and sent to another, a data sharing agreement is in place between The Cardiology Department, CHI at Crumlin Hospital, and APC Microbiome Ireland, in Cork. This research is fully compliant with the guidelines as set out in The General Data Protection Regulation (GDPR), the Irish Data Protection Acts 1988 to 2018 including Protection Act 2018 (Section 36(2)) (Health Research) Regulations 2018.

#### **Data Statement**

Once collected, the anonymised demographic, clinical and laboratory analysis data as well as statistical codes will be uploaded to the open access Research Repository University College Dublin.

### **Sample collection and analysis**

#### **Faecal samples**

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3 Stool samples will be collected at the following time points: within 24 hours after birth  
4 (timepoint (TP) 1), within 24 hrs pre surgery (TP 2), 1 week post-surgery (TP 3) 4 weeks post-  
5 surgery (TP 4), 24 weeks post-surgery (TP 5) at 52 weeks (TP 6) and 2 years of age (TP 7).  
6 Information about antibiotic therapy administered before or during the stool collection will  
7 be recorded.  
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11 As the study site is the National Centre for Paediatric Cardiology, all infants diagnosed with  
12 CHD antenatally are transferred to the study site from the Maternity Unit for management  
13 of CHD. The first study stool specimen is typically obtained after the infant is transferred to  
14 the study site and informed consent obtained, which is typically within 24 hours of delivery.  
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18 The sample will be collected by the bedside nurse or the parent/carers and transferred to  
19 the laboratory upon receipt of the sample during the weekdays or weekends. At night, the  
20 sample will be kept in the dedicated fridge and transferred to the laboratory within 4-5  
21 hours for appropriate storage at -80°C until further analysis. A standard operating procedure  
22 (SOP) for sample collection when participant is no longer an in-patient is provided in  
23 Appendix 2.  
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## 27 28 Urine Samples

29  
30 Testing the urinary metabolomics of study participants will allow the potential identification of  
31 altered metabolomic profiles, and explore the role of microbiota in such alterations. Mirroring  
32 the INFANTMET study, urine samples will be collected at 4-8 weeks post-surgery for  
33 metabolomic analysis using Sterisets Uricol Urine Collection Pack (MedGuard, Ireland). The  
34 urine sample will be collected from the urinary catheter if the participant is catheterised.  
35 Alternatively, a pad will be placed in the diaper and used to collect an unsoiled urine sample  
36 from the infant. The pad will then be placed in a biohazard bag and frozen immediately at -  
37 80 °C prior to processing. After all the sample collections are complete, they will be shipped  
38 to Teagasc Food Research, Moorepark, Ireland, using DHL overnight service for microbiome  
39 and metabolomics analyses. Styrofoam Saf-T-Pak STP-309 shipper box or equivalent will be  
40 used. DNA extraction will be performed on stool samples using the modification of the  
41 Repeated Bead Beating Plus Column (RBB+C) method.[20] LC-MS will be utilised for  
42 metabolomics analysis of urine.[19]  
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## 50 Sample collection for discharged participant

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52 Parents/carers will receive a sample collection discharge pack and a parent diary/instruction  
53 served as reminder to collect due samples at different time-points prior to discharge home.  
54 They will receive a follow up text message or phone call to remind them on the due sample.  
55 The sample collection discharge pack consists of urine/stool collection containers with study  
56 code, sterile pad, syringe, zip-lock bag, gloves, biohazard bag and cooler bag. Parents/carers  
57 are asked to keep the collected sample at the dedicated section of their home freezer. They  
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3 will transport the collected sample in the cooler bag provided when attending out-patient  
4 department for appointments. They will ask a member of the project team to transfer the  
5 collected sample to the laboratory upon arrival at the hospital. The study researcher is  
6 available at the dedicated contact number for any queries.  
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### 10 **Adverse events and participant well-being**

11  
12 There are no expected safety concerns related to the study. All study participants will be  
13 under the care of the cardiology team at the hospital with access to psychological support,  
14 as well as nursing and medical professionals, social workers and chaplaincy.  
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### 18 **Data collection and management**

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20 The study diaries, study dataset, and paper/digital CRF systems will be used for recording  
21 data from each study participant. All the data collected in this study is pseudonymised, as  
22 each of the participants will be assigned a specific study code and upon receipt, data will be  
23 referred to the study code. All study staff responsible for entering data into the CRF system  
24 received training in advance of the study commencement. This training included familiarity  
25 with the study diaries, study dataset, and paper/digital CRF system and have completed  
26 good clinical practice (GCP) in research training. They have individual access to the password  
27 protected study shared drive within the hospital. The study team will monitor the  
28 data/sample collection process. Any inconsistencies identified during the study will be  
29 presented as queries at the regular project team meeting.  
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### 36 **Comparison Data**

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38 Data collected as part of the INFANTMET Study will serve as a healthy control comparison  
39 for this study. INFANTMET compared the gut microbiota development of breastfed infants  
40 born via C-section or vaginally at full –term or preterm at Cork University Maternity  
41 Hospital. Ethical approval for sample collection by Cork University Hospital Research Ethics  
42 Committee (reference number ECM(w) 07/02/2012). One hundred and ninety two infants  
43 were recruited to the INFANTMET study and stratified according to delivery mode and  
44 gestational age at birth. Faecal samples were collected from the infants at 1, 4, 8 and 24  
45 weeks of age and year 1, 2 and 4 of life and stored under controlled conditions. Urine  
46 samples were collected at 4 weeks of age for metabolomic analysis and stored in a freezer  
47 at -80°C prior to processing. Samples were analysed in accordance with the analysis  
48 proposed for the GuMIBear study. Although INFANTMET study participants did not have  
49 CHD, they nonetheless serve as a valuable comparison group. The stool and urine samples  
50 collected as part of the GuMIBear study will be as closely time matched as possible to the  
51 INFANTMET study samples to capture the major developmental period of the early life gut  
52 microbiota.  
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## Bioinformatics and Statistical analysis

### Sample size justification

Published research in this area is lacking. However one case control study by Salomon et al. (2021) included 17 cases and 12 control participants and was sufficiently powered to determine a statistically significant difference in beta diversity in cases versus controls ( $F=5.6$ ,  $p<0.001$ ). Our study proposes to include 50 patients with CHD undergoing surgery with CPB and age matched controls, almost three times the Solomon et al. (2021) study. We therefore anticipate that the proposed sample size is justified.

### Demographic and Clinical Data

Demographic and clinical data and laboratory information will be tested for normality using the Shapiro-Wilk test. Descriptive statistics will be used to describe normally distributed data, and expressed as mean  $\pm$  standard deviation (SD). Continuous data not normally distributed will be reported as median and interquartile ranges. Categorical variables will be expressed as counts and percentages. Groups will be compared using chi square tests for categorical variables and independent-samples student's  $t$ - tests for normally distributed continuous variables. For variables not normally distributed, the Mann-Whitney  $U$  test will be used. Comparison will include subgroup analysis of participants who experienced post-operative complications including NEC with those that did not. Comparisons will also include cyanotic versus acyanotic heart disease subgroup analysis, as well as mode and type of feeding pre and post-operatively.

### Microbiome analysis

Metagenomic shotgun sequencing data will be analysed using bioBakery suite of tools ([https://huttenhower.sph.harvard.edu/biobakery\\_workflows/](https://huttenhower.sph.harvard.edu/biobakery_workflows/)). Trimmed and human reads filtered using KneadData (v0.7.2) with the default parameters. Quality controlled data will be taxonomically profiled at the species level with relative abundance by MetaPhlAn2. Functional profiling will be performed using HUMAnN3 and strain profiling using StrainPhlAn.

For alpha diversity analysis, samples will be rarefied to even depth and phyloseq::estimate richness will be used to calculate Chao1, Shannon and Simpson indices. Alpha diversity indices between groups will be univariately compared using the Wilcoxon rank sum test. A beta-diversity ordination will be generated using the Aitchison distance and visualised using Principal Component Analysis (PCA) plot. The Adonis function in the vegan package will be used to implement a permutational multivariate analysis of variance to test whether samples cluster beyond that expected by sampling variability. MaAsLin2 (Multivariate Associations with Linear Models) will be used to investigate multivariable associations

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3 between sequencing data and clinical metadata. MaAsLin2 performs boosted, additive,  
4 linear models to detect associations while adjusting for confounding factors. Sparse  
5 canonical correlation analysis (sCCA) will be used to calculate the overall correlation  
6 between metabolites and microbes, and to identify strongly associated biomarkers. Pairwise  
7 spearman rank correlation analysis will also be performed.  
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## 11 **Discussion**

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14 Despite an increasing awareness of the early-life critical window of microbiome  
15 development on the health and wellness of infants, there remains much to learn about the  
16 interactions of the microbiome with the infant host with CHD undergoing surgery involving  
17 CPB. This study is designed to address this knowledge gap, and incorporates a sound  
18 methodology with particular strengths enhancing the value of its findings. The specimen  
19 collection strategy occurring at multiple time points over a 2 year period in the proposed  
20 study will deepen our understanding of the temporal dynamics of the colonising microbiota,  
21 and their interactions with host physiology. The study design will account for maternal and  
22 perioperative variables to determine changes to the microbiome. Access to existing  
23 microbiome data from healthy age matched infants provides a valuable opportunity to  
24 present high quality comparative information. A limitation of this study may include the  
25 failure to recruit infants with CHD not identified antenatally, despite active fetal screening  
26 services. While multi-centre trials capturing sufficient case numbers of NEC cases will offer  
27 robust conclusions, this study will offer valuable evidence to support the influence of CHD  
28 and CPB on the microbiome and intestinal epithelial barrier dysfunction (EBD). Future  
29 research can build on existing studies, and explore treatment strategies including  
30 recommendations for efficacious probiotic strain administration, including the supplements  
31 to promote a diverse gut microbiota to improve outcomes for this vulnerable population.  
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## 40 **Ethics and Dissemination**

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43 This research study is ethically approved by the Clinical Research Ethics Committee of  
44 Children's Health Ireland (REC REF No: GEN/826/20). Study results will be available to  
45 patients with CHD and their families, carers, support networks, paediatric cardiology and  
46 microbiome societies and other researchers. Study findings will provide a deeper  
47 understanding of the gut microbiota of infants with CHD and inform perioperative  
48 management options including strategies to prioritise the integrity of the gut microbiota.  
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## 54 **Status of Study**

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56 The trial is ongoing and as of 5<sup>th</sup> February 2023 , 84 % of the participants have been  
57 recruited. Laboratory analysis has been carried out on 25% of study samples.  
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## Competing interests

The authors declare no association with commercial entities, either financial or non-financial.

## Author contributions

CM, CJMcM, CS, KM devised the project, and the main conceptual ideas. CJMcM, CM, CS, RPR, FK, DJ, MHT were involved in the study design and writing of the manuscript. MOT, JB, NM, SD, CJMcM are involved in consenting participants, collecting samples and acquiring data. DJ, CS, FK and RPR are responsible for analysing study samples. All authors read manuscript revisions, approved the final manuscript and accept accountability for the accuracy and integrity of the work.

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## Appendix 1: Case Report Form PDF Version

Appendix 2: SOP for Obtaining a Stool Sample when Participant is no longer Inpatient

## References

1. Skillington O MS, Gupta A, Mayer EA, Gill CIR, Del Rio D, O'Riordan KJ, Cryan JF, Ross RP and Stanton C The contrasting human gut microbiota in early and late life and implications for host health and disease. *Nutrition and Healthy Ageing*. 2012;6:157-78.
2. Cilieborg MS, Boye M, Sangild PT. Bacterial colonization and gut development in preterm neonates. *Early human development*. 2012;88 Suppl 1:S41-9.
3. Healy DB, Ryan CA, Ross RP, Stanton C, Dempsey EM. Clinical implications of preterm infant gut microbiome development. *Nature microbiology*. 2022;7(1):22-33.
4. Ohland CL, Jobin C. Microbial activities and intestinal homeostasis: A delicate balance between health and disease. *Cellular and molecular gastroenterology and hepatology*. 2015;1(1):28-40.
5. Cabrera-Perez J, Badovinac VP, Griffith TS. Enteric immunity, the gut microbiome, and sepsis: Rethinking the germ theory of disease. *Experimental biology and medicine*. 2017;242(2):127-39.
6. Halter J, Steinberg J, Fink G, Lutz C, Picone A, Maybury R, et al. Evidence of systemic cytokine release in patients undergoing cardiopulmonary bypass. *The journal of extra-corporeal technology*. 2005;37(3):272-7.

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  - 60
7. Centers for Disease Control and Prevention (CDC). Data and Statistics on Congenital Heart Defects. 2022 [updated January 24th 2022; 26th July 2022]. Available from: <https://www.cdc.gov/ncbddd/heartdefects/data.html> Accessed 26<sup>th</sup> July 2022.
8. van der Linde D, Konings E, Slager M, Witsenburg M, Helbing W, Takkenburg J, Roos-Hesselink J. Birth Prevalence of Congenital Heart Disease Worldwide: A Systematic Review and Meta-Analysis. *Journal of the American College of Cardiology*. 2011;58(21):2241-2247.
9. Martin R, Makino H, Cetinyurek Yavuz A, Ben-Amor K, Roelofs M, Ishikawa E, et al. Early-Life Events, Including Mode of Delivery and Type of Feeding, Siblings and Gender, Shape the Developing Gut Microbiota. *PloS one*. 2016;11(6):e0158498.
10. Siano E, Lauriti G, Ceccanti S, Zani A. Cardiogenic Necrotizing Enterocolitis: A Clinically Distinct Entity from Classical Necrotizing Enterocolitis. *European journal of pediatric surgery : official journal of Austrian Association of Pediatric Surgery*. 2019;29(1):14-22.
11. Kelleher ST, McMahon CJ, James A. Necrotizing Enterocolitis in Children with Congenital Heart Disease: A Literature Review. *Pediatric cardiology*. 2021;42(8):1688-99.
12. Murphy K, O'Donovan AN, Caplice NM, Ross RP, Stanton C. Exploring the Gut Microbiota and Cardiovascular Disease. *Metabolites*. 2021;11(8).
13. Carlo WF, Kimball TR, Michelfelder EC, Border WL. Persistent diastolic flow reversal in abdominal aortic Doppler-flow profiles is associated with an increased risk of necrotizing enterocolitis in term infants with congenital heart disease. *Pediatrics*. 2007;119(2):330-5.
14. McElhinney DB, Hedrick HL, Bush DM, Pereira GR, Stafford PW, Gaynor JW, et al. Necrotizing enterocolitis in neonates with congenital heart disease: risk factors and outcomes. *Pediatrics*. 2000;106(5):1080-7.
15. Miller TA, Minich LL, Lambert LM, Joss-Moore L, Puchalski MD. Abnormal abdominal aorta hemodynamics are associated with necrotizing enterocolitis in infants with hypoplastic left heart syndrome. *Pediatric cardiology*. 2014;35(4):616-21.
16. Nino DF, Sodhi CP, Hackam DJ. Necrotizing enterocolitis: new insights into pathogenesis and mechanisms. *Nature reviews Gastroenterology & hepatology*. 2016;13(10):590-600.
17. Typpo KV, Larmonier CB, Deschenes J, Redford D, Kiela PR, Ghishan FK. Clinical characteristics associated with postoperative intestinal epithelial barrier dysfunction in children with congenital heart disease. *Pediatric critical care medicine : a journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies*. 2015;16(1):37-44.
18. Salomon J, Ericsson A, Price A, Manithody C, Murry DJ, Chhonker YS, et al. Dysbiosis and Intestinal Barrier Dysfunction in Pediatric Congenital Heart Disease Is Exacerbated Following Cardiopulmonary Bypass. *JACC Basic to translational science*. 2021;6(4):311-27.
19. Hill CJ, Lynch DB, Murphy K, Ulaszewska M, Jeffery IB, O'Shea CA, et al. Evolution of gut microbiota composition from birth to 24 weeks in the INFANTMET Cohort. *Microbiome*. 2017;5(1):4.
20. Yu Z, Morrisson M. Improved extraction of PCR-quality community DNA from digesta and fecal samples. *Biotechniques*. 2004;36(5):808-12.



^999 – missing data; 0 – no data in medical notes

**CASE RECORD FORM**

**Stool Sample Collections**

Period/ Time Frame	Projected Date	Sample Date	Sample Collected	Comments
1. Within 24h of Birth			<input type="checkbox"/> YES <input type="checkbox"/> NO	
2. Pre-operatively			<input type="checkbox"/> YES <input type="checkbox"/> NO	
3. Week 1 of life/Post-Op			<input type="checkbox"/> YES <input type="checkbox"/> NO	
4a. Week 4 to 8 life/Post-op			<input type="checkbox"/> YES <input type="checkbox"/> NO	
4b. <b>Urine</b> Sample Week 4 to 8			<input type="checkbox"/> YES <input type="checkbox"/> NO	
5. Week 24 of life/Post-Op			<input type="checkbox"/> YES <input type="checkbox"/> NO	
6. Week 52 of life/Post-Op			<input type="checkbox"/> YES <input type="checkbox"/> NO	
7. Year 2 of life/Post-Op			<input type="checkbox"/> YES <input type="checkbox"/> NO	

**PATIENT DEMOGRAPHIC**

Date of Enrolment:		Date of Birth:		Cardiac Classification Group No:
Gender:	<input type="checkbox"/> Male <input type="checkbox"/> Female <input type="checkbox"/> Other/Ambiguous	Gestational Age:		Multiple <input type="checkbox"/> Yes <input type="checkbox"/> No
		APGAR at ① ⑤ min	<input type="text"/> <input type="text"/>	Birth Order:
Reason for admission:				
Comorbidities:				
Mode of Delivery:		Weight at Birth: _____ . _____ KGs		
SVD: <input type="checkbox"/> Yes <input type="checkbox"/> No		Head Circumference: _____ cms		
LSCS: <input type="checkbox"/> Yes <input type="checkbox"/> No, if Yes: <input type="checkbox"/> Elective/ <input type="checkbox"/> Emergency		Timing of cardiac diagnosis:		
Antibiotics to Infant post-delivery; <input type="checkbox"/> Yes <input type="checkbox"/> No		Postnatal <input type="checkbox"/> Antenatal <input type="checkbox"/>		
If Yes, list:				
Significant Antenatal Events:				

**MATERNAL INFORMATION**

Maternal Age (years) at Birth:	Gestational Age at Booking Appt:
Antibiotics given Pre-Delivery: <input type="checkbox"/> Yes <input type="checkbox"/> No	Maternal Probiotics taken during pregnancy:
List:	<input type="checkbox"/> Yes <input type="checkbox"/> No List:
Maternal Smoking during Pregnancy: <input type="checkbox"/> Yes <input type="checkbox"/> No	Other Household Members Smoking during Pregnancy: <input type="checkbox"/> Yes <input type="checkbox"/> No



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### SURGERY INFORMATION

Surgery Date:			
Surgery Performed:			
Pre-Op Antibiotics <sup>1?</sup> List:		Post-Op Antibiotics <sup>1?</sup> List:	
1. Abs <48hrs pre sampling		1. Abs <48hrs pre sampling	
2. Abx <72hrs pre sampling		2. Abx <72hrs pre sampling	
3. Abx in last 7 days/during sample		3. Abx in last 7 days/during sample	
ABG	pH	PO <sup>2</sup>	
First Pre-Op ABG:		Cardiopulmonary Bypass Duration:	
First Post-Op ABG:		Aortic Cross Clamp Duration:	
Significant Intraoperative Events:			

<sup>1</sup>Antibiotic treatment at time of stool sampling as below. Important not to include antibiotics which were started post the stool sampling

- 1) Abs < 48hrs pre sampling
- 2) Abx < 72 hrs pre sampling
- 3) Abx in previous 7 days/ during sample collection

### POST-OPERATIVE INFORMATION

PIM3 Score:		No. of days in ICU (1 <sup>st</sup> adm):	
Mechanical Vent:	<input type="checkbox"/> Yes <input type="checkbox"/> No	No of Days on ECLS:	
No of Days Vent:		No of Days on RRT:	
Agent	Day 1	Day 2	Day 3
Milrinone			
Epinephrine			
Norepinephrine			
Vasopressin			
Midazolam			
Morphine			
Clonidine			
Others			
Fluid Balance:			
BUN:			
Creatinine:			
HCT:			
Hgb:			

### FEEDING INFORMATION

Mode of Feeding (note <b>date initiated</b> and <b>date d/c</b> ):		
Breastmilk:	Infant Formula:	Other:
Prebiotics given to Infant: <input type="checkbox"/> Yes <input type="checkbox"/> No	Type and Date Given:	
Excessive Infantile Crying (cried for $\geq 3$ Hrs for 3 Days in one week): <input type="checkbox"/> Yes <input type="checkbox"/> No		

# GuMIBear Study



Study ID: GMB: \_\_\_\_\_

1 ^999 – missing data; 0 – no data in medical notes

Date Trophic Feeds Commenced:		Type of Feed Used:	
Time to Establishment of full feed <sup>2</sup> :			
Development of NEC <sup>3</sup> :			
Days post-op when developed NEC?			
Gut stasis:	Not applicable		
Management Strategy:			

2 Full feed – No longer requires parenteral nutrition or intravenous fluids supplement regardless the TFI.

3 NEC – Initiation of triple IV antibiotic therapy and nil by mouth for at least 5 days, based on a full surgical review including clinical presentation, radiological and laboratory data.

## DISCHARGE INFORMATION

Date of Discharge			
Ward:	Home:	RIP:	

## READMISSION TO ICU

Total ICU Readmission days:				
	Date of Admission	Date of Discharge	Total ICU stays	Reason for admission
1.				
2.				
3.				
4.				
5.				

## DATA ENTRY BY (NAME)

Admission	Date	Paper	Date	G-Drive
First				
Second				
Third				

Participant Withdrawal from Study: <input type="checkbox"/> Yes <input type="checkbox"/> No
GCP Procedure Followed: <input type="checkbox"/> Yes <input type="checkbox"/> No
See Study Folder Appendix 4. Signed: _____ Date: _____





## The Gut Microbiota of Infants with Complex Congenital Heart Disease Undergoing Cardiopulmonary Bypass



( GuMiBear)

### STANDARD OPERATING PROCEDURE FOR STOOL SAMPLE COLLECTION AS AN OUTPATIENT

**Purpose:** To collect infant stool samples while the infant is an out-patient from CHI at Crumlin

**Objective:** To collect infant stool samples for the study in a uniform manner and under a set of conditions, so that they can be processed by the laboratories to achieve optimal results.

**Procedure:**

1. Ensure that at least one legal guardian has provided written informed consent for their infant to participate in the study and that they are happy for their infant to remain in the study.
2. Ensure that in the hospital chart of the infant that it is noted that he/she is participating in the study and contact details of the study team.
3. Ensure the parent/guardian has been supplied with a study pack containing the requisites for the collection of the stool sample
  - a. Completed labels
  - b. Disposable Gloves
  - c. Stool collection containers
  - d. Bio-hazard bags
4. Explain to the legal guardian that a minimum of a teaspoon of stool has to be collected. Explain to the legal guardian when the samples have to be collected as close as possible to the next out-patient appointment.
5. Explain to parent/guardian how to collect the sample as follows:
  - a. Have requisites for collection at the ready.(Gloves, sample container, red biohazard bag & labels)
  - b. Place appropriate label on the sample bottle.
  - c. Wear disposable gloves
  - d. Unscrew cap of sample bottle





## The Gut Microbiota of Infants with Complex Congenital Heart Disease Undergoing Cardiopulmonary Bypass



( GuMiBear)

- e. Spoon in stool sample (1 teaspoon in volume)
  - f. Screw cap on tightly
  - g. Place in red hazard bag
  - h. Remove disposable gloves
  - i. Dispose with soiled nappy
  - j. Wash hands
  - k. Place stool sample in fridge
  - l. Text research nurse that sample is ready for collection.
6. Label the sample bottles with Subject Number, Date of Birth, Initials, Sample Number, Date of Collection
  7. Ask the parent/guardian to attach the appropriate label to the sample container and immediately place container in freezer.
  8. Text the parent/guardian the night before the sample is to be collected. Meet the parent/guardian in out-patients to collect the stool sample and store the sample in the study container in the designated study freezer.
  9. Update the study spreadsheet to indicate sample has been obtained.