A PROSPECTIVE, COHORT PILOT DESIGN THESIS: FAST I(n)DENTIFICATION OF PATHOGENS IN NEONATES (FINDPATH-N)

A PROSPECTIVE, COHORT PILOT DESIGN THESIS: FAST I(n)DENTIFICATION OF PATHOGENS IN NEONATES (FINDPATH-N)

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A thesis submitted to the School of Graduate Studies in partial fulfillment of the requirements for the degree Master of Science in Health Research Methodology

Degree: Master of Science (2020), McMaster University, Hamilton, Ontario

Title: A prospective, cohort pilot design thesis: Fast I(n)Dentification of PATHogens in Neonates (FINDPATH-N)

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Number of pages: 73

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Abstract

Introduction: Sepsis is a major source of morbidity and mortality in neonates; however, identification of the causative pathogens can be challenging. Next generation sequencing (NGS) is a high-throughput, parallel sequencing technique for DNA. Pathogen-targeted enrichment followed by NGS has the potential to be more sensitive and faster than current gold-standard blood culture. In this pilot study, we will test the feasibility and pathogen detection patterns of pathogen-targeted NGS in neonates with suspected sepsis. Additionally, the distribution and diagnostic accuracy of cell-free DNA and protein C levels at two time points will be explored.

Methods: We will conduct a prospective, pilot observational study. Neonates over 1 kg with suspected sepsis from a single tertiary care children's hospital will be recruited for the study. Recruitment will be censored at 200 events or 6 months duration. Two blood study samples will be taken: the first simultaneous to the blood culture (time = 0 hr, for NGS and biomarkers) via an exception to consent (deferred consent) and another 24 hours later after prospective consent (biomarkers only). Neonates will be adjudicated into those with clinical sepsis, culture-proven sepsis and without sepsis based on clinical criteria. Feasibility parameters (e.g. recruitment) and NGS process time will be reported.

Analysis: NGS results will be described in aggregate, compared to the simultaneous blood culture (sensitivity and specificity) and reviewed via expert panel for plausibility. Pilot data for biomarker distribution and diagnostic accuracy (sensitivity and specificity) for distinguishing between septic and non-septic neonates will be reported.

Study amendment and interim results: After obtaining ethics approval, study enrolment started October 15, 2020. Interim feasibility results showed successful deferred consent, but low enrolment. A study amendment was used to increase enrolment, create pre-packaged blood kits and implement a substitute decision maker Notification form.

Acknowledgements

I would like to express my sincere gratitude to my supervisor Alison Fox-Robichaud. I have been enthralled by her passion for translational research, embraced by the research team she has built and eased by her unwavering support.

I would also like to thank my other committee members, Salhab el Helou and Melissa Parker, for their expertise, enthusiasm and dedication to this project and my Master's degree. Their wise counsel was often needed and always appreciated. In addition, I am very grateful to have the thoughtful input of Hendrik Poinar, Michael Surette and Jeffrey Pernica.

I appreciate the funding support of CIHR and the McMaster Department of Pediatrics. Funding for research is so critical in transforming our questions into action.

I would like to thank my family for their ongoing support throughout this process and especially my mother Jane Ann Klowak, who has been my diligent proof-reader for many years.

Lastly, I would like to thank the other members of both Alison Fox-Robichaud's and Patricia Liaw's labs. We have celebrated each other's research victories, commiserated and troubleshot the difficulties, and shared in our passion for science.

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List of Abbreviations

cfDNA cell-free DNA

CFU colony-forming unit

CoNS coagulase negative staphylococcus

CRP C-reactive protein

DNA deoxyribonucleic acid

EDTA ethylenediaminetetraacetic acid

ELISA enzyme-linked immunosorbent assay

HiREB Hamilton Integrated Research Ethics Board

MCH McMaster Children's Hospital

MRSA methicillin-resistant *Staph. aureus*

NETs neutrophil extracellular traps

NGS next-generation sequencing

NICU neonatal intensive care unit

NRC Neonatal Research Committee

PCR polymerase chain reaction

PRRG Pediatric Resident Research Grant

SDM substitute-decision maker

CHAPTER 1: INTRODUCTION

1.1 Sepsis

Sepsis definition

Sepsis is defined in adults as a "life-threatening organ dysfunction caused by a dysregulated host response to infection" by the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3).[1] This consensus definition was published in 2016, addressing the lack of specificity of previous definitions. However, defining and understanding sepsis is an ongoing field of research. Sepsis is a broad syndrome of abnormalities induced by infection without a true gold standard test. Even the recent Sepsis-3 definition has received critique in regards to adequate early identification of sepsis.[2]

Sepsis pathophysiology

Key pillars of sepsis pathophysiology are presence of infection, a dysregulated host response and the consequent negative circulatory, cellular and metabolic effects. Host factors affecting both the risk and response to sepsis include an immunocompromised state, cancer, age, biologic sex, diabetes, obesity and instrumentation (e.g. intubated or central line in place).[3] First is the infection, which serves as a trigger and may be fungal, viral or bacterial. The most common sources worldwide are respiratory and abdominal.[4] Second is the dysregulated host response, which exists as a spectrum of severity. The response is propagated by a complex interplay of signalling molecules such as chemokines

and cytokines. Key signalling molecules include interleukin-1, interleukin-8 and tumour necrosis factor alpha.[3] The inflammatory signalling molecules create a self-propagating cycle whereby they increase the number and activation of other immune cells, further increasing inflammatory signalling. This auto-amplifying process is termed cytokine storm.[5] At the level of the endothelium, there is vasodilation, increased leukocyte adhesion and lowered barrier function.[6] The previously balanced coagulation system shifts to procoagulant, and both microthrombi and larger thrombi can form. Neutrophils release cell-free DNA (cfDNA) in the form of extracellular traps (NETs), which contributes to coagulation and platelet aggregation.[7,8] Overconsumption of coagulation factors may result in disseminated intravascular coagulation, which can cause both bleeding and clotting.

These cellular, microvascular and metabolic changes result in larger-scale, hemodynamic alterations and inadequate end-organ perfusion. In adults, sepsis is most commonly a ‘warm shock’ with low blood pressure and increased cardiac output. In children and neonates, sepsis is more commonly ‘cold shock’ with a low cardiac output and higher systemic vascular resistance.[9] The inadequate perfusion can negatively affect every organ in the body, causing organ dysfunction and ultimately, death.

1.2 Neonatal sepsis

Defining neonatal sepsis

Defining neonatal sepsis is a challenge due to the presence of non-specific clinical features, overlap with other diagnoses and uncertainties in the pathophysiology. There is no consensus definition or standard for neonatal sepsis.[10] One limitation is that pathogens are infrequently identified in blood culture, despite clinical evidence of sepsis.[11,12] The use of biomarkers for the diagnosis of neonatal sepsis has been studied extensively, but so far there is no single reliable biomarker or scoring system that has been demonstrated to reliably identify sepsis.[13] Due to the lack of a rapid and accurate diagnostic test or clear definition of neonatal sepsis, neonates are at risk for overtreatment with antimicrobials and their associated toxicities or inadequate antimicrobial treatment. These factors create an ongoing need for a rapid and sensitive diagnostic test for neonatal sepsis.[13,14]

Neonatal sepsis pathophysiology and pathogens

In neonates, sepsis is a major source of mortality and morbidity.[15] Neonatal sepsis may present with subtle features such as irritability, changes in feeding patterns, fever, vomiting or tachycardia. The clinical presentation may also be severe, such as altered level of consciousness, shock, seizures or respiratory failure.

Neonatal sepsis is divided into early onset, defined as before 3 to 7 days of life, and late onset sepsis, defined as after 3 to 7 days of life.[13] Common organisms are *E. coli* and group B streptococcus (GBS) in early onset sepsis, which are thought to be transmitted in utero, via a transplacental route or from the vaginal environment.[13,15] In contrast, the most common causes of late onset sepsis in the neonatal intensive care unit (NICU) are coagulase negative staphylococcus (CoNS) and *Staph. aureus*, which are both common skin flora.[16] Differentiating between a true CoNS infection and a contaminated blood culture is difficult because contamination is common and symptoms of sepsis can be subtle.[17] Moreover, CoNS can form protective layers called biofilms on plastic such as central lines, and neonatal CoNS sepsis has been associated with poor outcomes.[17,18] The organisms in late onset sepsis, such as CoNS, are transmitted through neonates' interaction with their environment and caretakers, with hand contact being the most common source.[13] Most cases of late onset sepsis are inpatients, typically due to prematurity, but it also occurs in neonates who have been discharged home.[19] Unfortunately, NICUs are susceptible to outbreaks and colonization with methicillin-resistant *Staph. aureus* (MRSA), which has been associated with later MRSA infection.[20]

Risk factors for neonatal sepsis include prematurity (born at less than 37 weeks of gestation), low birth weight, colonization of the mother's vaginal tract with GBS, chorioamnionitis and premature or prolonged rupture of membranes.[15]

Additional risk factors for late onset sepsis include longer duration of intubation, use of central lines and treatment with histamine-2 receptor antagonists.[21–23]

Neonatal immune system

Neonates' increased risk of infection is partly due to an ineffective immune system.[24] The etiology of the reduced immunity is not fully understood, but is thought to be due to an impairment in immune response.[24,25] For example, newborns produce less overall pro-inflammatory cytokines and have less of a Toll-like receptor-induced response compared to adults.[26] The immune system of full-term neonates (at least 37 weeks of gestation) was previously termed immature, but recent evidence indicates that it is rather an "evolutionarily adaptive phenotype".[27] Neonatal neutrophils are particularly important and distinct in their function: they are essential for a neonate's *adaptive* immune function and can tolerate hypoxic conditions without an inflammatory response.[28] However, premature infants (less than 37 weeks of gestation) are at an even higher risk of infection due to true immaturity of both the innate and adaptive immune systems. In particular, maturation of the antimicrobial pattern recognition receptors occurs up to a gestational age of approximately 33 weeks.[29] The neonatal immune system is an ongoing area of research, for example exploring whether the different microbiome in neonates compared to adults may affect mucosal immunity and the risk of sepsis.[29]

Burden of neonatal sepsis

Schrag *et. al* assessed the incidence of early onset sepsis from 2005-2014 in the United States and found that early onset sepsis continues to be a significant problem, with an estimated incidence of 0.79 per 1000 live births.[30] Screening and intrapartum antibiotic prophylaxis for GBS has led to a small decrease in the incidence of GBS early onset sepsis. However, the overall incidence of early onset sepsis has remained the same.[30] Canadian data demonstrate a similar reduction in GBS early onset sepsis over time with a trend towards increase in other organisms.[31] Late onset sepsis has an estimated incidence of 0.86 (0.76-0.97) per 1000 live births for hospital-acquired disease and 0.28 (0.23-0.34) per 1000 live births for community-acquired disease.[32] Very low birth weight infants (401-1500 g) have a particularly high burden of late onset neonatal sepsis, with 21% having at least one episode of culture-positive late onset sepsis during their NICU stay.[33]

In a meta-analysis of predominately high-income countries, the mortality rate of neonatal sepsis is 11-19% and is higher with lower weight, prematurity and pathogen (especially *E. coli*).[34] In particular, GBS infection can cause serious long-term morbidity in survivors, namely cerebral palsy, intellectual disability, epilepsy, hearing loss and visual impairment.[35,36] Therefore, neonatal sepsis is a common, life-threatening condition with potential for lifelong sequelae.

Treatment of neonatal sepsis

Key principles of the treatment of neonatal sepsis are early antimicrobial therapy and supportive care within a critical care setting.[37] The recommended empiric antibiotic choice is intravenous ampicillin and an aminoglycoside or cephalosporin with review of local resistance patterns.[13] Antifungals and antivirals are not used empirically, but rather depending on the risk profile with input from infectious disease specialists.[13] Neonates who are immunocompromised or have had a previous candida infection would raise concern for fungal infection, while a neonate who has vesicles or known exposure to the herpes simplex virus may require empiric antivirals. In late onset neonatal sepsis, consideration should be given to coverage of coagulase negative staphylococcus.[13,38] Antibiotics dose and coverage are later modified based on detected pathogens, requirement of cerebral spinal fluid penetration and drug levels. Despite extensive research in neonates and adults, there is no effective treatment that specifically targets the maladaptive host response in sepsis.[13,39]

1.3 Reference standard blood culture

Blood culture is the traditional diagnostic test for blood stream infection and considered the gold standard.[13] In neonatal sepsis, samples are taken from normally sterile sites such as the blood or cerebrospinal fluid and ‘cultured’, or incubated to assess for growth over a period of days. The median time until

growth for a positive blood culture in true neonatal sepsis is estimated at 9-18 hours; however CoNS may often take longer.[11] If a pathogen grows, it is assessed with a gram stain followed by further laboratory testing to identify the species and antimicrobial resistance pattern. Blood cultures can therefore take days to identify a pathogen.

In neonatal sepsis, pathogens are infrequently identified in blood culture, in some cases despite clinical evidence of sepsis.[11,12] This has raised concerns about the sensitivity of blood culture, especially because small blood volumes (e.g. 0.5 ml) are often used for blood culture in neonates. The volume of blood drawn and the number of cultures taken (e.g. taking two blood cultures simultaneously) have been shown to affect the sensitivity of blood culture in both neonates and adults.[40–42] In an *in vitro* study using inoculated blood, a culture volume of 0.5 ml was inadequate for detecting low colony count, defined as less than 4 colony-forming units (CFU/ml). Cultures of at least 1 ml had high sensitivity for low, but not ultralow bacteremia (<1 CFU/ml).[42] However, obtaining larger blood samples from small neonates can be mechanically challenging and also poses the risk of causing iatrogenic anemia. The term ‘culture-negative sepsis’ is used to describe clinical findings of sepsis with a negative culture. Culture-negative sepsis is a variably defined term with an unclear etiology: low or ultralow bacteremia, sepsis with improper culture technique (e.g. cultured after antibiotics), viral infection or non-infectious causes.[43] As demonstrated in two studies assessing the

diagnostic accuracy of physicians for sepsis, physicians may have difficulty distinguishing sepsis from other neonatal or pediatric conditions.[44] Comparing molecular methods to culture-based methods has the potential to help rapidly and sensitively identify bacteria and improve our understanding and treatment of neonatal sepsis, especially that which is culture-negative.

1.4 Newer diagnostic methods for bloodstream infection

Next generation sequencing

Next generation sequencing (NGS) is a high-throughput, parallel sequencing technique for DNA and RNA. NGS is faster and less expensive than the previous sequencing method, Sanger sequencing. NGS follows a series of processes: i) DNA extraction, ii) library preparation (shearing, adding adaptors and amplification), iii) template preparation; and iv) sequencing.[45] Sequencing results are compared to known genomic libraries: this therefore allows NGS to identify all species of bacteria and/or multiple bacteria at once.

The theoretic clinical benefits of NGS include increased speed compared to standard blood culture,[46] and fast antibiotic-resistant gene identification. Since detection is not based on growth, detection may be possible even after antibiotic administration and identification of polymicrobial infections may be improved. NGS can identify viral, bacterial and fungal DNA in one test, unlike blood culture. NGS has correctly identified pathogens in septic adults.[46,47] Grumaz *et al.*

assessed blood plasma from 60 patients with septic shock, 30 healthy volunteers and 30 patients postoperative from abdominal surgery. Using a 'sepsis indicating quantifier' formula to normalize and interpret the sequencing NGS results, matching bacteria were detected in all blood culture-positive patients as well as additional pathogens in blood culture-negative samples. No NGS results from postoperative uninfected patients were positive.[46] The vast majority (96%) of the NGS-positive results were independently deemed plausible, which is suggestive of increased sensitivity of NGS relative to blood culture for pathogen identification in septic adults.[46,48] Gosiewski *et al.* detected bacterial DNA using NGS in 23 healthy volunteers.[49] The taxonomy of the bacteria, predominately intestinal microbiota of the order *Bifidobacteriales* in the healthy volunteers, was significantly different than the septic patients. *Bifidobacteriales* has been reported to modulate the host immune response in a protective manner.[50] These findings indicate that NGS has potential to generate new knowledge, but also requires stringent controls and interpretation for clinical application.

Polymerase chain reaction

Multiplex polymerase chain reaction (PCR) has also been studied for rapid pathogen detection in sepsis; however, limitations exist. These limitations include a lack of quantitative output for some PCR methods, variable sensitivity, and a restricted range of pathogen recognition. A meta-analysis of 41 studies on a multiplex PCR system demonstrated a low summary sensitivity (68%) relative to

blood culture, although poor study quality was noted.[51] A 2017 Cochrane review of 35 studies comparing several PCR methods (e.g. broad-range or multiplex PCR) to blood culture in neonatal sepsis reported a summary sensitivity of 90% (95% CI 0.82-0.95) and specificity 93% (95% CI 0.89-0.96) with moderate quality evidence.[52] However, the summary estimates for late onset sepsis only had low sensitivity at 79% (95% CI 0.69-0.86).

NGS offers the advantage of semi-quantitative output and a larger range of pathogen recognition over PCR-based methods. If initial studies in application and interpretation are successful, the potential increased sensitivity and speed of NGS relative to BC could aid in diagnostic clarity and decisions around antibiotic duration.[53] Antibiotic-resistant gene identification could offer physicians valuable information required for targeted antibiotic therapy. NGS has significant clinical potential in neonatal sepsis and, to our knowledge, has not been studied in this population.

Novel pathogen-targeted NGS system

The relatively large amount of human DNA present in blood samples compared to pathogen DNA can result in a low signal-to-noise ratio during sequencing. Our group has designed novel biotin-labelled pieces of RNA, termed ‘baits’, which are around 80 base pairs long. These baits ‘map’ to, or align with, unique regions (‘Kmers’) of DNA on a wide variety of fungal, viral and bacterial species in a

hierarchical fashion. Pathogen DNA is enriched (increased in relative concentration) through hybridization with the baits. The biotin-labelled bait and hybridized pathogen DNA are pulled out of solution using streptavidin-coated magnetic beads.[54] This additional enrichment step is performed prior to sequencing and increases the relative amount of pathogen versus human DNA.

1.5 Biomarkers in neonatal sepsis

Existing literature on biomarkers in neonatal sepsis

Biomarkers are an appealing research target for neonatal sepsis because, if reliable, rapid quantification could allow for clinical decisions around antibiotic therapy and hospital admission. Numerous biomarkers such as C-reactive protein and procalcitonin have been studied extensively in neonatal sepsis.[14] However, there is not yet a biomarker with the ideally high sensitivity, specificity, reliability and short turnaround time desired.[14] The positive predictive values of inflammatory markers, such as cytokines, are generally low because other causes of stress and inflammation in the neonatal period are possible, such as a difficult birth.[11] Procalcitonin-guided treatment has been shown to reduce antibiotic duration in early onset neonatal sepsis, but requires serial measurements.[55]

Cell-free DNA

Cell-free DNA (cfDNA) is freely-circulating DNA and can be found in the blood plasma of healthy individuals. The cfDNA found in plasma is predominantly from host blood cells.[56] Typically, cfDNA is released from cells by apoptosis (programmed cell death), necrosis (cell death due to injury) and NETosis (the production of extracellular traps directed at pathogens by neutrophils). NETosis by neutrophils is part of the body's immune response during sepsis.[57] Levels of cfDNA have shown prognostic utility in adult trauma,[58] cancer[59] and sepsis.[60–62] In a case-control study of 27 very preterm infants, the level of cfDNA was elevated at late onset sepsis diagnosis and trended higher even days prior to the onset of necrotizing enterocolitis.[63]

Protein C

Protein C is a glycoprotein with anticoagulant activity through regulation of thrombin activity. Protein C has been studied as a potential treatment for severe neonatal sepsis using activated protein C concentrate, although there is no clear evidence of benefit.[64] Protein C levels are significantly higher in healthy controls compared to septic neonates, and protein C has been described as a useful biomarker in severe sepsis.[65] Protein C has also demonstrated prognostic utility for mortality in septic low birth weight neonates.[66] The diagnostic capability of protein C levels to identify neonatal sepsis has not been investigated.

1.6 Rationale

Both NGS and the biomarkers cfDNA and protein C have potential to improve care for neonates with sepsis through, respectively, rapid and sensitive identification of pathogens and improved diagnostic accuracy. The described pilot study will test the feasibility and pathogen detection patterns of NGS in neonates with suspected sepsis. Additionally, cfDNA and protein C levels at two time points will be analyzed for diagnostic capability of clinical and culture-proven sepsis. Pilot studies are crucial to inform larger trials.[67] For this research question and design, a pilot study is particularly necessary because of unclear consent rates with deferred consent, uncertainties of performing a novel methodology on small samples of neonatal blood and the need to ensure the ability of clinical staff to identify eligible neonates rapidly.

CHAPTER 2: STUDY DESIGN AND METHODS

2.1 Objectives

The objectives of this study are shown in **Table 1**. The primary outcomes are related to feasibility, as this is a pilot study.[67] The study protocol was published in *BMJ Paediatrics Open.*[68]

Table 1: Objectives, outcome measures and methods of analysis for FINDPATH-N pilot study

Objective	Outcome measure	Method of analysis
Co-primary objectives		
1. Recruitment	Successful recruitment is defined as ≥80% of eligible patients	Proportion
2. Sample collection	Successful sample collection is defined as ≥80% of the blood samples for recruited patients at the first time point	Proportion
3. Ability to perform NGS on blood samples of premature and term neonates at MCH with suspected sepsis	Description of mechanical or process issues	Descriptive only
Secondary objectives		
1. To describe the blood NGS pathogen output in order to gain a preliminary understanding of the potential clinical role of NGS testing in neonates with suspected sepsis	NGS pathogen output (taxonomy, reads, plausibility from panel review)	Descriptive statistics ± case discussions
2. To describe the plasma levels and diagnostic accuracy of biomarkers cfDNA and protein C at 0 and 24-hour time points between neonates with clinical sepsis, culture-proven sepsis and without sepsis	Levels of blood cfDNA and protein C, sensitivity and specificity (%), likelihood ratios	Descriptive statistics, diagnostic accuracy measures with confidence intervals
3. To compare the sensitivity and specificity of NGS for bacterial identification compared to gold-standard aerobic blood culture	Sensitivity and specificity (%)	Proportion and confidence intervals
4. To determine blood sample NGS laboratory process time	Process time from thawing sample to sequence acquisition (hours)	Descriptive only
5. To determine consent rate using an exception to prior consent (deferred consent)	The target consent rate is ≥80% of families approached	Proportion

Abbreviations: MCH (McMaster Children's Hospital), NGS (Next Generation Sequencing), cfDNA (cell-free DNA)

2.2 Study design

FINDPATH-N is a pilot, observational single-centre cohort study in Hamilton, Ontario, Canada. The study design is shown in **Figure 1**. Major considerations in study design included the need to compare the index test NGS directly to the reference standard (blood culture), to rapidly identify eligible patients and obtain samples, and to draw only a safe, small amount of blood relative to patient size. Preliminary diagnostic studies are often designed using a case-control approach.[69] However, obtaining the reference standard (blood culture) in healthy 'control' neonates created the potential ethical concern of exposure to unnecessary treatment should the blood culture result positive from a contaminant. In addition, some preliminary work in adults with bacteremia using NGS has already been performed.[45,46,48] Lastly, case-control designs tend to overestimate measures of diagnostic accuracy due to spectrum bias by only including patients at opposite ends of a single disease spectrum (e.g. completely well neonates and neonates with severe sepsis).[70] A cohort design was therefore chosen because it allowed both simultaneous acquisition of the reference and index tests, while also allowing assessment of index test performance in the true clinical population of interest: neonates with suspected sepsis.

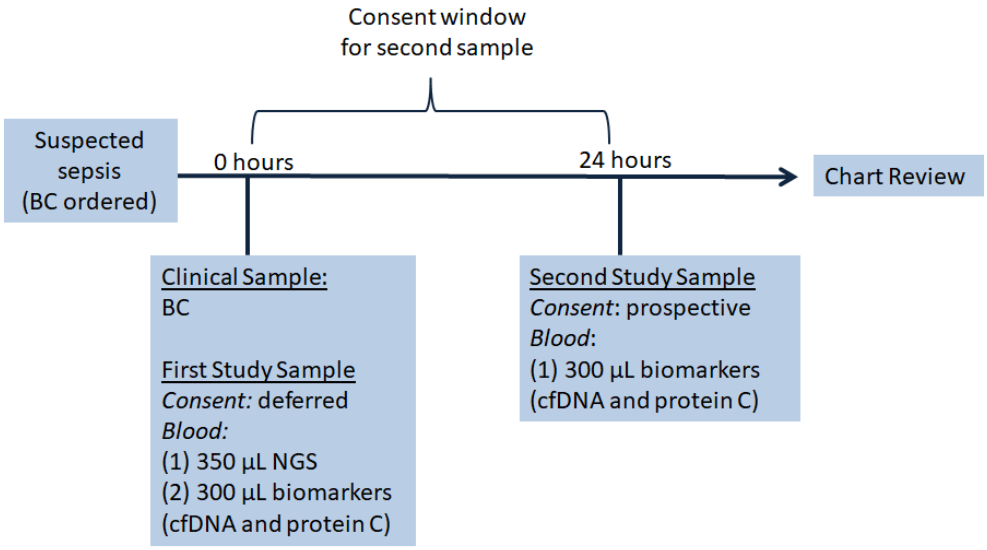

Figure 1: FINDPATH-N study workflow
Abbreviations: BC (blood culture), NGS (next generation sequencing), cfDNA (cell-free DNA)

The point of entry into the study is suspected sepsis, defined as an order for a blood culture. The choice was pragmatic by creating a clear mental trigger (blood culture order) for the clinical team to consider study eligibility. A minimum participant weight of 1 kg was created as an inclusion criterion for several reasons. Firstly, the initial blood volume taken via exception to prior consent (further explained in section **2.6 Ethics**) would be less than 1% of the smallest participant's estimated blood volume (0.65 ml / 85 ml = 0.8%). Secondly, the removal of this volume of blood was considered to be very minimal to no risk by consensus decision between two neonatologists and agreement from the Neonatal Research Committee. Lastly, the 1 kg mark was also a simple number so that application of study eligibility criteria was clear. Exclusion of known

Jehovah's witnesses was a precautionary choice to avoid even a very small potential contribution to anemia in this population. Children apprehended by the Children's Aid Society were excluded because of the anticipated futility of taking a blood sample given our centre's experience with later being unable to obtain consent for research.

2.3 Study method

Study setting

Potential patients will be from the level II and III neonatal intensive care units (NICUs) in a pediatric, tertiary care hospital (McMaster Children's Hospital) in Hamilton, Ontario, Canada. The level IIIb NICU, the highest level of NICU across the province of Ontario, is capable of caring for any gestational age including providing mechanical ventilation, on site surgical capability and access to subspecialists.[71] The level II NICU at McMaster Children's Hospital generally cares for infants with gestational ages over 32 weeks who have mild illness. Participants may be born at McMaster Children's Hospital or transferred to McMaster Children's Hospital from regional referral centres.

Eligibility criteria

Inclusion Criteria

(1) Patient in level II or level III NICU with physician or nurse practitioner order to draw blood culture

(2) Current or birth weight over 1 kg

Exclusion Criteria

(1) Substitute decision maker (SDM) has previously declined consent for FINDPATH-N

(2) Patient apprehended by Children's Aid Society

(3) SDM is a known Jehovah's witness

Study processes

Patient recruitment and first sample

Study education sessions and posters will increase awareness and inform staff about study processes. For the six-month study period, a blood culture being ordered will act as a cue for nursing staff to check eligibility. Chart inserts and stickers will be used to identify patient consent status. If eligible, 650 μL of blood will be collected via an exception to prior consent (deferred consent, section **2.6 Ethics**) in two separate study tubes (one 350 μL EDTA tube for NGS and one 300 μL EDTA tube for biomarkers) at the same time as the blood culture.

Consenting and second sample

Study personnel will approach SDMs for consent in person or via telephone within 24 hours of the first blood sample. Before approaching the family, study personnel will make a reasonable effort to ensure that a medical update has been given and obtain permission to approach via a member of the circle of care.

If consent is obtained for a second sample (300 µL EDTA tube for biomarker analysis), study personnel will write a 'suggest order' in the chart for this to be drawn 24 ±4 hours from the time of the first sample. If the SDM is not able to be reached within 24 hours, the second sample will not be collected. Later attempts to contact the SDM will be made so that the first sample may still be used. If consent is not obtained for inclusion in the study, the blood sample collected via an exception to prior consent will be destroyed. Study personnel will maintain a master list to log consent and sample acquisition throughout the study period. Automated online searches for use of the study code by nursing staff will serve as a backup to notification from the clinical team.

Patient data collection methods

We have developed a detailed case report form (**Appendix A**). Trained data abstractors will review the charts of participants and record patient demographic data, timing of blood culture and sample collection, treatment details (antibiotics, vasopressors), vital signs, laboratory data and clinical outcomes data.

Sample size

We will censor the study at 200 events of suspected sepsis or 6 months duration. In general, pilot studies do not require sample size calculations.[67] The 200 events will allow a confidence interval estimate of the primary feasibility outcomes with a

margin of error less than 0.1 when expected rates are 0.8.[67] Results from this study could serve to inform the sample size of a larger trial.

In order to understand expected frequency of eligible patients, we used the McMaster Children's Hospital Data Management Team to acquire historical, aggregate data on blood cultures in the NICU (**Table 2**). Among babies born at 28 weeks of gestational age and over admitted to the McMaster NICU during the 2017-2018 fiscal year, there were 497 blood cultures drawn. Blood culture data by weight was not available; however the average weight of a neonate born at 28 weeks gestation is 1 kg for females and 1.1 kg for males based on the 2013 Fenton premature growth charts.[72] Although there are data limitations including a lack of known weight, we estimated approximately 250 eligible events over the six-month study period.

Table 2: Gestational age at birth and number of blood cultures from aggregate 2017-2018 data[1]

Gestational age at birth	Number of BC drawn[2]
28	36
29	28
30	30
31	38
32	29
33	19
34	44
35	29
36	35
37	31
38	54
39	53
40	41
41	29
42	1
All gestational ages (total)	497

[1]Includes all patients admitted to the McMaster NICU during the 2017-2018 fiscal year

[2]Anytime during the patient's NICU stay, even if the date of blood culture was not within the 2017-2018 fiscal year. Multiple blood cultures may be from the same patient.

Definitions and adjudication

There is currently no consensus definition for neonatal sepsis.[12] Study definitions were made by adapting previous literature and consensus decision among a neonatologist, pediatrician and pediatric infectious disease specialist.[73,74] Patients will be adjudicated into those with and without sepsis (sub-classifications: culture-proven and clinical, see **Table 3**). NGS-positive results will be reviewed for plausibility by a panel, which will include a neonatologist, pediatrician and pediatric infectious disease specialist, and majority voting will be used.

Table 3: Study definitions

Term	*Study definition*
Clinical changes consistent with sepsis	(1) temperature ≥38 or ≤36.5°C (2) new marked tachycardia (>180 bpm) or bradycardia <80 bpm (including episodes of bradycardia increased from baseline) (3) new apnea (4) extended capillary refill time (≥4 seconds) (5) new metabolic acidosis (pH ≤ 7.25 or bicarbonate ≤ 18 mmol/L) (6) new hyperglycemia (glucose >10 mmol/L) (7) change in energy, change in level of consciousness or seizure
Laboratory changes consistent with sepsis	(1) new thrombocytopenia (platelets <100 /nl) (2) CRP >15 mg/L (3) immature/total neutrophil ratio >0.2 (4) white blood cell count under 5/nl
Suspected sepsis	neonate with physician or nurse practitioner order to draw a blood culture
Culture-proven sepsis	(1) at least one clinical or laboratory change consistent with sepsis, and (2) a positive blood or cerebrospinal fluid culture of a pathogenic species (other than CoNS)
CoNS sepsis	(1) two or more clinical or laboratory changes consistent with sepsis, (2) blood culture positive for CoNS, and (3) an indwelling catheter present
Clinical sepsis	(1) two or more clinical or laboratory changes consistent with sepsis, (2) treatment with antibiotics for ≥ 5 days, and (3) no apparent better explanation
Non-septic	patients who do not meet criteria for sepsis as outlined above

Abbreviations: CoNS (coagulase-negative staphylococci), CRP (C-reactive protein)

Data management

Data with personal identifiers will be stored on an encrypted USB in a locked drawer in a locked institution. Data will be de-identified using a study code. REDCap, a secure web application, will be used to build and manage our study database.[75] Range checks will be performed for all continuous data. Data will be destroyed after 10 years.

Monitoring

A data safety and monitoring committee was deemed unnecessary due to the short study duration and this being an observational, minimal risk study. The primary investigator will monitor recruitment, consent processes and sample collection on a weekly basis.

2.4 Storage and processing of index tests

Storage

Samples for NGS will be frozen as whole blood at -20 °C. Biomarker samples will be frozen as plasma at -80 °C. Any remaining blood will be stored for up to 10 years for future pathogen and biomarker analysis.

Biomarker analysis

Levels of cfDNA and protein C will be quantified from plasma via Qiagen cfDNA extraction kit as previously described[60] and an enzyme-linked immunosorbent assay (ELISA) respectively.

NGS library preparation

Total DNA is placed into double- and single-stranded DNA libraries using methodology designed at McMaster University (H. Poinar personal communication) and modified from previous work.[76] DNA libraries are subsequenty barcoded using indexed primers for each individual blood sample. These indexed libraries are then subjected to targeted enrichment.

Pathogen-targeted enrichment

We will use biotin-labelled RNA baits (80 bp) corresponding to unique regions in bacterial, fungal and viral species genomes. The baits are manufactured using myBaits® (Arbor Biosciences, Michigan, USA). The long list of pathogens was created via consensus decision with infectious disease specialist input. Indexed library samples will undergo hybridization with the pathogen baits for between 2-12 hours (final sensitivity testing pending) at 55-65 °C followed by magnetic purification using streptavidin-coated magnetic beads to enrich the level of pathogen versus human DNA in the sample.[77]

Sequencing

Enriched samples will undergo NGS using an Illumina HiSeq 1500flx sequencing platform in the Farncombe Family Digestive Health Research Institute, McMaster University.[47,78,79] We will use three biological replicates per sample group where possible with a minimum of two technical repetitions. Sequences will be analyzed using a proprietary pipeline that trims, merges and collapses sequences for final comparison using metagenomic analysis software. Analysis software includes DUDes, Kraken2, DIAMOND, MegaBlast and direct mapping using Burrows-Wheeler Alignment.[80] Healthy adult blood samples will serve as negative controls, and blood samples spiked with multiple bacterial strains with variable genome size and guanine or cytosine content will serve as positive controls.

Quality controls

For the cfDNA and protein C analysis, any thick, red plasma samples will be excluded due to likely hemolysis. The purity of cfDNA isolate will be assessed and reported using an absorbance (260 nm/280 nm) ratio via spectrophotometer. The Protein C ELISA will have duplicate samples where possible and concentration standards.

For every five sequencing reactions, we will include an extraction, an indexing, a library preparation and an enrichment control. All sequences found within the controls are used as a decontamination database to assess potential contamination in our clean room facilities.

Collection tubes

Our laboratory has previous experience with using citrate as the blood collection tube type for cfDNA. However, the McMaster NICU did not routinely stock a citrate blood collection tube in an appropriately small size (a microtainer), but did have an EDTA (ethylenediaminetetraacetic acid) microtainer (**Figure 2**). QIAGEN, the nucleic extraction kit producer, recommends EDTA as the collection tube type.[81] To confirm this, we analyzed the cfDNA levels of plasma in four healthy volunteers using both EDTA and citrate collection tubes. There was no significant difference in the level of cfDNA extracted between the two collection tube types (p = 0.95, paired t-test using IBM SPSS Statistics© Version 23).

Figure 2. Blood collection tubes for the FINDPATH-N study. An EDTA BD Microtainer® was selected for the biomarker collection (left). An EDTA BD Vacutainer® was selected for the NGS collection because of its sterility (right).

2.5 Analysis

Co-primary outcomes

Co-primary outcomes are described in **Table 1**.

Secondary outcomes

(1) NGS pathogen output

The types of bacteria, fungi and viruses identified will be described using both species-level terminology and higher-level taxonomy. The higher-level taxonomy patterns will be compared in relation to the adjudicated clinical subgroups (culture-proven sepsis, clinical sepsis and not septic) with descriptive statistics. Plausibility of NGS results by panel review will be reported using descriptive statistics as well as individual cases where relevant. Individual case data have the potential to suggest increased sensitivity of NGS if a blood culture-negative patient is NGS-positive for a corresponding pathogen found in a culture of the patient's cerebrospinal fluid or urine. Quantitative or semi-quantitative NGS output will be reported with descriptive statistics.

(2) cfDNA and Protein C distribution and diagnostic accuracy

Levels of cfDNA and protein C at 0 and 24 hours will be described with descriptive statistics by clinical groups. Levels of cfDNA and protein C at 0 and 24 hours will be assessed for ability to discriminate between patients who have clinical or culture-proven sepsis and those who do not. Sensitivity and specificity will be reported for multiple potential cut-off values. If there are adequate data

available, receiver operator curves will be created. Likelihood ratios will also be reported.

(3) Sensitivity and specificity of NGS vs. gold-standard aerobic blood culture

The number of species-corresponding NGS-positive and blood culture-positive samples will be divided by the total number of blood culture-positive samples to calculate the sensitivity. The sensitivity and specificity of NGS versus blood culture will be reported as a percent with an associated 95% confidence interval.

(4) *NGS laboratory process time*

The process time from thawing the sample to sequence acquisition will be recorded in hours.

(5) *Consent rate using initial exception to prior consent (deferred consent)*

Our target consent rate is ≥80% of families approached for consent.

2.6 Ethics

Exception to prior consent (deferred consent)

Informed consent is a key foundation in research ethics and is tightly regulated to protect potential research participants. Informed consent is traditionally obtained prior to inclusion in the research study. The *Tri-Council Policy Statement for the Ethical Conduct for Research Involving Humans* supports alteration to consent

requirements in particular circumstances where otherwise it would be "impossible or impracticable" to perform the research.[82] As written in the Tri-Council Policy Statement, a research ethics board must ensure the following when considering an alteration to consent requirements:

a. the research involves no more than minimum risk to the participants;
b. the alteration to consent requirements is unlikely to adversely affect the welfare of participants;
c. it is impossible or impracticable to carry out the research…if prior consent is required;
d. …the precise nature and extent of any proposed alteration is defined; and
e. the plan to provide a debriefing (if any) that may also offer participants the possibility of refusing consent and/or withdrawing data and/or human biologic materials, shall be in accordance with Article 3.7B.[82]

Deferred consent is an alteration in consent process where the informed consent happens following enrolment in the study, including collection of biological materials if applicable. Survey data and qualitative research have shown that the public is generally supportive of deferred consent; however deferred consent requires a high level of methodological and ethical rigor as well as an explanation of the rationale for deferred consent to the SDMs.[83–86]

Ethical considerations for study design

An exception to prior consent (deferred consent) will be used for the first biologic specimen because prospective consent is not feasible, sample acquisition poses very minimal harm, and research on neonatal sepsis has significant potential

future benefit. Prospective consent is not feasible because the NGS sample must be simultaneous to the current gold-standard blood culture, which is typically acquired in a timely fashion prior to antibiotic administration. Administration of antibiotics in suspected sepsis should be within one hour.[87] In the best interest of the patient, antibiotic administration should not be withheld until informed consent is obtained. Additionally, in a prospective consent model, SDMs would likely be under emotional stress and not able to optimally listen to the study team members seeking urgent informed consent.

This study poses very minimal harm to the neonate. Exception to prior consent is for a single study sample of 650 µL. Our smallest study participant would be 1 kg, making this less than one percent of our smallest study participant's estimated total blood volume. When using an exception to prior consent, there will be no additional venous pokes for the study.

Ethics approval

This study was approved by the McMaster Neonatal Research Committee on April 5, 2019 and given final approval by the Hamilton Integrated Research Ethics Board (HiREB) on August 2, 2019 (project #5869).

2.7 Funding

This work was supported by a McMaster University Pediatric Resident Research Award (Hamilton, Ontario, Canada). JAK was supported by a CIHR Canada Graduate Scholarship – Master's. AFR is supported by a Collaborative Health Research Program (CIHR/NSERC 146477) grant to develop a point of care cfDNA device. HP is supported by funding from the Boris Family. MP is supported by a CBS/CIHR New Investigator Award.

2.8 Timeline and Dissemination

Timeline

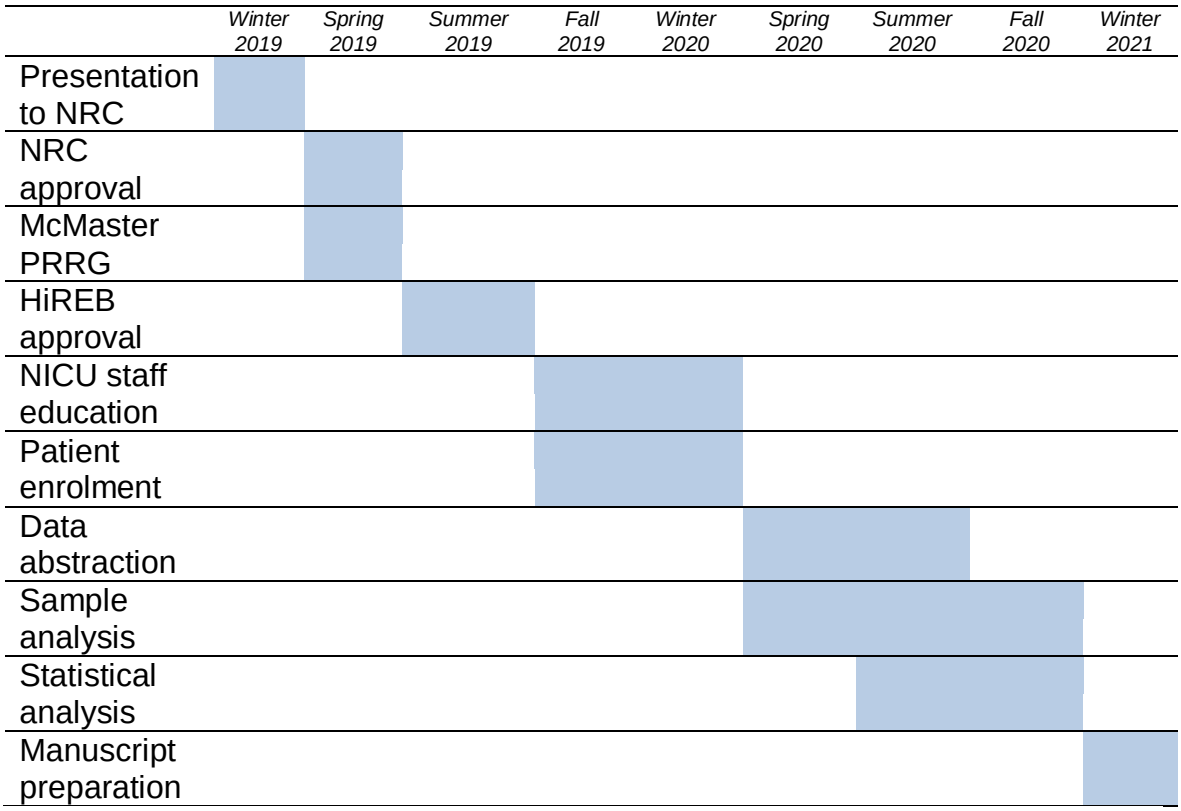

Figure 3: Research timeline
Abbreviations: NRC (Neonatal Research Committee), PRRG (pediatric resident research grant), HiREB (Hamilton Integrated Research Ethics Board)

Dissemination

We will seek publication in a peer-reviewed journal, presentation at conferences and share these data within the Canadian Critical Care Translational Biology Group. The protocol was published in *BMJ Paediatrics Open.*[68]

CHAPTER 3: STUDY AMMENDMENT AND INTERIM RESULTS

3.1 Study amendment timeline

Recruitment started on October 15, 2019 and will be complete by April 14, 2020. A study amendment was submitted to the HiREB on November 29, 2019 based on the identified issues described below. This amendment was designed to improve recruitment and improve family and nursing engagement in the study. The amendment was approved on January 2, 2020. Dates are summarized in **Figure 4**.

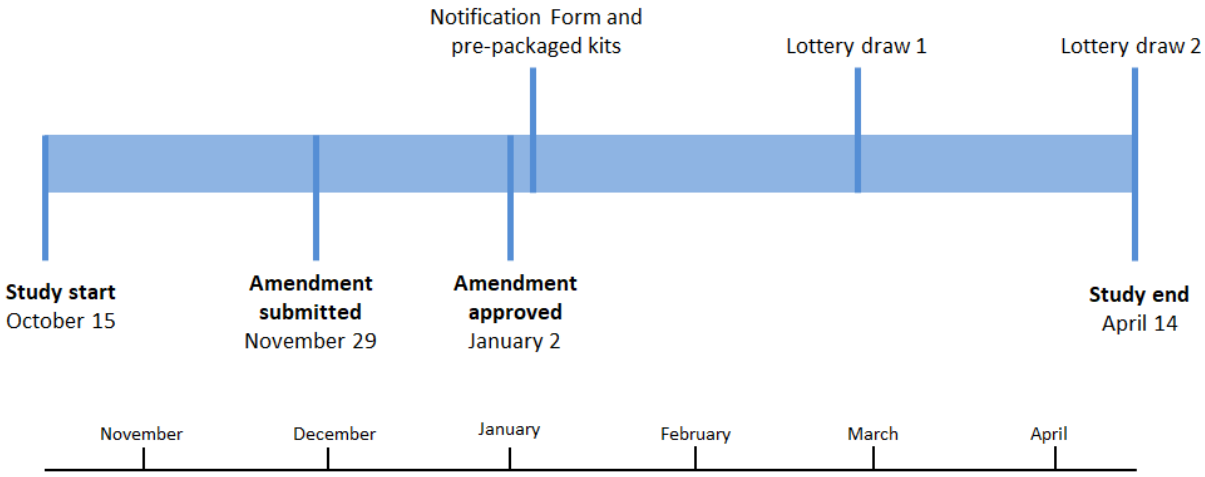

Figure 4. Timeline of study modifications

3.2 Notification form

During the first two months of the study, we received feedback from a NICU nurse and physician that in certain circumstances, it would be beneficial to have

an optional Notification Form for the first blood sample. This form would serve to give the parents some information about the study in the time between the first blood sample (collected using deferred consent) and being contacted by the study team. Specifically, if parents are at the baby's bedside during the study blood sample acquisition, which is the minority of cases, they may hear the clinical team discuss the study. The optional Notification Form (**Appendix B**) was developed with multidisciplinary involvement and approved by HiREB on January 2, 2020. The Notification Form was available in the NICU with the pre-packaged blood collection kits starting January 8, 2020 for distribution to SDMs at the discretion of the clinical team.

3.3 Incentivization for enrolment

As of November 27, 2019, our total number of first blood samples collected was 17, of which 15 were successfully consented. There was a decline in identification since study start in October. This prompted a study amendment request to perform intermittent incentivization of enrolment using a lottery system. Following approval on January 2, 2020, we announced two lottery draws for a $50 gift card amongst clinical staff who collected a first blood sample for an eligible patient. There continued to be no financial incentivization for study participants or their SDMs.

3.4 Pre-packaged blood collection kits

The initial design strategy was to exactly mirror the blood collection process with that of normal blood work. However, several phone calls to our research coordinator indicated that there was confusion around which blood collection tubes to use despite study education and the nursing information sheet. With input from the NICU nurse educator, we created pre-packaged blood collection kits for the first sample that include the two blood collection tubes, optional Notification Form and instructions for blood collection. These kits were placed in all intravenous access carts in the NICU starting January 8, 2020.

3.5 Interim feasibility results

Interim results were collected up to February 24, 2020, which corresponds to 73% study completion (19/26 total planned study weeks). The flow of potentially eligible events, starting with the total blood cultures drawn in the NICU, through the study is shown in **Figure 5**. In two cases, the study team was unable to approach the SDM within 24 hours. In one case, the neonate was discharged from hospital prior to 24 hours. In the second case, there was a screening error by the research team resulting in missing the 24-hour window.

The interim feasibility outcomes from study processes October 15, 2019 to February 24, 2020 are summarized in **Table 4.** The overall interim recruitment is below the target rate (80%) at 30/192 (16%) of eligible events. The rate of recruitment over time is shown graphically in **Figure 6**. In feedback from a NICU

fellow and the NICU nurse educator, the two primary reasons for low recruitment were missing an eligible event (due to busy clinical load, not remembering the study, etc.) and technical issues with blood collection (e.g. unable to get adequate blood for study samples) in equal amount. There were no instances of clinician refusal directly discussed with the study team.

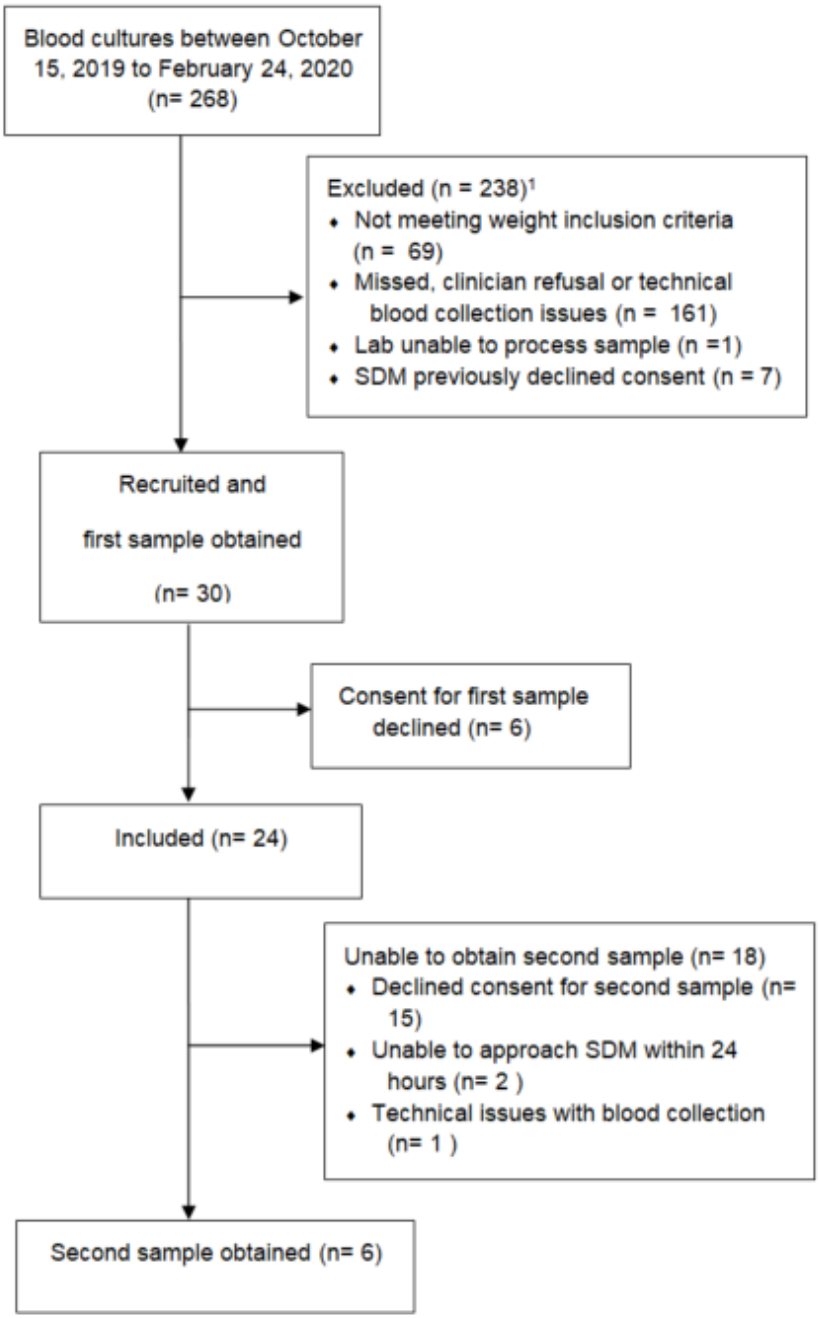

Figure 5. Study flow diagram of interim results
Abbreviations: SDM (substitute decision maker)
[1]Two of the exclusion criteria, patient apprehended by CAS and known Jehovah's witness, were not assessed retrospectively in the interim study flow diagram because they are relatively rare. These criteria will be assessed in the final analysis.

Table 4: Interim feasibility outcomes

Outcome	Definition	No. (%)
Recruitment	Proportion of eligible events that were identified and first sample collected	30/192 (16%)
Deferred consent	Proportion of successful consents from unique families approached for consent	21/25 (80%)
SDM unable to be reached within 24 hours	Proportion of events where the SDM was unable to be reached within 24 hours from total recruited events	2/30 (7%)

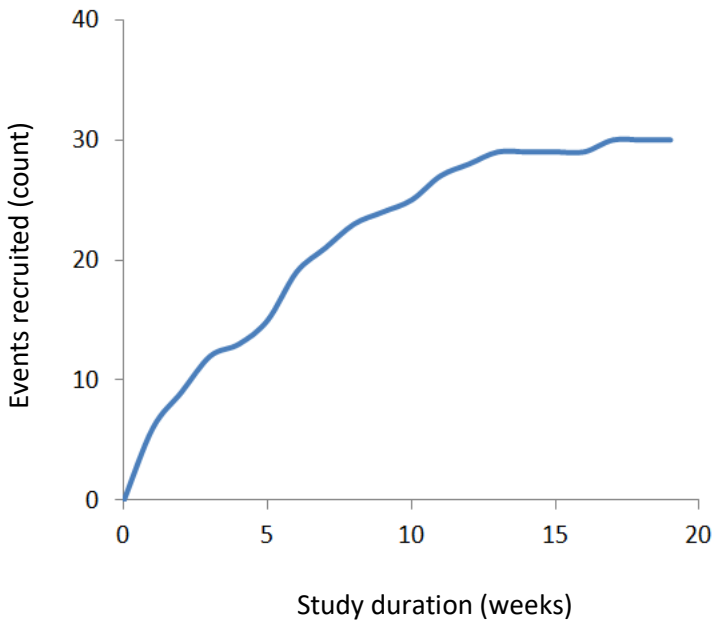

Figure 6: Recruitment over study duration using interim results

3.6 Interim patient demographics and microbiology

As of February 24, 2020, there are 24 included events from 21 unique patients. The baseline characteristics and microbiology data for these events are presented in **Table 5** and **Table 6** respectively.

Table 5: Baseline characteristics of included events from interim results

Characteristic	**Included events (n = 24)**
Day of life, days, no. (%)	
≤ 3	10/24 (42)
4-30	7/24 (29)
≥ 30	7/24 (29)
Gestational age, no. (%)	
<28 weeks	7/24 (29)
28-33+6 weeks	10/24 (42)
34-36+6 weeks	4/24 (17)
37-41+6 weeks	3/24 (13)
≥42 weeks	0/24 (0)
Corrected gestational age, no. (%)	
<28 weeks	0/24 (0)
28-33+6 weeks	16/24 (67)
34-36+6 weeks	2/24 (8)
37-41+6 weeks	6/24 (25)
≥42 weeks	0/24 (0)
Weight, kilograms, mean (SD)	1.80 (1.07)
Gender, male, no. (%)	13/24 (54)

Table 6: Clinical microbiology data of included events from interim results

Microbiology culture	**Event result (n = 24)**
Blood	
Negative	20/24 (83)
E. coli	2/24 (8)
Staph. epidermidis	1/24 (4)
Staph. aureus	1/24 (4)
Urine	
Not performed	13/24 (54)
Negative	11/24 (46)
Cerebral spinal fluid	
Not performed	21/24 (88)
Negative	3/24 (12)

CHAPTER 4: DISCUSSION AND CONCLUSION

4.1 Discussion

There is an ongoing need for a rapid and accurate diagnostic test for neonatal sepsis, which is an important cause of morbidity and mortality. Faster and more accurate testing including pathogen identification may allow for earlier identification, earlier treatment initiation, narrower antimicrobial coverage and reduced exposure to unnecessary antimicrobials in sepsis-negative cases. A foundational design consideration in the described study was to assess the diagnostic accuracy of two types of index tests (NGS and biomarkers) for neonatal sepsis. However, in this particular case, a pilot study was necessary because of unclear consent rates with deferred consent, uncertainties of performing a novel methodology on small samples of neonatal blood and the need to ensure the ability of clinical staff to identify eligible neonates rapidly. Pilot studies are used to answer scientific questions or assess the feasibility of research processes, resources and management.[67] Although commonly used to inform interventional trials, pilot studies can also be very valuable for observational studies.[88] Therefore, the co-primary objectives were to assess the feasibility of recruitment, sample collection and ability to perform NGS on blood samples of premature and term neonates with suspected sepsis.

The design, objectives and methods of this prospective, pilot cohort study have been presented. The study obtained ethics approval, started recruitment on October 15, 2019 and will be complete by April 14, 2020. An amendment was

made to the study design to address early low enrolment and implement clinical staff feedback for an optional Notification Form when using deferred consent. Interim results as of February 24, 2020 (19/26 total planned study weeks) show low recruitment at 30/192 (16%) events; however, deferred consent is at the target rate of 80% (21/25). The identified organisms in interim blood culture results (*E. coli, Staph. epidermidis and Staph. aureus*) are known pathogens in neonatal sepsis and will be clinically correlated following full data abstraction.

The design of this prospective, pilot cohort study has several strengths: (1) the study focuses on a very important topic in neonatal health with multidisciplinary input and novel methodology; (2) the study uses deferred consent to allow direct simultaneous comparison between blood culture and NGS; (3) the diagnostic accuracy measures are being assessed on the clinical population of interest, neonates with suspected sepsis; (4) the inclusion and exclusion criteria are simple and relatively objective; and (5) the biomarkers, which are often dynamic, are assessed at two time points.

Although deferred consent was required in the design to rapidly obtain blood samples, use of deferred consent requires thoughtful and rigorous implementation, monitoring, staff education and explanation to families. The study amendment to include a Notification Form highlights the importance of close communication so that both the NICU staff and families feel comfortable with the consent process. Unfortunately, the use of deferred consent and the fact

that eligible events occur any time of day or night create a reliance on rapid identification by non-study personnel such as nurses, nurse practitioners and doctors. In addition, enrolled patients must then be approached for consent within 24 hours so as not to miss the second sample, requiring a research coordinator to be available all days of the week. The interim rate of successful deferred consent was 80% (21/25), which is at our target of 80% and similar to reported rates of 83-84% in two pediatric trials using deferred consent.[89,90] Approaching a SDM later than the 24-hour window was a rare occurrence (2/30, 7%). This suggests successful implementation of our deferred consent design in this study. Future considerations include engaging parents at the study and ethics board level for studies using deferred consent. Though generally recommended, there is a paucity of evidence on the effect of parental engagement in neonatal or pediatric research with deferred consent.[91,92]

A potential limitation of the study design as a cohort study is that there are no blood samples from a group of completely healthy, asymptomatic neonates. Careful analysis and interpretation of NGS output is required because blood culture is an imperfect gold standard, the presence of pathogen DNA does not imply infection, and NGS is likely a highly sensitive test.[49] Prior to neonatal sample analysis, our team will complete work to calibrate the NGS output using healthy and septic adult blood samples. Planned analyses for this study include comparing the type of pathogens between septic and non-septic neonates, comparing NGS results to blood culture, and also presenting any cases that

suggest increased sensitivity and true infection (e.g. blood culture negative, urine positive, blood NGS positive to corresponding urine pathogen). In keeping with this being a pilot study, the primary outcomes are feasibility. This work may generate hypotheses into the etiology of some cases of 'culture negative sepsis', but ultimately would require further research that may include healthy neonatal controls and larger study design to confirm and answer this important question.

Including neonates with both late and early onset sepsis allows assessment across a larger spectrum of patients and increases the pool of eligible patients; however, there are pathophysiological differences in these two entities that may result in different test performance. A subgroup analysis will be performed, should numbers allow. An additional limitation is that participant blood culture volume was not recorded, which is the reference test. Lower blood culture volumes, especially less than 0.5 ml, have reduced sensitivity.[42] However, the McMaster NICU has a minimum blood culture volume of 0.5 ml in place and neonates with inadequate blood culture volumes would likely not have enough blood to also obtain a NGS sample from the same attempt.

Low enrolment is a common issue among prospective trials.[93] This study relies on identification from the clinical team. The McMaster NICU has a large group of nurses, making complete staff education difficult, and is a very busy clinical unit. In addition, obtaining adequate blood from neonates is challenging and there are no additional pokes via deferred consent. Technical issues with collecting the

blood samples were one of the reasons given by clinical personnel for non-enrolment of patients. Although never directly discussed with the research team or nurse educator, neonatal and pediatric nurses can have negative views towards research.[94] Research on low study recruitment has focused on both low consent success and also low eligible participant recruitment, the latter seen in this study's interim feasibility results. A thematic meta-synthesis identified the following as potential facilitators of recruitment: regular research reminders, recruitment incentives, providing additional time for recruitment, assigning labour-intensive parts of recruitment to research personnel, and appropriately training potential recruiters.[95] Many of these were addressed in the initial study design and amendment. The effect of incentivization and increased reminders on enrolment will be assessed in the final feasibility results. Further research could explore and quantify the barriers of recruitment involving rapid identification, neonatal blood sample collection and deferred consent in a busy NICU.

4.2 Conclusions

Neonatal sepsis is an important cause of morbidity and mortality. There remains a knowledge gap in our ability to rapidly and accurately identify neonatal sepsis. Studying diagnostic measures in neonatal sepsis is complicated by an imperfect reference standard (blood culture), lack of consensus definition of neonatal sepsis, nonspecific disease presentation and an evolving understanding of the etiology behind 'culture-negative sepsis'. Both NGS and the biomarkers cfDNA and protein C have potential to improve care for neonates with sepsis through, respectively, rapid and sensitive identification of pathogens and improved diagnostic accuracy. We have presented the design, amendments and interim feasibility results of a prospective, pilot cohort study to assess the feasibility and pathogen detection patterns of NGS in neonates with suspected sepsis. Interim results identify low enrolment; however, the rate of successful deferred consent met the target of 80%. Future directions are to complete the pilot study, followed by sample analysis, data abstraction and statistical analysis, with anticipated completion in winter 2021. The results of this study could serve as a first step to demonstrate the feasibility and value of NGS for the neonatal population.

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Appendix A: Case report form

Hamilton Health Sciences | McMaster University | FINDPATH-N

Case Report Form

Patient ID # ___ ___ ___

Issues/Notes:

By signing, I confirm that the data collected below are complete and accurate to the best of my ability.

Printed Name _____ Signature _____ Date _____

PATIENT DEMOGRAPHICS & VISIT DETAILS

1. Age at time of sample collection (days): _____ days
2. Corrected gestational age at time of sample collection: _____weeks and _____days
3. Current weight at time of sample collection: _____ grams
4. Sex: [ ] Male [ ] Female [ ] Other
5. Location of patient at time of sample collection?
   [ ] Level 2 NICU [ ] Level 3 NICU
6. NICU admission date for the stay associated with sample collection
   (dd/mmm(letters)/yy): __ __/__ __ __/__ __
7. NICU admission diagnosis: _____
8. Transferred from another institution? [ ] Yes [ ] No
9. Was the patient previously included in the FINDPATH-N study?
   [ ] Yes (previous ID# __ __ __, ID# __ __ __, ID# __ __ __) [ ] No
10. If new patient, was consent obtained for? [ ] sample 1 only OR [ ] ongoing consent

STUDY SAMPLE DETAILS

1. Samples collected: [ ] NGS (sample 1) [ ] biomarkers (sample 1)
   [ ] biomarkers (sample 2)

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Hamilton Health Sciences | McMaster University | FINDPATH-N

Case Report Form

Patient ID # ___ ___ ___

Sample 1

2. Date and time of sample 1: (dd/mmm(letters)/yy): __ __/__ __ __/__ __

   Time (24 hour clock, hh:mm): ____:____

3. Source blood sample 1: [ ] Arterial [ ] UVC [ ] venous poke [ ] capillary

Sample 2

4. Date and time of sample 2: (dd/mmm(letters)/yy): __ __/__ __ __/__ __

   Time (24 hour clock, hh:mm): ____:____

5. Time difference from sample 1 (hours to one decimal): _____ hours

6. Source blood sample 2: [ ] Arterial [ ] UVC [ ] venous poke [ ] capillary

CLINICAL MICROBIOLOGY DETAILS

Cultures and Virology (within day -7 to day +7)

1. Were any microbiology **cultures** ordered for the patient +/- 7 days from sample 1?

   [ ] Yes [ ] No

Cultured Organism Result ("Negative" if none)	Site of Culture	Date Culture Drawn (dd/mmm(letters)/yy)	Duration of Time until Growth (hours)
		__ __/__ __ __/__ __	
		__ __/__ __ __/__ __	
		__ __/__ __ __/__ __	
		__ __/__ __ __/__ __	
		__ __/__ __ __/__ __	
		__ __/__ __ __/__ __	
		__ __/__ __ __/__ __	

2. Were any **virology** tests (ex. nasopharyngeal swab, HSV, enterovirus, VZV) ordered for the patient +/- 7 days from sample 1? [ ] Yes [ ] No

Hamilton Health Sciences McMaster University FINDPATH-N **Case Report Form**

Patient ID # ___ ___ ___

Virus Identified ("Negative" if none)	Site of Swab	Date Swab Taken (dd/mmm(letters)/yy)
		__ __/__ __ __/__ __
		__ __/__ __ __/__ __
		__ __/__ __ __/__ __
		__ __/__ __ __/__ __

3. Was there a **urinalysis** ordered for the patient +/- 7 days from sample 1?

[ ] Yes [ ] No

Date Urine Taken (dd/mmm(letters)/yy)	Catheter specimen?	Leukocytes	WBC count (microscopy)
__ __/__ __ __/__ __	[ ] Yes [ ] No		
__ __/__ __ __/__ __	[ ] Yes [ ] No		
__ __/__ __ __/__ __	[ ] Yes [ ] No		

4. Was there a **CSF analysis** ordered for the patient +/- 7 days from sample 1?

[ ] Yes [ ] No

Date CSF Drawn (dd/mmm(letters)/yy)	Glucose	WBC count	RBC count
__ __/__ __ __/__ __			
__ __/__ __ __/__ __			

5. Was there a central line in place at any point in the day for the 48 hours around sample 1?

[ ] No central line [ ] Yes, within 24 hours before [ ] No, within 24 hours after

Hamilton Health Sciences McMaster University FINDPATH-N **Case Report Form**

Patient ID # ___ ___ ___

PERINATAL DETAILS

1. Birth Weight: _____grams
2. Gestational Age: _____weeks and _____ days
3. Multiple gestation: [ ] Yes [ ] No
4. APGARS: 1 minute: _____, 5 minutes: _____, 10 minutes: _____
5. Mom GBS + (vagina-rectal swab or history of GBS bacturia): [ ] Yes [ ] No
6. Prolonged rupture of membranes (>18 hours): [ ] Yes [ ] No
7. Mom chorioamnionitis: [ ] Yes [ ] No

COMORBIDITIES

1. HIE	Yes / No	Unable to assess
2. Intraventricular haemorrhage or cyst	Yes / No	Unable to assess
3. Other neurologic (ex. prior meningitis)	Yes / No	Unable to assess
4. Chronic lung disease	Yes / No	Unable to assess
5. Other lung disease (ex. pneumothorax)	Yes / No	Unable to assess
6. Congenital diaphragmatic hernia	Yes / No	Unable to assess
7. Necrotizing enterocolitis	Yes / No	Unable to assess
8. Other gastrointestinal (ex. omphalocele, severe GERD)	Yes / No	Unable to assess
9. Major cardiac structural anomaly (ex. TOF)	Yes / No	Unable to assess
10. Other cardiac (ex. arrhythmia, PDA, ASD)	Yes / No	Unable to assess
11. Major kidney malformation (ex. missing kidney, polycystic kidney)	Yes / No	Unable to assess
12. Other kidney problem (ex. nephrolithiasis)	Yes / No	Unable to assess
13. Hematologic or immune problem	Yes / No	Unable to assess
14. Metabolic problem	Yes / No	Unable to assess

Hamilton Health Sciences McMaster University FINDPATH-N **Case Report Form**

Patient ID # ___ ___ ___

CLINICAL CONCERNS

As per MD and RN documentation.

Concern (Y/N)	At Sample 1	Sample 1 to 24 hours after	24-48 hours after
Respiratory distress or apnea	Yes / No	Yes / No	Yes / No
Tachycardia or bradycardia	Yes / No	Yes / No	Yes / No
Hypotension or poor perfusion	Yes / No	Yes / No	Yes / No
Hypothermia or hyperthermia	Yes / No	Yes / No	Yes / No
Seizures	Yes / No	Yes / No	Yes / No
Hypotonia	Yes / No	Yes / No	Yes / No
Irritability or lethargy	Yes / No	Yes / No	Yes / No
Vomiting, feed intolerance or new bloody stools	Yes / No	Yes / No	Yes / No

DAILY DATA

All **procalcitonin (PCT)** values from Sample 1 to 48 hours later.

Value	**Date** dd/mmm(letters)/yy	**Time** 24 hour clock, hh:mm)	**Time after sample 1** hours

All **C-reactive protein (CRP)** values from Sample 1 to 48 hours later.

Value	**Date** dd/mmm(letters)/yy	**Time** 24 hour clock, hh:mm)	**Time after sample 1** hours

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Hamilton Health Sciences McMaster University FINDPATH-N **Case Report Form**

Patient ID # ___ ___ ___

All **white blood cell (WBC/LCKS)** values from Sample 1 to 48 hours later.

Value	**Date** dd/mmm(letters)/yy	**Time** 24 hour clock, hh:mm)	**Time after sample 1** (hours)

Sample 1 date and time: (dd/mmm(letters)/yy): __ __/__ __ __/__ __ (hh:mm): ____:____

Laboratory Data	24 hours before Sample 1	Sample 1 to 24 hours after	24-48 hours after
Leukocytes (LKCS) (highest)			
Leukocytes (LKCS) (lowest)			
Platelets (PLT) (lowest, not clotted)			
Creatinine (highest)			
Bilirubin (highest)			
Lactate (highest, non-hemolyzed)			
pH (lowest)			

Hamilton Health Sciences McMaster University FINDPATH-N **Case Report Form**

Patient ID # ___ ___ ___

Bicarbonate (lowest)			
Glucose (lowest)			
Glucose (highest)			

Vitals & Patient Status	24 hours before	Sample 1 to 24 hours after	24-48 hours after
15. Lowest temperature and source (Celcius)			
16. Highest temperature and source (Celcius)			
17. MAP (lowest)			
18. Highest systolic BP			
19. Lowest systolic BP			
20. Vasopressor use at any time?	Yes / No	Yes / No	Yes / No
21. CPR/arrest at any time?	Yes / No	Yes / No	Yes / No
22. Supplemental oxygen	Yes / No	Yes / No	Yes / No
23. FiO2 (highest, %)			
24. Positive pressure ventilation in any form at any time?	Yes / No	Yes / No	Yes / No
25. Mechanically ventilated and intubated at any time?	Yes / No	Yes / No	Yes / No
26. Glasgow coma scale score (lowest)			
27. Blood (PRBC) transfusion?	Yes / No	Yes / No	Yes / No
28. Any other blood product transfusion? (Y/N)	Yes / No	Yes / No	Yes / No

ANTIBIOTICS

1. Were antibiotics administered up to 48 hours *before* sample 1? [ ] Yes [ ] No

Hamilton Health Sciences McMaster University FINDPATH-N **Case Report Form**

Patient ID # ___ ___ ___

Antibiotic Administered	Date First Dose (dd/mmm(letters)/yy)	Date Last Dose (dd/mmm(letters)/yy)
	__ __/__ __ __/__ __	__ __/__ __ __/__ __
	__ __/__ __ __/__ __	__ __/__ __ __/__ __
	__ __/__ __ __/__ __	__ __/__ __ __/__ __
	__ __/__ __ __/__ __	__ __/__ __ __/__ __

2. Were antibiotics administered up to 7 days *after* sample 1? [ ] Yes [ ] No

Antibiotic Administered	Date First Dose (dd/mmm(letters)/yy)	Date Last Dose (dd/mmm(letters)/yy)
	__ __/__ __ __/__ __	__ __/__ __ __/__ __
	__ __/__ __ __/__ __	__ __/__ __ __/__ __
	__ __/__ __ __/__ __	__ __/__ __ __/__ __
	__ __/__ __ __/__ __	__ __/__ __ __/__ __
	__ __/__ __ __/__ __	__ __/__ __ __/__ __
	__ __/__ __ __/__ __	__ __/__ __ __/__ __
	__ __/__ __ __/__ __	__ __/__ __ __/__ __
	__ __/__ __ __/__ __	__ __/__ __ __/__ __

3. How long were any antibiotics continued after Sample 1?

[ ] 48 hours or less [ ] 2-4 days [ ] 5-7 days [ ] 8 or more days

[ ] no antibiotics after sample 1

INFECTION

1. Clinical team's *final* diagnos(es) (can pick multiple):

Diagnosis	YES	NO	Team unsure, "query"

Hamilton Health Sciences McMaster University FINDPATH-N **Case Report Form**

Patient ID # ___ ___ ___

1. Meningitis or encephalitis			
2. Sepsis			
3. Endocarditis			
4. Cellulitis/soft tissue infection or abscess			
5. Pneumonia			
6. Urinary Tract Infection			
7. Necrotizing enterocolitis			
8. Viral infection			

STATUS AT 30 DAYS

1. Discharged from McMaster NICU? [ ] Yes [ ] No
   Date ICU discharge (dd/mmm(letters)/yy): __ __/__ __ __/__ __
2. Discharged from hospital? [ ] Yes [ ] No
   Date hospital discharge (dd/mmm(letters)/yy): __ __/__ __ __/__ __
3. Final status at 30 days: [ ] Alive [ ] Deceased
4. Date of death, if within 30 days (dd/mmm(letters)/yy): __ __/__ __ __/__ __
5. Location of death: [ ] NICU [ ] Ward [ ] Other (specify: _____)

Appendix B: Notification form

FINDPATH-N

McMaster University

Fast Identification of Pathogens in Neonate (FINDPATH-N) Study

PARENT (OR DECISION MAKER) NOTIFICATION SHEET

We would like to inform you that your baby is a candidate for participation in a study called FINDPATH-N. This study has been approved by the Hamilton Integrated Research Ethics Board and the McMaster Neonatal Research Committee.

It is very important to know that this study will *not* affect the treatment your baby receives. The purpose of this study is to try to improve detecting infection in babies. The medical team taking care of your baby have ordered a test called a 'blood culture' to look for infection. An additional very small sample of blood (less than 1/6th of a teaspoon) was collected for this study at the same time as the 'blood culture'.

The blood sample collection is very time sensitive, meaning there was not enough time to fully discuss and explain the study before taking a small sample of blood. A member of our research team will contact you within the next day to provide more information and answer any and all questions. This process is called deferred consent and has also been approved by the Hamilton Integrated Research Ethics Board and the McMaster Neonatal Research Committee. Once you have been fully informed about the study, you will decide whether or not to allow your baby to be included in the study.

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November 29th, 2019. Version 1.0.