Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Protocol for: Chen RY, Mostafa I, Hibberd MC, et al. A microbiota-directed food intervention for undernourished children. N Engl J Med 2021;384:1517-28. DOI: 10.1056/NEJMoa2023294

This supplement contains the following items:

1. Original Protocol, Final Protocol, Summary of Changes-Protocol

2. Original Statistical Analysis Plan. No amendments made.

1. Original Protocol, Final Protocol, Summary of Changes-Protocol

Original Protocol

icddr,b	**RRC APPLICATION FORM**

RESEARCH PROTOCOL **Number: PR-18073** **Version No. 1.2** **Version date: 22-10-2018**	**FOR OFFICE USE ONLY**		
	RRC Approval:	☒ Yes	☐ No	Date: 13-09-2018
	ERC Approval:	☐ Yes	☐ No	Date: 23-10-2018
	AEEC Approval:	☐ Yes	☐ No	Date:
	External IRB Approval	☐ Yes	☐ No	Date:
	Name of External IRB: _____			

Protocol Title: * (maximum 250 characters including space**)**
Community-based clinical trial with microbiota-directed complementary foods (MDCFs) made of locally available food ingredients for the management of children with primary moderate acute malnutrition

Short Title: (maximum **100** characters including space)
Microbiota-directed complementary food (MDCF) trial

Key Words: * Microbiota, complementary food

Name of the Research Division Hosting the Protocol: *

- ☐ Health Systems and Population Studies Division (HSPSD)
- ☒ Nutrition and Clinical Services Division (NCSD)
- ☐ Infectious Diseases Division (IDD)
- ☐ Maternal and Child Health Division (MCHD)
- ☐ Laboratory Sciences and Services Division (LSSD)
- ☐ Other (specify) _____

Has the Protocol been Derived from an Activity: * ☒ No ☐ Yes (please provide following information):

Activity No. :
Activity Title:
PI:
Grant No.: Budget Code: Start Date: End Date:

icddr,b Strategic Priority/ Initiative (SP 2015-8):* (check all that apply**)**

- ☐ Reducing maternal and neonatal mortality
- ☐ Controlling enteric and respiratory infections
- ☒ Preventing and treating maternal and childhood malnutrition
- ☐ Detecting and controlling emerging and re-emerging infections
- ☐ Achieving universal health coverage
- ☐ Examining the health consequences of climate change
- ☐ Preventing and treating non-communicable diseases
- ☐ Others (specify) _____

Research Phase (4 Ds): * (check all that apply**)**

- ☒ Discovery
- ☒ Development
- ☒ Delivery
- ☐ Evaluation of Delivery

Anticipated Impact of Research: * (check all that apply and please provide details below**)**

- ☒ Knowledge Production
- ☒ Capacity Building
- ☒ Informing Policy
- ☒ Health and Health Sector Benefits
- ☐ Economic Benefits

Please provie details here:

Which of the Sustainable Development Goal This Protocol Relates to?:* (check all that apply**)**

- ☐ 1. End poverty in all its forms everywhere
- ☒ 2. End hunger, achieve food security and improved nutrition and promote sustainable agriculture
- ☒ 3. Ensure healthy lives and promote well-being for all at all ages
- ☐ 4. Ensure inclusive and equitable quality education and promote lifelong learning opportunities for all
- ☐ 5. Achieve gender equality and empower all women and girls
- ☐ 6. Ensure availability and sustainable management of water and sanitation for all
- ☐ 7. Ensure access to affordable, reliable, sustainable and modern energy for all
- ☐ 8. Promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all
- ☐ 9. Build resilient infrastructure, promote inclusive and sustainable industrialization and foster innovation
- ☐ 10. Reduce inequality within and among countries
- ☐ 11. Make cities and human settlements inclusive, safe, resilient and sustainable
- ☐ 12. Ensure sustainable consumption and production patterns
- ☐ 13. Take urgent action to combat climate change and its impacts
- ☐ 14. Conserve and sustainably use the oceans, seas and marine resources for sustainable development
- ☐ 15. Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss
- ☐ 16. Promote peaceful and inclusive societies for sustainable development, provide access to justice for all and build effective, accountable and inclusive institutions at all levels
- ☒ 17. Strengthen the means of implementation and revitalize the global partnership for sustainable development

Does this Protocol Use the Gender Framework:* (Please visit: http://shetu.icddrb.org/index.php?option=com_content&view=article&id=265&Itemid=677 for Gender Alanysis Tool with instructions)	☒ Yes (please complete Gender Analysis Tool) ☐ No
If 'no' is the response, its reason(s) in brief:	
Will this Research Specifically Benefit the Disadvantaged (economically, socially and/or otherwise**):**	☒ Yes ☐ No
Does this Protocol use Behaviour Change Communication:	☐ Yes ☒ No

Principal Investigator (Should be icddr,b staff):* Sex ☐ Female ☒ Male

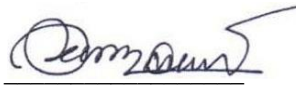
Dr Tahmeed Ahmed
MBBS, PhD
Senior Director and Senior Scientist
Email: tahmeed@icddrb.org
Do you have ethics certification? ☐ No ☒ Yes (please attach in your CV below)
Do you have RBM training certification? ☐ No ☒ Yes (please attach the certificate with CV below)

Primary Scientific Division of the PI

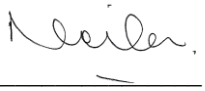
Nutrition and Clinical Services Division, icddr,b

Co-Principal Investigator(s) Internal: Sex ☐ Female ☒ Male **Dr Md. Munirul Islam** MBBS, PhD Scientist 880-2-9827001, Ext-2352 Email: mislam@icddrb.org Signature or written consent of Co-PI: _____ (electronic signature or email or any sort of written consent) [if more than one, please copy and paste this row for additional Co-PIs] Do you have ethics certification? ☐ No ☒ Yes (please attach in your CV below) Do you have RBM training certification? ☐ No ☒ Yes (please attach the certificate with CV below)	Primary Scientific Division/ Programme of the Co-PI Nutrition and Clinical Services Division, icddr,b _____ Approval of the Respective Senior Director/ Programme Head (Signature)
Co-Investigator(s) - Internal: Sex ☒ Female ☐ Male **Dr Sayeeda Huq** Associate Scientist MBBS, MIPH Email: sayeeda@icddrb.org Signature or written consent of Co-I: _____ (electronic signature or email or any sort of written consent) [if more than one, please copy and paste this row for additional Co-Is Do you have ethics certification? ☐ No ☒ Yes (please attach in your CV below) Do you have RBM training certification? ☐ No ☒ Yes (please attach the certificate with CV below)	Primary Scientific Division of the Co-I Nutrition and Clinical Services Division, icddr,b
Co-Investigator(s) – Internal: Sex ☐ Female ☒ Male **Dr Mustafa Mahfuz** MBBS, MPH Associate Scientist Email: mustafa@icddrb.org Signature or written consent of Co-I: _____ (electronic signature or email or any sort of written consent) [if more than one, please copy and paste this row for additional Co-Is]	
Co-Investigator(s) – Internal: Sex ☒ Female ☐ Male **Dr Ishita Mostafa** BDS, MPH Research Investigator, Ext-2313 Email: ishita.mostafa@icddrb.org Signature or written consent of Co-I: _____ (electronic signature or email or any sort of written consent) [if more than one, please copy and paste this row for additional Co-Is]	

Co-Investigator(s) – Internal: Sex ☐ Female ☒ Male

Dr Imteaz Mahmud
MBBS, MPH
Research fellow
Email: imteaz.mahmud@icddrb.org
Signature or written consent of Co-I: 
(electronic signature or email or any sort of written consent)
[if more than one, please copy and paste this row for additional Co-Is]

Co-Investigator(s) – Internal: Sex ☒ Female ☐ Male

Dr Nurun Nahar Naila
MBBS, MPH
Assistant Scientist
Email: nurun.nahar@icddrb.org
Signature or written consent of Co-I: 
(electronic signature or email or any sort of written consent)
[if more than one, please copy and paste this row for additional Co-Is]

Collaborating Institute(s): Please provide full official address

Institution # 1

Country	USA
Contact person	**Prof Jeffrey I. Gordon**
Department (including Division, Centre, Unit)	Director, Center for Genome Sciences & Systems Biology
Institution (with official address)	Washington University School of Medicine 4444 Forest Park Expressway Campus Box 8510 St. Louis, MO 63108 Phone: +1 314-362-7243 FAX: +1314-362-7047 Email: jgordon@wustl.edu
Directorate (in case of GoB i.e. DGHS)	N/A
Ministry (in case of GoB)	N/A

Institution # 2

Country	
Contact person	
Department (including Division, Centre, Unit)	
Institution (with official address)	
Directorate (in case of GoB i.e. DGHS)	
Ministry (in case of GoB)	

Institution # 3

Country	
Contact person	
Department (including Division, Centre, Unit)	
Institution (with official address)	
Directorate (in case of GoB i.e. DGHS)	
Ministry (in case of GoB)	

Note: If less than or more than three collaborating institutions, please delete or insert blocks as needed.

Contribution by the Members of the Scientific Team:

Members' Name	Contribution								
	Research idea/ concept	Study design	Protocol writing	Respond to external reviewers' comments	Defending at IRB	Developing data collection Tool(s)	Data Collection	Data analysis/ interpretation of results	Manuscript writing
Dr Tahmeed Ahmed	☒	☒	☒	☒	☒	☒	☐	☒	☒
Prof Jeffrey I. Gordon	☒	☒	☒	☒	☐	☒	☐	☒	☒
Dr Michael Barratt	☒	☒	☒	☒	☐	☒	☐	☒	☒
Dr Md Munirul Islam	☐	☒	☒	☒	☒	☒	☐	☒	☒
Dr Sayeeda Huq	☐	☐	☐	☒	☒	☒	☐	☒	☒
Dr Mustafa Mahfuz	☐	☐	☐	☒	☒	☒	☐	☒	☒
Dr Ishita Mostafa	☐	☐	☒	☒	☒	☒	☒	☒	☒
Dr Imteaz Mahmud	☐	☐	☒	☒	☒	☒	☒	☒	☒
Dr Nurun Nahar Naila	☐	☐	☒	☒	☒	☒	☒	☒	☒
	☐	☐	☐	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐	☐	☐	☐

Study Population: Sex, Age, Special Group and Ethnicity

Research Subject:
- ☒ Human
- ☐ Animal
- ☐ Microorganism
- ☐ Other (specify): _____

Sex:
- ☒ Male
- ☒ Female
- ☐ Transgender

Age:
- ☒ 0 – 4 years
- ☐ 5 – 10 years
- ☐ 11 – 17 years
- ☐ 18 – 64 years
- ☐ 65 +

Special Group:
- ☐ Pregnant Women
- ☐ Fetuses
- ☐ Prisoners
- ☐ Destitutes
- ☐ Service Providers
- ☐ Cognitively Impaired
- ☐ CSW
- ☐ Expatriates
- ☐ Immigrants
- ☐ Refugee
- ☐ Others (specify): _____

Ethnicity:
- ☒ No ethnic selection (Bangladeshi)
- ☐ Bangalee
- ☐ Tribal group
- ☐ Other (specify): _____

NOTE: It is icddr.b's policy to include men, women, children and transgender in its research projects involving participation of humans, unless there is strong justification(s) for their exclusion.

Consent Process: (Check all that apply**)**
- ☒ Written
- ☐ Oral
- ☐ Audio
- ☐ Video
- ☐ None

Language:
- ☒ Bangla
- ☒ English
- ☐ Other (specify: _____

Project/Study Site: (Check all that apply**)**
- ☐ Chakaria
- ☐ Bandarban
- ☒ Dhaka Hospital
- ☐ Kamalapur Field Site/HDSS
- ☒ Mirpur (Dhaka)
- ☐ Matlab DSS Area
- ☐ Matlab non-DSS Area
- ☐ Matlab Hospital
- ☐ Mirzapur
- ☐ Bianibazar (Sylhet)
- ☐ Kanaighat (Sylhet)
- ☐ Jakigonj (Sylhet)
- ☐ Other community in Dhaka
  Name:
- ☒ Other sites in Bangladesh
  Name: Kurigram
- ☐ Multi-national Study
  Name of the country_____

Project/Study Type: (Check all that apply)

- ☐ Case Control Study
- ☒ Clinical Trial (Hospital/Clinic/Field)*
- ☐ Community-based Trial/Intervention
- ☐ Cross Sectional Survey
- ☐ Family Follow-up Study
- ☐ Longitudinal Study (cohort or follow-up)
- ☐ Meta-analysis
- ☐ Programme Evaluation
- ☐ Programme (Umbrella Project)
- ☐ Prophylactic Trial
- ☐ Record Review
- ☐ Secondary Data Analysis
  Protocol No. of Data Source: _____
- ☐ Surveillance/Monitoring
- ☐ Systematic Review
- ☐ Other (specify): _____

***Note**: International Committee of Medical Journal Editors (ICMJE) defines Clinical Trial as "*Any research project that prospectively assigns human participants to intervention and comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome*".

PI of the RRC- and ERC-approved Clinical Trials should provide necessary information to IRB Secretariat (Research Administration) for registration and uploading into relevant websites (usually at the https://register.clinicaltrials.gov/). They should also provide relevant information to the IRB Secretariat in the event of amendment/modification after their approval by RRC and ERC.

Biological Specimen:

a) Will the biological specimen be stored for future use?	☒ Yes ☐ No ☐ Not applicable
b) If the response is 'yes', how long the specimens will be preserved?	5 years
c) What types of tests will be carried out with the preserved specimens?	Analyzing and examining the gut bacteria and their metabolites and enteropathogens in collected fecal, urine and plasma samples
d) Will the consent be obtained from the study participants for use of the preserved specimen for other initiative(s) unrelated to this study, without their re-consent?	☒ Yes ☐ No ☐ Not applicable
e) Will the specimens be shipped to other country/ countries? If yes, name of institution(s) and country/countries.	☒ Yes ☐ No ☐ Not applicable Gordon Lab, Washington University School of Medicine, Washington University in St. Louis, USA
f) If shipped to another country, will the surplus/unused specimen be returned to icddr,b? If the response is 'no', then the surplus/unused specimen must be destroyed.	☐ Yes ☒ No ☐ Not applicable
g) Who will be the custodian of the specimen at icddr,b?	Dr Tahmeed Ahmed Senior Director and Senior Scientist Nutrition and Clinical Services Division, icddr,b
h) Who will be the custodian of the specimen when shipped outside Bangladesh?	Prof Jeffrey I. Gordon Washington University School of Medicine 4444 Forest Park Expressway Campus Box 8510 St. Louis, MO 63108, USA Phone: +1 314-362-7243 Fax: +1314-362-7047 Email: jgordon@wustl.edu
i) Who will be the owner(s) of the specimens?	Dr Tahmeed Ahmed Senior Director and Senior Scientist Nutrition and Clinical Services Division, icddr,b
j) Has a MoU been signed with regards to collection, storage, use and ownership of specimen? If the response is 'yes', please attach a copy of the MoU.. If the response is 'no', appropriate justification should be provided for not signing a MoU.	☒ Yes ☐ No ☐ Not applicable

Proposed Sample Size:
Sub-group (Name of subgroup e.g. Men, Women) and Number

Name	Number	Name	Number
Arm 1: 12-18 months old children with primary MAM (WLZ <-2 to -3)	62		
Arm 2: 12-18 months old children with primary MAM (WLZ <-2 to -3)	62		
Total sample size	124		

Determination of Risk: Does the Research Involve (Check all that apply**)**

- ☐ Human exposure to radioactive agents?
- ☐ Foetal tissue or abortus?
- ☐ Investigational new device?
  Specify: _____
- ☐ Existing data available from Co-investigator?
- ☐ Human exposure to infectious agents?
- ☐ Investigational new drug?
- ☐ Existing data available via public archives/sources?
- ☒ Pathological or diagnostic clinical specimen only?
- ☐ Observation of public behaviour?
- ☐ New treatment regime?

	Yes	No
Will the information be recorded in such a manner that study participants can be identified from the information directly or through identifiers linked to the study participants?	☐	☒
Does the research deal with sensitive aspects of the study participants' sexual behaviour, alcohol use or illegal conduct such as drug use?	☐	☒

Could information on study participants, if available to people outside of the research team:

	Yes	No
a) Place them at risk of criminal or civil liability?	☐	☒
b) Damage their financial standing, reputation or employability, or social rejection, or lead to stigma, divorce etc.?	☐	☒

Do you consider this research: (check one)

☐ Greater than minimal risk	☒ No more than minimal risk	☐ Only part of the diagnostic test

Note: Minimal Risk: The probability and the magnitude of the anticipated harm or discomfort to participants is not greater than those ordinarily encountered in daily life or during the performance of routine physical, psychological examinations or tests, e.g. the risk of drawing a small amount of blood from a healthy individual for research purposes is no greater than when the same is performed for routine management of patients.

Risk Group of Infectious Agent and Use of Recombinant DNA	
a) Will specimens containing infectious agent be collected?	☒ Yes ☐ No ☐ Not applicable
b) Will the study involve amplification by culture of infectious agents?	☒ Yes ☐ No ☐ Not applicable
c) If response to questions (a) and/or (b) is 'yes', to which Risk Group (RG) does the agent(s) belong? (Please visit http://shetu.icddrb.org/index.php?option=com_content&view=article&id=265&Itemid=677 to review list of microorganism by Risk Group)	☒ RG1 ☐ RG2 ☐ RG3 ☐ RG4
d) Does the study involve experiments with recombinant DNA?	☒ Yes ☐ No ☐ Not applicable

Does the study involve any biohazards materials/agents or microorganisms of risk group 2, 3, or 4 (GR2, GR-3 or GR4)?

☐ Yes ☒ No

[If the response is 'yes'] I, (print name of the PI) affirm that we will use the standard icddr,b laboratory procedures for biosafety of the hazardous materials/agents or microorganisms in the conduction of the study.

_____ _____

Signature of the Principal Investigator **Date**

Dissemination Plan: [please explicitly describe the plans for dissemination, including how the research findings would be shared with stakeholders, identifying them if known, and the mechanism to be used; anticipated type of publication (working papers, internal (institutional) publication, international publications, international conferences/seminars/workshops/ agencies. [Check all that are applicable]

Dissemination type	Response		Description (if the response is a yes)
Seminar for icddr,b scientists/ staff	☐ No	☒ Yes	The research findings will be presented in a seminar for icddr,b scientist/staffs along with other partners
Internal publication	☒ No	☐ Yes	
Working paper	☒ No	☐ Yes	
Sharing with GoB (e.g. DGHS/ Ministry, others)	☐ No	☒ Yes	The final results will be shared with Government of Bangladesh /Directorate General of Health Services through seminars
Sharing with national NGOs	☒ No	☐ Yes	
Presentation at national workshop/ seminar	☐ No	☒ Yes	The research findings will be presented in a seminar organized by icddr,b before GoB and other national and international agencies
Presentation at international workshop/ conference	☐ No	☒ Yes	The findings will be shared in international conferences within the region and elsewhere
Peer-reviewed publication	☐ No	☒ Yes	The research findings will be published in peer reviewed journal (s)
Sharing with international agencies	☐ No	☒ Yes	Any publications arising out of this work will be shared with international agencies
Sharing with donors	☐ No	☒ Yes	The final report will be shared with Bill and Melinda Gates Foundation
Policy brief	☒ No	☐ Yes	
Other			
Other			

Funding:

Is the protocol fully funded?	☐ Yes	☒ No
If the answer is yes, please provide sponsor(s)'s name	1.	
	2.	
Is the protocol partially funded?	☐ Yes	☒ No
If the answer is yes, please provide sponsor(s)'s name	1.	
	2.	

If fund has not been identified:

Is the proposal being submitted for funding?	☒ Yes	☐ No
If yes, name of the funding agency	1. Bill and Melinda Gates Foundation	
	2.	

Conflict of interest:
Do any of the participating investigators and/or member(s) of their immediate families have an equity relationship (e.g. stockholder) with the sponsor of the project or manufacturer and/or owner of the test product or device to be studied or serve as a consultant to any of the above?

☒ No ☐ Yes (please submit a written statement of disclosure to the Executive Director, icddr,b)

Proposed Budget:

Dates of Proposed Period of Support

(Day, Month, Year - DD/MM/YY)

Beginning Date: 1 Nov 2018

End Date: 30 June 2021

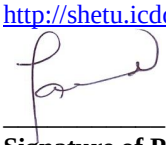
Cost Required for the Budget Period ($)

Years	Direct Cost	Indirect Cost	Total Cost
Year-1			0
Year-2			0
Year-3			0
Year-4			0
Year-5			0
Total	[redacted]	[redacted]	[redacted]

Certification by the Principal Investigator:

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept the responsibility for the scientific conduct of the project and to provide the required progress reports including updating protocol information in the NAVISION if a grant is awarded as a result of this application.

I also certify that I have read icddr,b Data Policies and understand the PIs' responsibilities related to archival and sharing of research data, and will remain fully compliant to the Policies. (Note: The Data Policies can be found here: http://shetu.icddrb.org/index.php?option=com_content&view=article&id=273&Itemid=685)



_____ ____12.09.2018_____

Signature of PI **Date**

Approval of the Project by the Division Director of the Applicant:

The above-mentioned project has been discussed and reviewed at the Division level.

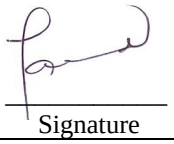
Dr Tahmeed Ahmed		12.09.2018
Name of the Division Director	Signature	Date of Approval

Table of Contents

☒ Check here if appendix is included

Project Summary

[The summary, within a word limit of 300, should be stand alone and be fully understandable.]

Principal Investigator: Dr Tahmeed Ahmed
Research Protocol Title: Community-based clinical trial with microbiota directed complementary foods (MDCFs) made of locally available food ingredients for the management of children with primary MAM
Proposed start date: 1 Nov 2018 Estimated end date: 30 June 2021

Background (brief):

Burden: A total of 52 million children under 5 are suffering from acute malnutrition globally, of whom 33 million have moderate acute malnutrition (MAM). In Bangladesh, more than 2 million children suffer from MAM. According to Bangladesh Demographic Health Survey 2014 26%, 25% and 17% of children aged less than two years are stunted, underweight and wasted respectively.

Knowledge gap: We have already demonstrated that children with SAM have immature gut microbiota that is partially corrected with treatment. Children with MAM have an increased risk of mortality, infections and impaired physical and cognitive development compared to well-nourished children. Although the global caseload of MAM is much greater than that of SAM, the condition has not received the same level of attention or priority. Through our previous and ongoing research we now know about the members of the gut microbiota that can promote growth in children and also about certain food ingredients that promote the proliferation of such beneficial microbiota. However, this knowledge needs to be applied on a sufficiently powered community-based clinical trial.

Relevance: The rationale for this study is to assess whether long-term administration of complementary food made of locally available food ingredients can stimulate the proliferation of growth promoting members of the gut microbiota and have a positive impact on child growth. Such a food (the microbiota directed complementary food; MDCF-2) has been identified through our recently concluded Pre-proof of concept trial done on children with primary MAM. We would now like to do a clinical community-based trial of this potential MDCF-2 in the management of children with primary MAM.

Hypothesis (if any): Complementary foods made of locally available food ingredients that stimulate the proliferation of growth promoting gut microbiota (MDCF-2) will improve clinical outcomes.

Objectives: To investigate the efficacy of complementary food made of locally available food ingredients that can stimulate the proliferation of growth promoting gut microbiota (MDCF-2) in

(i) promoting repair of microbiota immaturity
(ii) promoting proliferation of beneficial members of the gut microbiota
(iii) improving both ponderal and linear growth in children
(iv) improving the metabolomic profile in children with MAM

Methods: We will conduct a proof of concept (POC) clinical trial in 12-18 months old children with primary MAM (Weight-for-Length Z-score, WLZ between -2 and -3). This study will be conducted at Bauniabadh, Radda MCH clinic, Gabtoli of Mirpur area and possibly at the Special Nutrition Unit run by Terre des Hommes in Kurigram. We will produce MDCF-2 at the icddr,b Food Processing Laboratory or nutrition centre established at the site in sufficient quantities for clinical study. This formulation will be matched in energy

density and micronutrient content of ready-to-use supplementary foods (RUSFs) used for MAM in Bangladesh and other countries, and will meet all other requirements for a complementary/supplementary food for 12-18 months old children with MAM. We will test MDCF-2 and the current RUSF standard of care for primary MAM to see the effect on growth, proteomics and metabolomics of an intervention for 12 weeks, with a 4-week post-intervention phase.

Outcome measures will include the following:

- Ponderal growth (rate of weight gain, primary outcome variable), measured at different time points by anthropometry
- Linear growth (LAZ), measured at different time points by anthropometry
- Proteomic profile, assayed by SomaLogic scan
- Morbidity, assessed by daily records
- Change in microbiota-for-age Z score

Description of the Research Project

Hypothesis to be tested:

In a hypothesis testing research proposal, briefly mention the hypothesis to be tested and provide the scientific basis of the hypothesis, critically examining the observations leading to the formulation of the hypothesis.

Does this research proposal involve testing of hypothesis: ☐ No ☒ Yes (describe below)

Complementary foods made of locally available food ingredients that stimulate the proliferation of growth promoting gut microbiota (MDCF) will provide a new way to improve clinical outcomes, for example by improving growth of children with MAM.

Specific Objectives:

Describe the specific objectives of the proposed study. State the specific parameters, gender aspects, biological functions, rates, and processes that will be assessed by specific methods.

To investigate the efficacy of complementary food made of locally available food ingredients that can stimulate the proliferation of growth promoting gut microbiota (Microbiota-Directed Complementary Food; MDCF-2) in

(i) promoting repair of microbiota immaturity
(ii) promoting proliferation of beneficial bacteria
(iii) improving both ponderal and linear growth in children
(iv) improving the metabolomic profile with MAM

Background of the Project including Preliminary Observations:

Provide scientific validity of the hypothesis based on background information of the proposed study and discuss previous works on the research topic, including information on sex, gender and diversity (ethnicity, SES) by citing specific references. Critically analyze available knowledge and discuss the questions and gaps in the knowledge that need to be filled to achieve the proposed aims. If there is no sufficient information on the subject, indicate the need to develop new knowledge.

Moderate acute malnutrition (MAM), a major global health problem, is defined as wasting (i.e. weight-for-height between < -2 and -3 Z-scores of the WHO Child Growth Standards) and/or mid-upper-arm circumference (MUAC) greater or equal to 115 mm and less than 125 mm. According to the Global Nutrition Report 2017, 8% or 52 million under-five children were acutely malnourished globally in 2016. Stunting affected 23% or 155 million children. Approximately one in 6 children under 5 years in South Asia suffered from MAM in 2013 (i.e. 17%).[1] These children are at increased risk of severe acute malnutrition (SAM), and have a three times higher

risk of mortality from common communicable diseases than the well-nourished peers.[2] Bangladesh has one of the highest childhood malnutrition burdens in the world. According to Bangladesh Demographic Health Survey (BDHS) 2014, the prevalence of stunting among under-five children is 36%, among them 12% suffer from severe stunting (LAZ <-3).[3] Around 15% of children are wasted (WLZ <-2); more than 2 million children suffer from MAM, while 3% or 450,000 children suffer from the deadly form of SAM. Malnutrition costs Bangladesh an estimated US $1 billion a year.[4]

According to WHO recommendations, infants and children aged 6-59 months with MAM need to consume nutrient-dense foods to meet their extra needs for weight and height gain and functional recovery. Currently there are no evidence-informed recommendations on the composition of supplementary foods used to treat children with MAM. In situations of food shortage, supplementary foods have been used to treat children with moderate acute malnutrition. Interventions to address undernutrition should therefore include a strong component of MAM management. MAM prevention should be taken into consideration in food security and other development strategies as the situation becomes critical in populations where food insecurity is rampant. Food insecurity has become a worldwide concern due to the increasing number of people who remain undernourished amounting to 842 million, approximately 12% of the total world's population. From the National Micronutrient Status Survey in Bangladesh which we conducted in 2011-12, severe insecurity of food was found most commonly in slum settlements (17.2 %), compared to 12.3% at the national level, 12% in rural areas and 12.4% in urban areas of the country. Since food insecurity cannot be overcome quite readily, it is important therefore to develop interventions that depend upon locally available food ingredients and are able to harness the beneficial power of the gut microbiota on infant and child growth.[5]

One of the major factors limiting the impact of nutrition intervention is the inability of the malnourished children to increase their intake to meet increased metabolic demands. In collaborative studies between icddr,b and the Gordon Lab at Washington University in St. Louis during the Jumpstart Phase of the Breast Milk, Microbiota and Immunity (BMMI) Project, we applied Random Forests, a machine-learning-based approach, to bacterial 16S rRNA datasets generated from monthly fecal samples obtained from a birth-cohort of children living in an urban slum of Dhaka, Bangladesh. These children exhibited consistently healthy growth (WLZ -0.32+0.98). Bacterial strains were identified whose proportional representation defines a healthy gut microbiota as it assembles during the first 2-3 postnatal years. In a randomized clinical trial at icddr,b of two therapeutic foods (imported ready-to-use therapeutic food [RUTF, Plumpy'Nut] versus locally prepared rice/lentil-based Khichuri and Halwa) in Bangladeshi children with severe acute malnutrition (SAM), it was observed that the microbiota immaturity is incompletely and only transiently improved, with children remaining markedly stunted and underweight throughout the follow-up period. Bangladeshi children with MAM also exhibited significant microbiota immaturity, although less severe than children with SAM. Microbiota immaturity thus serves as a potential biomarker to identify infants at risk for undernutrition and to monitor treatment and prevention strategies.[6] Microbiota maturity indices provide a microbial measure of human postnatal development, a way of classifying malnourished states, and a parameter for judging therapeutic efficacy. SAM is associated with significant relative microbiota immaturity that is only partially ameliorated following two widely used nutritional interventions. Immaturity is also evident in less severe forms of malnutrition and correlates with anthropometric measurements. More prolonged interventions with existing or new therapeutic foods and/or addition of gut microbes required to achieve enduring repair of gut microbiota immaturity in childhood malnutrition and improve clinical outcomes.

We recently developed ready-to-use therapeutic foods using locally available food ingredients-rice, lentil, and chickpeas that are culturally relevant and acceptable. We found through a double-blind RCT that chickpea-based

and rice-lentil-based RUTF were as effective as the commercial peanut based-RUTF and well accepted by children with SAM.[7] Through a combination of the above mentioned RCT and clinical translational studies, we have identified growth promoting age-discriminatory beneficial microbiota and locally available food ingredients that support proliferation of these beneficial microbiota. Besides, results of earlier studies done on gnotobiotic animals in Washington University Centre for Genome Science has led us to suggest that a combination of food ingredients (chickpea, soy flour, peanut and green banana) will be worth studying with respect to the diet's impact on stimulating proliferation of growth-discriminatory microbiota as well as cost and sustainability. To assess the degree to which the results obtained from the gnotobiotic mouse and piglet models translate to humans, we recently performed a RRC and ERC approved study, icddr,b protocol (PR-16099) 'Pre-Proof of Concept clinical trials to optimize lead microbiota-directed complementary food (MDCF) prototypes for their ability to repair microbiota immaturity and establish their organoleptic acceptability 'and successfully completed the study. This study was designed to test the effects of three locally produced MDCF prototypes (MDCF-1, MDCF-2 and MDCF-3) and a locally produced rice-lentil-based RUSF. The objective of this pilot study was to demonstrate a Pre-proof of Concept that certain complementary foods would have a beneficial effect on young children suffering from moderate acute malnutrition by stimulating the proliferation of particular members of the gut microbiota that are known for their growth promoting effect. In that pilot study we investigated microbiota-for-age Z score as well as the impact of the proliferation of good members of the gut microbiota on certain body systems. The results of the Pre-POC pilot trial conclusively showed that one of the three microbiota directed complementary foods namely (MDCF-2; composed of a combination of chickpea, soy flour, peanut, green banana, oil, sugar and micronutrients) was associated with increased levels of certain amino acids, that have a key role in development of the long bones, development of the brain and increased production of IGF-1. This was done using the state-of-the-art DNA aptamer based SomaLogic scan. And the results suggest that this candidate MDCF-2 is effective in stimulating the growth of growth promoting members of the gut microbiota, for example *Faecalibacterium prausnitzii*. Thus, MDCF-2 promotes gut microbiota that induces the hormone insulin-like growth factor 1 (IGF-1), which promotes bone growth and remodelling.[8] In our previous study approved by the ERC (PR-09023), we compared the gut microbiota of healthy children living in slum of Mirpur with children hospitalized for the treatment of severe acute malnutrition. The microbiota was assessed by 16S Ribosomal RNA sequencing. We found that the gut microbiota of children with SAM lags chronologically behind microbiota of healthy children. For example, a two year old child with SAM may have the gut microbiota similar to that of a one year old healthy child. This is known as immaturity of the gut microbiota. We have developed two metrices to represent this gut immaturity – the relative microbiota maturity index and the microbiota-for-age Z score; these results have been published in the journal Nature in 2014. SAM is associated with significant relative microbiota immaturity that is only partially ameliorated following nutritional interventions. Immaturity is also evident in less severe forms of malnutrition and correlates with anthropometric measurements.

Based on this evidence we would now like to do a much larger clinical trial using the most promising MDCF which is MDCF-2 with the primary end point being linear growth. This trial would be on children with primary MAM.

Research Design and Methods

Describe the research design and methods and procedures to be used in achieving the specific aims of the research project. If applicable, mention the type of personal protective equipment (PPE), use of aerosol confinement, and the need for the use BSL2 or BSL3 laboratory for different part of the intended research in the methods.. Define the study population with inclusion and exclusion criteria, the sampling design, list the important outcome and exposure variables, describe the data collection methods/tools, and include any follow-up plans if applicable. Justify the scientific validity of the methodological approach (biomedical, social, gender, or environmental).

Also, discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them.

We will conduct a clinical trial among 12-18 months old children with primary MAM (WLZ <-2 to -3).

Study design: Randomized controlled intervention trial.

Study site: This study will be conducted at Bauniabadh, RADDA MCH clinic in Mirpur area and Gabtoli of Dhaka city, and possibly at the Special Nutrition Unit run by Terre des Hommes in Kurigram.

Study participants: The study participants will be 12-18 months old children of either sex with MAM (WLZ <-2 to -3).

Initial screening and enrolment: Children will be screened and enrolled through household surveys by Field Research Assistants (FRAs) following pre-specified inclusion criteria. Fulfilling the enrolment criteria and upon receiving the consent for study participation from the parents or legal guardians, the children with the respective mother/caregiver will be enrolled and randomly assigned to one of the two arms according to computer-generated random numbers. The code of assigned type of diet will be kept in closed opaque envelopes for each individual, and will be opened only when the caregiver signs the consent form.

Study design:

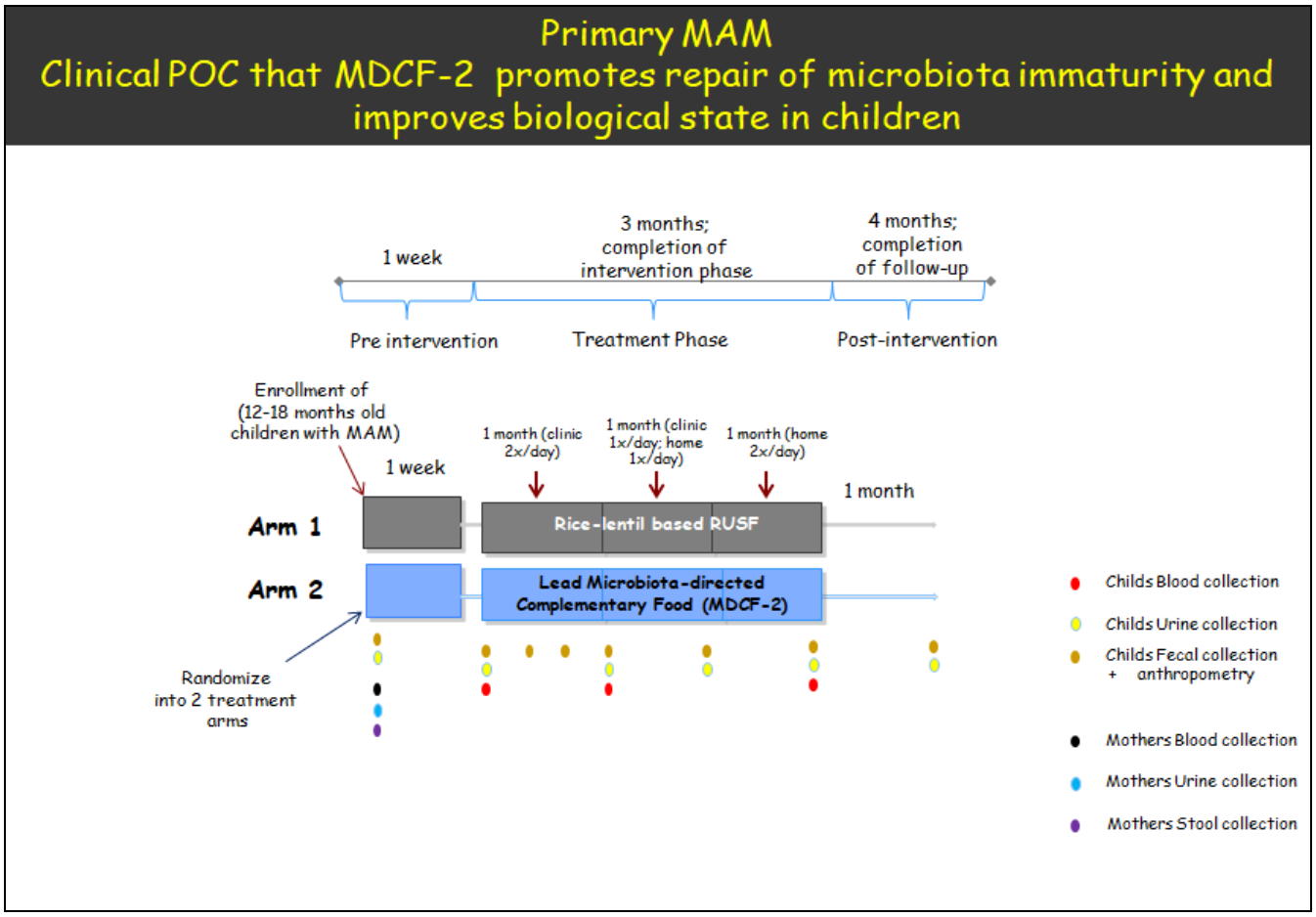


Figure: Study design for the community-based clinical trial among children with primary MAM

Arm 1 - Rice-lentil based RUSF (rationale: reference standard of care for MAM; based on knowledge of its effects on the gut microbiota or microbiota immaturity)

Arm 2 – MDCF2 with four complementary food ingredients (rationale: lead with evidence from Pre-POC clinical trials to optimize lead microbiota-directed complementary food prototypes for their ability to repair microbiota immaturity and positive effects on growth)

Fecal sample collection

Weekly fecal samples (1-2g) will be collected within 30 minutes of excretion (at home and transported back to the icddr,b) using the nitrogen dry shipper specimen collection SOP used in the recently concluded Pre-POC clinical trial (Appendex-A). Specimens will be stored at -80°C before being sent to the Gordon Lab analysis of microbiota maturity (measured before, during and after cessation of treatment with MDCF and RUSF) and PCR-based assessment of enteropathogen burden in fecal samples (measured before and after MDCF treatment).

1-2 gm of fecal samples will be collected from each child at enrollment, weekly during the 1st month of intervention, and fortnightly during the 2nd and 3rd month of intervention and post-intervention phases. Assuming 62 study participants /arm, a total of 992 fecal specimens will be collected in this study. 2 gm of faecal samples will be collected from mothers at the time of enrolment.

Urine sample collection

2 mL of urine samples will be collected from each child at enrollment, one week after enrolment and monthly once in the intervention and post-intervention phases. Assuming 62 study participants /arm, a total of 744 urine samples will be collected in this study. 5 mL of urine samples will be collected from mothers at the time of enrolment.

Blood sample collection

2 mL of blood samples each will be collected from each child prior to intervention, end of first month of intervention and just after the intervention is complete. A total of 372 plasma samples will be collected in this study. 5 mL of blood samples will be collected from mothers at the time of enrolment.

*Maternal nutritional status is associated with child nutritional status, as shown through the results of our eight country MAL-ED study. In addition, neonatal and other maternal factors were early determinants of lower length-for-age, and their contribution remained important throughout the first 24 months of life.[9] The other maternal factors, we believe, would include maternal gut microbiota as well as maternal blood amino acid profile. Initiatives to address childhood stunting should also consider improvements to the composition of complementary foods (i.e., higher protein) and strategies to reduce gut pathogen exposure. As such, we will record maternal height and weight. In order to understand the biological state of nutrition of the mother, enrolment samples of stool, blood and urine will be asked of the mother. These samples will be analyzed for gut microbiota, and proteomics, and the results correlated with those of the enrolled children.

Table 1: Summary of the work plan for the Clinical Trial

Work Plan for the Clinical Study												
Week	0	1	2	3	4	5	6-7	8-9	10-11	12-13	14-15	16-17
		1 month (clinic 2x/day)					1 month (clinic 1x/day; home 1x/day)		1 month (home 2x/day)			

Study activity	Enrolment & randomization in treatment arms	Nutritional therapy starts	ITN	ITN	ITN	ITN	ITN	ITN	ITN	Nutritional therapy ends	Follow up	Follow up
Food Frequency questionnaire	X	X				X		X		X		X
Anthropometry	X	X		X		X	X	X	X	X	X	X
Urine sample (2 ml)	X	X				X		X		X		X
Fecal sample (1-2)g	X	X	X	X	X			X		X		X
Blood sample (2 ml)		X				X				X		

Feeding sessions

The children and mothers/caregivers will be requested to come to the nutrition centers established at the sites preferably between 9-11 am and 3-5 pm on day 1. The mothers will be requested not to give any food and breast milk in the 2 hours preceding the observed meal time. The child will be offered 25 grams of MDCF or RUSF as decided by random allocation in each of the two meals between 9-11 am and 3-5 pm. The mothers will be asked to spoon feed the pre-weighed diets to their children until s/he refuses to eat, as described below. After a two-minute pause, the same diet will be offered a second time until s/he refuses again. After a second two-minute pause, the diet will be offered a third time until refused again. After this third refusal, the feeding episode will be considered as 'terminated'. The duration of the feeding (excluding the intervening 'rest periods') will be recorded by stopwatch, and the total duration of the feeding will be noted. This feeding episode will last for maximum 60 minutes. Measured volumes of plain water will also be given and the amount of water taken during this meal period of 60 minutes will be measured. The feeding episode will take place under the direct supervision of trained study personnel. Children will be considered as refusing further intake if they move their head away from the food, cry, clamp the mouth or clinch the teeth, or become agitated, spit out the food or refuse to swallow. The amount of MDCF/RUSF actually ingested will be calculated by subtracting the left over from the offered amount. Pre-weighed napkins will be provided; any food that is regurgitated, vomited or spilled will be swabbed, weighed and subtracted from the amount offered. The amount of consumed food (g), energy (kcal) and category of acceptability will be analyzed. The enrolled children will be monitored daily by Field Research Assistants for any possible side effects/adverse events (e.g. rash, urticaria due to food allergy or any significant changes in clinical status) for a week. If any side effects/adverse events are observed, they will be treated according to standard of care. A standardized production procedure will be followed to control the quality of RUSF and MDCF following international standard protocol. RUSF and MDCF will be prepared at the food processing laboratory. Preparation of food under different steps, that is, roasting, particle size reduction, homogeneous blending, and supplying to the nutrition centres will be monitored by icddr,b investigators. Food will be prepared everyday to ensure that no unexpected contamination and nutrient losses occur during preparation. Although raw food ingredients will be very carefully procured from the local market and stored in reasonable quantities, we will prepare, dispense, and feed the children the same day the MDCF and RUSF are prepared. Every child will be offered 25gm of the diet twice daily at the feeding center for the first 4 weeks. In the following month, the child will be offered 25gm of the diet at the feeding center and additional 25gm will be provided in a clean container to

feed at home. In the third month, two separate containers containing 25gm diet will be provided every day to each enrolled child at participant's home.

In this study nutritional status will be assessed through anthropometry, comparing with WHO growth reference standards. At the beginning of the study, information will be sought on the demographic characteristics (families' wealth, standard of housing, family structure and parental characteristics etc.), and FRAs will record the children's weight using a digital scale with 2g precision (Seca, model 728, Germany), length (using infantometer, Seca, model 416, Germany), and mid upper-arm circumference to the nearest mm (using a non-stretch insertion tape). Anthropometrics will be done according to the standard procedures and all measurements will be taken thrice and the middle one will be recorded.

All interventions in each study will be administered to children at the Mirpur health clinic/RADDA clinic or in Kurigram. Mothers/primary caregivers will be advised to maintain their child's current dietary and breastfeeding practices.

We will complete enrolment within 12 months and the follow up as well as data analysis within an additional 6 months. However, this trial on primary MAM will continue simultaneously with the other clinical trial on Post SAM-MAM that will have a longer period of duration.

Inclusion criteria

All of the following criteria must be met for a child to be eligible to participate in the study:

- Parent(s) willing to sign consent form
- Child age 12-18 months and no longer exclusively breast fed
- WLZ (<-2 to -3) without bilateral pedal edema at the time of randomization
- Parent(s) willing to bring the child to the feeding center twice daily for 4 weeks for nutritional therapy, once daily for next 4 weeks and provide feeding once daily at home for 4 weeks and twice daily for next 4 weeks.
- The informed consent document will explicitly request permission to use the collected fecal samples for future studies, including but not limited to culturing component bacterial strains

Exclusion criteria

- Medical conditions: Children with tuberculosis (diagnosis based on WHO 2014 guidelines which have been incorporated in the national TB control guidelines of Bangladesh). The guidelines depend upon the following five diagnostic principles (three out of five should be positive): 1. Specific symptoms of TB, 2. Specific signs, 3. Chest X-ray, 4. Mantoux test, and 5. History of contact. [10] or any congenital/acquired disorder affecting growth i.e. known case of trisomy-21 or cerebral palsy; children on an exclusion diet for the treatment of persistent diarrhea; having known history of soy, peanut or milk protein allergy
- Antibiotic use within the last 15 days
- Receiving concurrent treatment for another condition
- Severe anemia (<8mg/dl) will be assessed by Hemocue (Model no. Hemocue Hb 301)
- Failure to obtain informed written consent from parents or caretakers

Recruitment, Screening and Consenting

Census, screening, enrolment of study participants will be done in the catchment areas of the sites in Dhaka city and in Kurigram. Parents of children who meet the MAM criteria (for Primary MAM trial) will be approached

about enrolment into the study. A Field Research Assistant will explain the study in detail, answer any questions from the parent(s), and invite the parent(s) to enroll the child in the study.

At the beginning of the study, information will be sought on the demographic characteristics (families' wealth, standard of housing, family structure and parental characteristics etc.), and FRAs will record the children's weight using a digital scale with 2 g precision (Seca, model 728, Germany), length (using infantometer, Seca, model 416, Germany), and mid-upper-arm circumference to the nearest mm (using a non-stretch insertion tape). Study participants will be asked to come directly to the nutrition center for nutritional therapy. They will be provided with a cell phone number to reach the clinic staff, and a member of the staff may visit a family's household for directly observed nutritional therapy with prior arrangement if needed.

Preparation of MDCF 2 and RUSF

Based on compatible combinations of complementary food ingredients identified in the Pre-POC study described above, we will produce MDCF2 as well as Rice-lentil RUSF at the icddr,b Food Processing facility in Mirpur and in Kurigram (to be established in both places) in sufficient quantities for clinical study. The two diets will be matched in energy density and micronutrient content. The energy density of MDCF is 125 kcal/25 g (per serving), and caloric distribution is targeted to be 45-50 percent from fat and 8-10 percent from protein. Experiments in development of the MDCF prototypes and assessment of the organoleptic properties have been done during the Pre-POC clinical trial. After receiving the raw materials (rice/lentil/chickpea) we will take out the foreign materials/grains or seeds (if there is any), and then in an open pan the raw materials will be roasted. We will maintain the temperature at 120-130°C for roasting. Usually it takes 8-10 minutes for roasting 100g of each raw material. Continuous stirring is essential to ensure roasting of single seeds/grains. After completion of roasting we will keep it aside for cooling and then we will grind. We will take the powder and strain it using a strainer. After straining for 4 to 5 times, we will take the fine powder for mixing with the other ingredients (oil for both MDCF2 and RUSF, and milk powder only for RUSF). We will also grind sugar and the fine powder will be used for mixing. Finally we will add the pre-weighed premix powder. The processing of whole green banana for inclusion in MDCF2 is different from the other ingredients. Green banana with skin will be placed in a deep pan in boiling water (100°C-110°C) and boiled for about 17-20 minutes until they are cooked and tender. The skin of the green banana will be peeled off and the edible white part would be taken and grated into small pieces. Then they will be taken in a pot and allowed to cool. We will smash the small pieces of banana with spoon/hand crusher. The weights of all other ingredients will be recorded. Recipes will be produced in small batches by mixing all ingredients in an electric blender. A small amount (1 percent) of soy lecithin shall be added to the recipe in order to improve the consistency and prevent oil separation.

Anthropometry

The age of the child will be verified against documentation (birth certificate or immunization card, if available) or caregiver's report of the child's birth date. Length will be measured by a infantometer sensitive to 0.1 cm (SECA 416, Hamburg, Germany). Body weight will be measured by a balance sensitive to 2g (SECA 728, Hamburg, Germany). Length-for-age (LAZ), weight-for-length (WLZ) and Weight-for-Age (WAZ) Z-scores will be calculated following the Multicentre Growth Reference Study (MGRS) WHO growth standards[11]. Edema will be examined by pressing the upper side of both feet for 3 seconds. Mid-upper arm circumference (MUAC) will be measured using TALC MUAC tape (UK). Regular standardization of the measuring equipments will be done using standards.

Analyses of plasma and fecal samples

Plasma samples collected from this trial will be sent to Dr Jeffrey Gordon's lab in the Center for Genome Sciences and Systems Biology at Washington University in St. Louis. Advanced mass spectroscopic- and immunoassay-based methods will be used to obtain new knowledge about the role of gut microbiota immaturity and the effects of attempting acute repair of this immaturity with lead microbiota-directed complementary food (MDCF) on biomarkers and mediators of healthy growth. Comparisons will be made with the control group (i.e., those consuming reference RUSF standard). Targeted Ultra Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS) and Gas Chromatography-Mass Spectrometry (GC-MS) will be used to profile analytes of specific interest in plasma and/or fecal samples including bile acids and short chain fatty acids (SCFAs); markers of mitochondrial function (e.g., β-hydroxybutyrate, acylcarnitines/acylCoAs, TCA cycle intermediates); amino acids in serum plus fecal samples [branch chain amino acids, tryptophan and tryptophan metabolites related to growth and inflammatory status, including those produced by bacterial tryptophan metabolism (e.g. indole acetic acid derivatives)]. Key mediators/biomarkers of linear growth (e.g., growth hormone and IGF-1), energy utilization (insulin, leptin), and bone biology [IL-6, osteoprotegerin, the C-terminal peptide of type I collagen (CTX, a marker of osteoclast activity/bone resorption), and the amino-terminal propeptide of Type 1 procollagen (P1NP, a marker of osteoblast activity/bone formation)], and systemic inflammation (CRP, AGP) will be quantified using established ELISA/Luminex assays. Proteins in blood will be identified using the SOMAlogic scan that permits identification of more than 1300 different proteins. The proteomic study done on plasma samples from children with SAM in our previous Pre-POC trial done in Dhaka has already demonstrated a number of significant and clinically relevant associations between certain proteins and clinical phenotypes using the SOMAlogic scan.

Information gathered will be used to select human fecal samples for transplantation into germ-free animals in the Gordon Lab in St Louis; these animals, who will be fed the diets of their human microbiota donors, will be used to further characterize the mechanisms that link MDCF prototypes, the gut microbiota, and host physiology/metabolism. In addition, these plasma and fecal biomarkers will be used to determine that MDCF promotes repair of microbiota immaturity and improves biological state in children.

Sample Size Calculation and Outcome (Primary and Secondary) Variable(s)

Clearly mention your assumptions. List the power and precision desired. Describe the optimal conditions to attain the sample size. Justify the sample size that is deemed sufficient to achieve the specific aims.

In the pre-POC trial of different MDCFs, the baseline weight-for-length Z score of children who received MDCF2 was -2.2 and after one month of supplementation was -1.7. If we consider the WLZ -2 at baseline and -1.7 at end line, pooled SD as 0.53 then the sample size is 49 in each arm at 80% power and 5% level of significance.

With 20% attrition 62 children will be required to be enrolled in each arm. Therefore, for the Primary MAM trial, 62 children will receive MDCF2 and 62 children will receive Rice-lentil RUSF.

Data Analysis

Describe plans for data analysis, including stratification by sex, gender and diversity. Indicate whether data will be analysed by the investigators themselves or by other professionals. Specify what statistical software packages will be used and if the study is blinded, when the code will be opened. For clinical trials, indicate if interim data analysis will be required to determine further course of the study.

The two groups of children with MAM will be compared at baseline and at different time points as shown in the illustration on trial design. The clinical outcome variables for comparison will include rate of weight gain,

anthropometric indices, and morbidity. All analytes mentioned in the section 'Analyses of plasma and fecal samples' will be compared between MDCF2 and RUSF groups.

Data Safety Monitoring Plan (DSMP)

All clinical investigations (research protocols testing biomedical and/or behavioural intervention(s)) should include the Data and Safety Monitoring Plan (DSMP). The purpose of DSMP is to provide a framework for appropriate oversight and monitoring of the conduct of clinical trials to ensure the safety of participants and the validity and integrity of the data. It involves involvement of all investigators in periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of trial sites, and other factors that can affect study outcome.

Data collection tools for this study will include case report forms, laboratory worksheets and source documentation. Complete source documentation (study visits, laboratory reports, etc.) will be kept for each study participant in individual study charts. All laboratory specimens, reports, study data collection and administrative forms will be identified by coded number to maintain study participant confidentiality and to enable tracking throughout the study.

Forms, lists, logbooks, appointment books, and any other listings that link study participant ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access. All information regarding study participants will be kept in password-protected computer files or in locked file cabinets that can be accessed only by authorized study personnel. Chart information and information from study records will not be released without written permission from the study participant's parent(s). However, records may be reviewed by representatives from the Research Review Committee and Ethical Review Committee of icddr,b.

The study investigators are responsible for ensuring complete and accurate documentation for the study and for each study participant, including: medical records, records detailing each study participant's progress through the study, laboratory reports, Case Report Forms (CRFs), signed informed consent forms, correspondence with IRB(s), adverse event reports, and information regarding participant discontinuation and completion of the study. All required data will be clearly and accurately recorded in the CRFs by authorized study personnel. Only designated study-site personnel who have received appropriate training will record or change data in a CRF. The investigators are responsible for procuring the data and for quality of data recorded in the CRFs. Data entry and management will be performed at icddr,b.

Ethical Assurance for Protection of Human rights

Describe the justifications for conducting this research in human participants. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how participants' rights will be protected, and if there would be benefit or risk to each participants of the study. Discuss the ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in sick children, use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Discuss procedures safeguarding participants from injuries resulting from study procedures and/or interventions, whether physical, financial or social in nature. [Please see Guidelines]

The study will be started after obtaining IRB approval by the icddr,b Research Review Committee and Ethical Review Committee. Before enrolment in the study, informed written consent will be taken from the legal guardian of the study participants. The privacy, anonymity and confidentiality of data/information identifying the study participants will be strictly maintained. Personal identifications taken during enrolment and other study procedures will be kept under lock and key. None other than the study personnel will have access to information of personal identification and other sensitive information.

Expected risks/adverse events for this protocol are those related to blood sample collection, fecal sample collection and feeding of microbiota directed complementary food. None of these qualify as a serious adverse event (SAE). Expected Adverse Events (EAEs) related to blood draw are as follows:

- discomfort
- pain
- introduction of infection
- bleeding
- fainting or bruising

- Precaution will be taken to avoid introduction of infection by disinfecting the site of venipuncture and using sterile equipment
- The risk of bleeding and bruising will be minimized by immediate application of pressure after venipuncture
- The participant (child) will be in the sitting or supine position during blood draws to avoid injuries from fainting

All possible adverse events will be treated appropriately. These will include:
- Vomiting
- Diarrhoea
- Skin rash
- Urticaria from food allergy
- Abdominal distension
- Pain abdomen

Assessment of Adverse Events

Both serious and non-serious adverse events will be assessed for severity; relationship to study participation; actions taken; and outcomes. All SAEs will be reported to the ERC of icddr,b within 24 hours of the site's awareness of the event that will in turn be distributed to the sponsor. This will be done by direct telephone communication, fax or e-mail.

Each category for AE assessment will be coded according to the following grading systems:

Severity:

1. Mild
2. Moderate
3. Severe

Relationship to Study Participation:

1. Definitely related: Clear cut temporal association, no other possible cause
2. Possibly related: Less clear cut temporal association, other causes possible
3. Unrelated: Independent of study, evidence exists that event is definitely related to another etiology

Actions:

None
Remedial therapy (more than one dose of medicine required)
Permanently discontinued from study participation
Hospitalization
Other

Use of Animals

Describe if and the type and species of animals to be used in the study. Justify with reasons the use of particular animal species in the research and the compliance of the animal ethical guidelines for conducting the proposed procedures.

Not applicable

Collaborative Arrangements

Describe if this study involves any scientific, administrative, fiscal, or programmatic arrangements with other national or international organizations or individuals. Indicate the nature and extent of collaboration and include a letter of agreement between the applicant or his/her organization and the collaborating organization.

This project is a collaborative effort between the investigators in the Washington University School of Medicine and the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b). The clinical work, field activities will be undertaken in Dhaka, Bangladesh, under the direct supervision of the Principal Investigator whereas the laboratory analyses will be conducted at the Gordon Lab at Washington University in St. Louis. All research units and the collaborating investigators have long histories of international collaborations. All investigators have communicated during development of this project and are committed to its successful implementation.

Facilities Available

Describe the availability of physical facilities at site of conduction of the study. If applicable, describe the use of Biosafety Level 2 and/or 3 laboratory facilities. For clinical and laboratory-based studies, indicate the provision of hospital and other types of adequate patient care and laboratory support services. Identify the laboratory facilities and major equipment that will be required for the study. For field studies, describe the field area including its size, population, and means of communications plus field management plans specifying gender considerations for community and for research team members.

icddr,b has a well equipped Food Processing Lab that will facilitate preparations of diet recipes. We have ongoing studies in the Baoniabad area in Mirpur. A number of project offices are located there. More importantly, we have an excellent rapport with the community. The community elite and elders are invited every year to a dissemination meeting so that they are aware of the research being conducted.

Center for Genome Sciences, Washington University in St. Louis:

The stool samples will be aliquoted at icddr,b. Stool samples will be sent to Prof Jeffery Gordon's Lab in St Louis for the high throughput 16S ribosomal RNA gene sequencing. The presence/abundances of a broad range of enteropathogens in stool samples before and after MDCF treatment will be determined using a PCR-based assay. Stool samples may also be used to culture constituent bacterial strains for use in future studies in gnotobiotic mice in the Gordon Lab. It is pertinent to mention here that the assays and equipment to carry out those are currently available only in a few labs in the world, including the one at St Louis. The Center is home to an interdisciplinary, multi-departmental, multi-generational team of investigators from multiple schools that focus on comparative genomics, statistical genomics, and systems biology It serves as 'proving ground' for developing new strategies for educating students and faculty who wish to work at the interface of the biological, physical, computational and engineering sciences**.**

Literature Cited

Identify all cited references to published literature in the text by number in parentheses. List all cited references sequentially as they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list of Literature Cited. There is no page limit for this section, however, exercise judgment in assessing the "standard" length.

1. Development Initiatives, 2017. Global Nutrition Report 2017: Nourishing the SDGs. Bristol, UK: Development Initiatives.

2. James P, Sadler K, Wondafrash M, Argaw A, Luo H, and Geleta B et al. Children with moderate acute malnutrition with no access to supplementary feeding programmes experience high rates of deterioration and no improvement: results from a prospective cohort study in rural Ethiopia. PloS one. 2016 Apr 21;11(4):e0153530.

3. National Institute of Population Research and Training (NIPORT), Mitra and Associates, and ICF International. 2016. Bangladesh Demographic and Health Survey 2014. Dhaka, Bangladesh, and Rockville, Maryland, USA: NIPORT, Mitra and Associates, and ICF International.

4. Save the children (2015), malnutrition in Bangladesh: Harnessing social protection for the most vulnerable (2015).

5. Blanton LV, Barratt MJ, Charbonneau MR, Ahmed T, Gordon JI. Childhood undernutrition, the gut microbiota, and microbiota-directed therapeutics. Science 2016 Jun 24;352(6293):1533

6. Subramanian S, Huq S, Yatsunenko T, Haque R, Mahfuz M, Alam MA, Benezra A, DeStefano J, Meier MF, Muegge BD, Barratt MJ. Persistent gut microbiota immaturity in malnourished Bangladeshi children. Nature. 2014 Jun; 510(7505):417.

7. Choudhury N, Ahmed T, Hossain MI, Islam MM, Sarker SA, Zeilani M, Clemens JD. Ready-to-Use Therapeutic Food Made From Locally Available Food Ingredients Is Well Accepted by Children Having Severe Acute Malnutrition in Bangladesh. Food and nutrition bulletin. 2018 Mar; 39(1):116-26.

8. Yan J, Herzog JW, Tsang K, Brennan CA, Bower MA, Garrett WS, Sartor BR, Aliprantis AO, Charles JF. Gut microbiota induce IGF-1 and promote bone formation and growth. Proc Natl Acad Sci U S A. 2016 Nov 22;113(47):E7554-63.

9. MAL-ED Network Investigators. Childhood stunting in relation to the pre-and postnatal environment during the first 2 years of life: The MAL-ED longitudinal birth cohort study. PLoS medicine. 2017 Oct 25;14(10):e1002408.

10. World Health Organization. Guidance for national tuberculosis programmes on the management of tuberculosis in children. World Health Organization; 2016.

11. de Onis M, Garza C, Victora CG, Bhan MK, and Norum KR. The WHO Multicentre Growth Reference Study (MGRS): Rationale, planning, and implementation. Food and Nutrition Bulletin 2004;25(supplement 1):S3-S84.

Clinical Pathology tests (USD 7400), clinical biochemistry tests (USD 7400), and clinical microbiology tests (USD 7400) budgeted for the required laboratory investigations advised by the physicians.

Food safety tests (USD 40,000): This amount is budgeted for food safety tests of the intervention products at regular intervals.

Fuel for electric generator: USD 19,600 is budgeted for the fuel required to run the electric generators.
Sub-contracts: USD 160,000

Name	Corporate Entity Name	Mailing Address
Executive Director	Radda MCH-FP Centre	Plot 324, Road 6, Block B, Mirpur-10, Dhaka-1216, Bangladesh
Country Representative, Dhaka, Bangladesh, Terre des Hommes	Terre des Hommes, Bangladesh	House 141, Flat 5A, Road 4, Block-A, Banani, Dhaka-1213, Bangladesh

We have worked successfully with both RADDA and Terre des Hommes in our previous trial on the efficacy of local RUTFs, the samples from which have been extensively used to generate data for developing the MDCFs. These organizations have nutrition clinics where we will enroll children with Primary MAM and Post-SAM MAM in addition to icddr,b Dhaka Hospital. Contracts will be signed by the Executive Directors of icddr,b and these organizations following prescribed rules of icddr,b

Other Support

Describe sources, amount, duration, and grant number of all other research funding currently granted to PI or under consideration.

Not applicable

Reviewer Comments and Response

Reviews by the Bill and Melinda Gates Foundation

Application ID: INV-000247
Proposer: Dr. Tahmeed Ahmed

Reviewer 1

External and Internal Reviewer Feedback
Overall comment by the Bill and Melinda Gates foundation:

Response: Attached

Note: Comments of three reviewers were compiled in this single table. Multiple bullet points in a row mean that multiple reviewers have had overlapping or related comments in this area

Comments:	Response and Modifications Made:
Power & Statistical Analysis: • Two sites are important for reducing recruitment time (Mirpur and Kurigram), but this interjects heterogeneity. Has this been accounted for in the statistical analysis?	• Heterogeneity is of course there. We compared data of children from Mirpur and Kurigram. The groups were differently placed chronologically, about 3 years apart. Being urban and rural, SES and other demographics are different, income is more for the urban households.
Recruitment: • We wonder if it would be possible to condense primary MAM recruitment to 6 months, recognizing that MAM post SAM will take longer given a smaller patient population. Aim would be to have • What data will be collected regarding the past SAM and acute infection history of children? Number of previous hospitalizations (cause, length of stay) for example might be particularly important in predicting outcome.	• As mentioned in the revised proposal shared with you, we would now like to do the enrolment and most of the follow up within 12 months. Completing enrolment within 6 months would be too tight. Estimate is half the sample size. Let's start and we will devote maximum effort for expediting enrolment. • Yes, hospitalization will be uncommon but we will record all morbidities including use of antibiotics.
Intervention: • Please provide more details around food preparation in the home. How will this be accomplished and monitored? • Please clarify if the feed timings are chosen so as to minimize displacement of normally eaten meals? • How will normally consumed food diversity be measured and analyzed? • Milk powder is mentioned in the protocol. Is this part of the rice-lentil recipe, or a typo? • Could you provide a table of the breakdown of micronutrients present in MDCF2 vs. Rice Lentil intervention? • Can you clarify what is meant by "measured water"?	• MDCF will be prepared by us in bulk at the field site in Mirpur as well as Kurigram, it will never be prepared in the households by the mothers. So, there will be enough control. • Yes, spacing as done in the pilot trial will take care of the displacement. • We will serve food frequency questionnaire. • Milk powder is a constituent of RUSF. • We will provide more details. • This is drinking water, given to offset the issues related to osmolarity of the food. In retrospect, this reference to measured water intake can be deleted
Outcomes: • In the trial, please clarify why there is no planned blood collection at the end of follow-up. It may help to gauge sustainability of outcomes to have blood collection at the end.	• We can keep blood at the end. • Caregivers are always counseled on progress of the child. However, we have no answer yet to your question about ownership of microbiota. We will certainly need to think about it.

• What plans are in place to report back on the study to participants? Do participants maintain ownership of their microbiota in the event that there is commercial potential in the future? • Please clarify the rationale for recording the middle anthropometric measure out of three. • Please specify a timeline for data transfer to BMGF. Three months after collection is complete may be reasonable.	• This should be the mean. The proposal will be corrected reflecting the standard anthropometric method. • 3-4 months should be fine.
Budget: • Please clarify which budget (Washington University or icddr,b) will cover the bio specimen analysis.	• Bio specimen analysis will be covered by Washington University.

Reviewer 2

Title:
Community-based clinical trial with microbiota-directed complementary foods (MDCFs) made of locally available food ingredients for the management of children with primary moderate acute malnutrition

Summary of Referee's Opinions: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.

	Rank Score		
	High	Medium	Low
Quality of project	√		
Adequacy of project design	√		
Suitability of methodology	√		
Feasibility within time period	√		
Appropriateness of budget	Not	provided	
Potential value of field of knowledge	√		

CONCLUSIONS

I support the application:

a) without qualification ☐

b) with qualification

- on technical grounds ☑
- on level of financial ☐

support I do not support the ☐

application

Name of Referee: Dr Mohammad Mushtuq Husain

Signature:  Date: 29 Aug 2018

Position: Coordinator, Coordination & Support Centre (CSC)

Institution: Directorate General of Health Services (DGHS)

Detailed Comments

Title: **Community-based clinical trial with microbiota-directed complementary foods (MDCFs) made of locally available food ingredients for the management of children with primary moderate acute malnutrition**

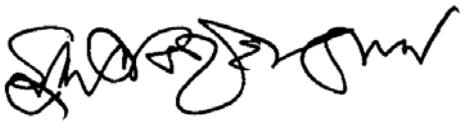
PI: **Dr Tahmeed Ahmed**

Reviewer: Dr Mohammad Mushtuq Husain

The following should be addressed before accepting the protocol:

a) Possible study site at Kurigram Terr des Hommes: Finalization of study site is needed before acceptance of protocol
b) Preparation of intervention and control foods: where will the MFCF and RUSF be produced? How quality will be controlled?
c) Method of preparing RUSF should be detailed as like MDCF
d) CONSENT FORM
    i. The term 'special food' should be avoided. Please replace it with 'one type of food' and 'other type of food', to avoid sense of discrimination
    ii. Please omit: "Children will be benefitted by receiving free special food ……."
    iii. Please write in more clear language: A total of 6 mL of blood in three occasions, with 2 mL each on every occasion (*cÖwZ ev‡i 2 wgwj/ †dvuUv K‡i †gvU 6 wgwj/‡dvuUv).* It will help to avoid apprehension of parents for bleeding too much.

Thank you



(Dr Mohammad Mushtuq Husain)

Date: 29 Aug 2018

Comments	Response
a)Possible study site at Kurigram Terr des Hommes: Finalization of study site is needed before acceptance of protocol	Collaborative agreement with Tdh will be done before commencement of the field work.
b)Preparation of intervention and control foods: where will the MDCF and RUSF be produced? How quality will be controlled?	Food processing laboratory will be established at the Mirpur area following standard procedures. We have already developed such standard laboratory during the "Pre-Proof of Concept (Pre-POC) phase of this protocol. RUSF and MDCF will be prepared at the food processing laboratory. A standardized production procedure will be followed to control the quality of RUSF and MDCF following international standard protocol. Food will be prepared everyday to ensure that no unexpected contamination and nutrient losses occur during preparation. Preparation of food under different steps, that is, roasting, particle size reduction, homogeneous blending, and supplying to feeding centre will be monitored by icddr,b investigators.
c) Method of preparing RUSF should be detailed as like MDCF	It is now incorporated
d) i. CONSENT FORM i. The term 'special food' should be avoided. Please replace it with 'one type of food' and 'other type of food', to avoid sense of discrimination	Replaced as per suggestion
ii. Please omit: "Children will be benefitted by receiving free special food ……."	Deleted as per advice
iii. Please write in more clear language: A total of 6 mL of blood in three occasions, with 2 mL each on every occasion. It will help to avoid apprehension of parents for bleeding too much.	Replaced as per advice

Biography of the Investigators

Provide biographical data in the following format for all key personnel including the Principal Investigator. Copy the same format for each of them.
Note: Biography of the External Investigators may, however, be submitted in the format as convenient to them..

1. **Name:** Dr Tahmeed Ahmed

2. **Present Position:** Senior Director, Nutrition & Clinical Services Division, icddr,b and Professor of Public Health Nutrition, James P. Grant School of Public Health, BRAC University

3. Educational background:

Degree	Institution	Year
PhD	University of Tsukuba, Japan	1996
MBBS	University of Dhaka	1983
Training	Clinical training in Pediatrics, University of Tsukuba Hospital	1990-1992
Training	Residential training in Pediatrics, Dhaka Shishu Hospital	1989-1990

4. Ethics Certification:

		Issuing Authority	Registration No	Valid Until
No ☐	Yes ☒	NIH	1933611	Issued on 12 August 2015

5. List of ongoing research protocols/ activities:

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time
MAL-ED	PI	Nov 2008	March 2017	40
PR-11005	PI	June 2011	June 2017	30
Aflatoxin	Co-I	February 2013	May 2016	5
Hypernatremia follow up	Co-I	January 2016	January 2017	-
PR-15101	Co-PI	January 2016	March 2018	10
PR-16007	PI	November 2015	November 2019	15
PR-16009	PI	July 2016	October 2018	20

6. Publications

Types of publications	Numbers
a. Original scientific papers in peer-review journals	182
b. Book chapters	18
c. Papers in conference proceedings	25
d. Letters, editorials, annotations, and abstracts in peer-reviewed journals	5
e. Working papers	10
f. Monographs	1

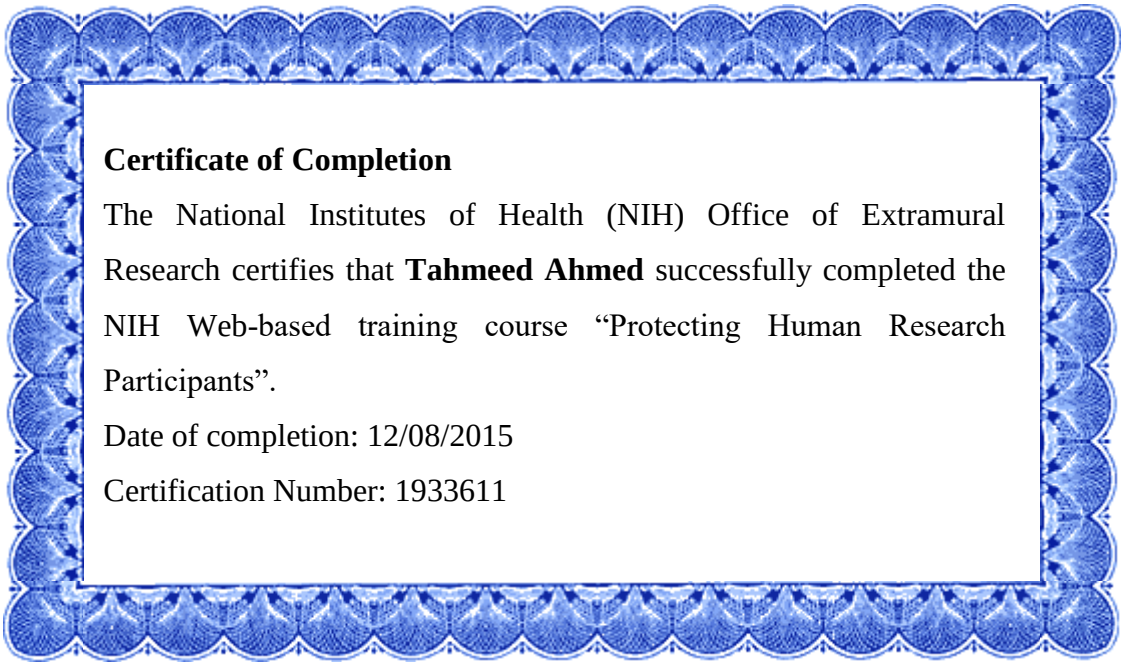
7. Five recent publications including publications relevant to the present research protocol

1. Ahmed T, Choudhury N, Hossain I, Tangsuphoom N, Islam MM, de Pee S, Steiger G, Fuli R, Sarker SA, Parveen M, West KP, Christian P. Development and acceptability testing of ready-to-use supplementary food made from locally available food ingredients in Bangladesh. BMC Pediatr 2014 Jun 27; 14:164.
2. Subramanian S, Huq S, Yatsunenko T, Haque R, Mahfuz M, Alam MA, Benezra A, DeStefano J,

Meier MF, Muegge BD, Barratt MJ, VanArendonk LG, Zhang Q, Province MA, Petri WA Jr, Ahmed T, Gordon JI. Persistent gut microbiota immaturity in malnourished Bangladeshi children. Nature 2014 doi: 10.1038/nature13421.

3. Bhutta ZA, Das JK, Rizvi A, Gaffey MF, Walker N, Horton S, Webb P, Lartey A, Black RE, The Lancet Nutrition Interventions Review Group (Bhutta ZA, Rizvi A, Das JK, Salam RA, Yousafzai A, Lassi ZS, Lenters L, McPhail C, Wazny K, Gaffey MF, Zlotkin S, Imdad A, Haider BA, Welch V, Martorell R, Black RE, Walker N, Tam Y, Ahmed T, and the Maternal and Child Nutrition Study Group (Black RE, Victora C, Walker S, Alderman H, Bhutta ZA, Gillespie S, Haddad L, Horton S, Lartey A, Mannar V, Ruel M, Webb P). Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost? Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost? Lancet 2013 Jun 6 [Epub ahead of print].
4. Ahmed T, Auble D, Berkley JA, Black R, Ahern PP, Hossain M, Hsieh A, Ireen S, Arabi M, Gordon JI. An evolving perspective about the origins of childhood undernutrition and nutritional interventions that includes the gut microbiome. Ann N Y Acad Sci 2014 Aug 12. [Epub ahead of print]
5. Chisti MJ, Salam MA, Smith JH, Ahmed T, Pietroni MAC, Shahunja KM, Shahid ASMSB, Faruque ASG, Ashraf H, Bardhan PK, Sharifuzzaman, Graham SM, Duke T. Bubble continuous positive airway pressure for children with severe pneumonia and hypoxaemia in Bangladesh: an open, randomized controlled trial. Lancet 2015 Aug 19

Ethics certificates



Certificate of Completion

The National Institutes of Health (NIH) Office of Extramural Research certifies that **Tahmeed Ahmed** successfully completed the NIH Web-based training course "Protecting Human Research Participants".

Date of completion: 12/08/2015

Certification Number: 1933611

Biography of the Investigators (1)

Provide biographical data in the following format for all key personnel including the Principal Investigator. Copy the same format for each of them.
Note: Biography of the External Investigators may, however, be submitted in the format as convenient to them..

1. **Name:** Dr Munirul Islam

2. **Present Position:** Scientist, Nutrition and Clinical Services Division, icddr,b

3. **Educational background:**

Degree	Institution	Year
PhD	PhD in Nutrition, Designated Emphasis in International Nutrition (University of California at Davis, California, USA)	2007
MBBS	Dhaka Medical College, University of Dhaka	1995

4. **Ethics Certification:**

		If Yes		
		Issuing Authority	Registration No	Valid Until
No ☐	Yes ☒	COLLABORATIVE INSTITUTIONAL TRAINING INITIATIVE (CITI)	ID:1306963	07/21/2017

5. **List of ongoing research protocols/ activities**

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time
PR-14036	Co-PI	2014	2017	30
PR-12082	Co-PI	2012	2015	25
PR-13008	PI	2015	2016	20
PR-2008-020	Co-I	2009	2015	10

6. **Publications**

Types of publications	Numbers
a. Original scientific papers in peer-review journals	44
b. Peer reviewed articles and book chapters	07
c. Papers in conference proceedings	20
d. Letters, editorials, annotations, and abstracts in peer-reviewed journals	0
e. Working papers	0
f. Monographs	1

7. **Five recent publications including publications relevant to the present research protocol**

1. Islam MM, Brown KH. Zinc transferred through breast milk does not differ between appropriate- and small-for- gestational-age, predominantly breast-fed Bangladeshi infants. J Nutr 2014; 144:771-6.
2. Baxter JA, Roth DE, Al Mahmud A, Ahmed T, Islam MM, Zlotkin SH. Tablets Are Preferred and More Acceptable Than Powdered Prenatal Calcium Supplements among Pregnant Women in Dhaka, Bangladesh. J Nutr 2014; 144: 1106-12.

3. Ahmed T, Mahfuz M, Islam MM, Mondal D, Hossain MI, Ahmed AMS, Tofail F, Gaffar SMA, Haque R, Guerrant RL, Petri WA. The MAL-ED Cohort Study in Mirpur, Bangladesh. CID 2014:59 (Suppl 4); S280-6.
4. Mahfuz M, Ahmed T, Ahmed AMS, Islam MM, Hossain MI. Weight Gain in Malnourished Children after 5 Months Food Supplementation in a Slum Setting in Bangladesh. FNS 2014, 5, 1365-1373
5. **Islam MM, Woodhouse LR, Hossain MB, Ahmed T, Huda MN, Ahmed T, Hotz C, Brown KH.** Zinc absorption from mixed diets containing either high-zinc rice or conventional rice, with or without additional exogenous zinc, among young Bangladeshi children. J Nutr. 2013;143:519-525

Ethics certificates

COLLABORATIVE INSTITUTIONAL TRAINING INITIATIVE (CITI)
BIOMEDICAL RESEARCH - BASIC/REFRESHER CURRICULUM COMPLETION REPORT
Printed on 07/22/2014

LEARNER	Md Munirul Islam (ID: 1306963)
DEPARTMENT	Centre for Nutrition and Food Security
PHONE	+880 2 9827 001-10; Ext. 2352
EMAIL	mislam@icddrb.org
INSTITUTION	University of California, Davis
EXPIRATION DATE	07/21/2017

BIOMEDICAL RESEARCHERS AND STAFF : Choose this group to satisfy CITI training requirements for Investigators and staff involved primarily in biomedical research with human subjects.

COURSE/STAGE:	Basic Course/1
PASSED ON:	07/22/2014
REFERENCE ID:	13537842

REQUIRED MODULES	DATE COMPLETED
Belmont Report and CITI Course Introduction	07/22/14
Basic Institutional Review Board (IRB) Regulations and Review Process	07/22/14
Informed Consent	07/22/14
Social and Behavioral Research (SBR) for Biomedical Researchers	07/22/14
Records-Based Research	07/22/14
Genetic Research in Human Populations	07/22/14
Research With Protected Populations - Vulnerable Subjects: An Overview	07/22/14
Vulnerable Subjects - Research Involving Children	07/22/14
Vulnerable Subjects - Research Involving Pregnant Women, Human Fetuses, and Neonates	07/22/14
FDA-Regulated Research	07/22/14
Research and HIPAA Privacy Protections	07/22/14
Conflicts of Interest in Research Involving Human Subjects	07/22/14
University of California, Davis	07/22/14

For this Completion Report to be valid, the learner listed above must be affiliated with a CITI Program participating institution or be a paid independent Learner. Falsified information and unauthorized use of the CITI Program course site is unethical, and may be considered research misconduct by your institution.

Paul Braunschweiger Ph.D.
Professor, University of Miami
Director Office of Research Education
CITI Program Course Coordinator

Biography of the Investigators

Provide biographical data in the following format for all key personnel including the Principal Investigator. Copy the same format for each of them.
Note: Biography of the External Investigators may, however, be submitted in the format as convenient to them..

1. **Name:** Dr. Mustafa Mahfuz

2. **Present Position:** Associate scientist, Nutrition and Clinical Services Division, icddr,b

3. **Educational background:**

	Institution	Year
MPH	University of Dhaka	2006
MBBS	University of Chittagong	2001

4. **Ethics Certification:**

		If Yes		
		Issuing Authority	Registration No	Valid Until
No ☐	Yes ☒	NIH	1973495	Issued 2016

Note: If the response is "no", please get certification from CITI or NIH before study initiation and submit a copy to the Committee Coordination Secretariat

2. **List of ongoing research protocols/ activities**

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time
PR-16007	Co-PI	15.12.2015	31.11.2019	60
PR-12096	PI	28.01.2013	30.06.2017	20
2008-20	Co-I	01.10.2008	31.08.2017	10
PR- 11005	Co-I	19.08.2011		10

3. **Publications**

Types of publications	Numbers
Original scientific papers in peer-review journals	50
Peer reviewed articles and book chapters	7
Papers in conference proceedings	4
Letters, editorials, annotations, and abstracts in peer-reviewed journals	7
Working papers	0
Monographs	0

4. **Five recent publications including publications relevant to the present research protocol**

1. **Mahfuz M**, Alam MA, Islam SB, Naila N, Chisti MJ, Alam NH, Sarker SA, Ahmed T. Treatment outcome of children with Persistent Diarrhoea admitted to an Urban Hospital, Dhaka during 2012-2013. BMC Pediatrics 2017; 17:142.
2. **Mahfuz M**, Das S, Mazumder RN, Rahman M, Haque R, Gordon JI, Ahmed T et al. Bangladesh Environmental Enteric Dysfunction (BEED) study: Protocol for a community-based intervention study to

validate non-invasive biomarkers of Environmental Enteric Dysfunction. BMJ Open 2017-017768 (accepted).

3. **Mahfuz M**, Alam MA, Islam MM, Mondal D, Hossain MI, Ahmed AMS, Choudhury N, Raihan MJ, Haque R, and Ahmed T. Effect of micronutrient powder supplementation for two and four months on hemoglobin level of children 6–23 months old in a slum in Dhaka: a community based observational study. BMC Nutrition 2016, DOI: 10.1186/s40795-016-0061-y. URL: http://www.biomedcentral.com/2055-0928/2/21
4. Subramanian S, Huq S, Yatsunenko T, Haque R, **Mahfuz M**, Alam MA, Benezra A, DeStefano J, Meier MF, Muegge BD, Barratt MJ, VanArendonk LG, Zhang Q, Province MA,. Petri WA, Ahmed T, Gordon JI. Persistent gut microbiota immaturity in malnourished Bangladeshi children (research letter). Nature 2014 Jun 19;510(7505):417-21.
5. Ahmed T, **Mahfuz M**, Islam MM, Mondal D, Hossain MI, and Ahmed AMS, et al. The MAL-ED Cohort Study in Mirpur, Bangladesh. Clin Infect Dis 2014 Nov;59 (Suppl 4):S280-6.


RBM/RA/2015-074

icddr,b

Awards this certificate to

Dr. Mustafa Mahfuz

for attending the Certificate Program on

Results Based Management (RBM)

held during 06 & 07 September 2015

Director, RAS

Director, HR

Executive Director

Research Administration, icddr,b
68, Shahid Tajuddin Ahmed Sharani
Mohakhali, Dhaka 1212, Bangladesh


Certificate of Completion

The National Institutes of Health (NIH) Office of Extramural Research certifies that **Mustafa Mahfuz** successfully completed the NIH Web-based training course "Protecting Human Research Participants".

Date of completion: 01/27/2016.

Certification Number: 1973495.

Biography of the Investigators

Provide biographical data in the following format for all key personnel including the Principal Investigator. Copy the same format for each of them.
Note: Biography of the External Investigators may, however, be submitted in the format as convenient to them..

1. Name: Dr. Sayeeda Huq

2. Present Position: Associate Scientist, Consultant Physician, Nutrition Ward, Nutrition & Clinical Services Division, icddr,b

3. Educational background:

	Institution	Year
MPH	University of Sydney, Australia	2007
MBBS	Bangladesh Medical College, Dhaka, Bangladesh	1996

4. Ethics Certification:

		If Yes		
		Issuing Authority	Registration No	Valid Until
No ☐	Yes ☒	NIH	1932845	Issued 12/07/2015

5. List of ongoing research protocols/ activities

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time
PR#15113	PI	25-03-16	31-05-17	6%
PR# 09023	PI	1-10-09	30-06-13	25%
PR#09038	Co-I	01-08-12	31-12-14	10%
PR #10039	Co-I	20-01-12	19-01-15	5%
PR#11005	Co-I	18-08-11	31-12-15	5%
PR#12082	Co-I	1-08-13	30-11-15	25%

6.. Publications

Types of publications	Numbers
Original scientific papers in peer-review journals	27
Peer reviewed articles and book chapters	2
Papers in conference proceedings	12
Letters, editorials, annotations, and abstracts in peer-reviewed journals	3
Working papers	
Monographs	1

7. Five recent publications including publications relevant to the present research protocol

7.1**. S Huq,** , M I Hossain, MA Malek, ASG Faruque and M A Salam. Hypoglycaemia in under five children with diarrhoea. Journal of Tropical Pediatrics; 2007 Jun;53(3):197-201.

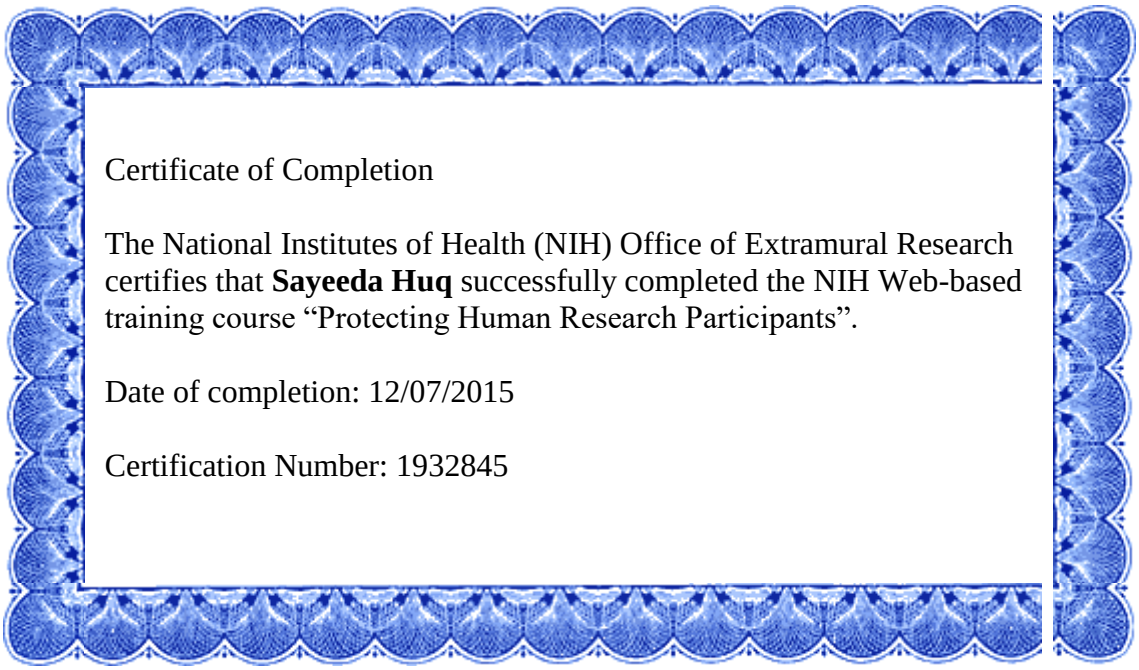
7.2. Sathish Subramanian, **Sayeeda Huq**,Tanya Yatsunenko,Rashidul Haque,Mustafa Mahfuz, Mohammed A.Alam,Amber Benezra,Joseph DeStefano,Martin F. Meier,Brian D. Muegge,Michael J. Barratt,Laura G. VanArendonk, Qunyuan Zhang, Michael A. Province,William A. Petri Jr,Tahmeed Ahmed,Jeffrey I. Gordon.

Persistent gut microbiota immaturity in malnourished Bangladeshi children. Nature http://dx.doi.org/10.1038/nature13421 (2014).

7.3. **S Huq**, Mark A.C. Pietroni, Hafizur Rahman, Mohammad Tariqul MA. Hereditary Spherocytosis. Journal of Health, Population and Nutrition , 2010 Feb; 28(1), 107-109.

7.4. Ahmed T, Islam M, Choudhury N, Hossain I, **Huq S**, Mahfuz M, Sarker SA.Results with complementary food using local food ingredients.Nestle Nutr Inst Workshop Ser. 2017;87:103-113. doi: 10.1159/000448960.2017 Mar 17.

7.5.Sumon Das, Jobayer Chisti, **Sayeeda Huq**, Mohammad Abdul Malek, Lana Vanderlee, Mohammed Abdus Salam, Tahmeed Ahmed, Abu Syed Golam Faruque, Abdullah Al Mamun. Changing trend of overweight and obesity and their associated factors in urban population of Bangladesh. Food and Nutrition Sciences, 2013,4,678-689.



Certificate of Completion

The National Institutes of Health (NIH) Office of Extramural Research certifies that **Sayeeda Huq** successfully completed the NIH Web-based training course "Protecting Human Research Participants".

Date of completion: 12/07/2015

Certification Number: 1932845

Biography of the Investigators

Provide biographical data in the following format for all key personnel including the Principal Investigator. Copy the same format for each of them.
Note: Biography of the External Investigators may, however, be submitted in the format as convenient to them..

1. **Name:** Dr. Ishita Mostafa

2. **Present Position:** Research Investigator, Nutrition & Clinical Services Division, icddr,b

3. **Educational background**: (last degree and diploma & training relevant to the present research proposal)

	Institution	Year
MPH	North South University	2011-13
BDS	University of Dhaka	2005-09
Training	Research Methodology and SPSS (icddr,b)	2013
Training	Post graduation training (ShSMC)	2011
Training	Internship	2009-10

4. **Ethics Certification:**

		If Yes		
		Issuing Authority	Registration No	Valid Until
No ☐	Yes ☒	**NIH**	**2246679**	**Issued 2016**

Note: If the response is "no", please get certification from CITI or NIH before study initiation and submit a copy to the Committee Coordination Secretariat

5. **List of ongoing research protocols/ activities**

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time
14003	PI	15.4.2014	14.4.2015	100%
16099	C0-I	4.1.2017	31.10.2018	100%

6. **Publications**

Types of publications	Numbers
g. Original scientific papers in peer-review journals	2
h. Peer reviewed articles and book chapters	
i. Papers in conference proceedings	1
j. Letters, editorials, annotations, and abstracts in peer-reviewed journals	
k. Working papers	2
l. Monographs	

7. **Five recent publications including publications relevant to the present research protocol**

7.1. Children living in the slums of Bangladesh face risks from unsafe food and water and stunted growth is common

7.2. The management of persistent diarrhoea at Dhaka Hospital of the International Centre for Diarrhoeal Disease and Research: a clinical chart review

Certificate of Completion

The National Institutes of Health (NIH) Office of Extramural Research certifies that **Ishita Mostafa** successfully completed the NIH Web-based training course "Protecting Human Research Participants".

Date of completion: 11/29/2016.

Certification Number: 2246679.

Biography of the Investigators

Provide biographical data in the following format for all key personnel including the Principal Investigator. Copy the same format for each of them.
Note: Biography of the External Investigators may, however, be submitted in the format as convenient to them..

1.Name: Dr. Imteaz Mahmud

2.Present Position: Research Fellow, Nutrition & Clinical Services Division, icddr,b

3.Educational background: (last degree and diploma & training relevant to the present research proposal)

	Institution	Year
MBBS	Dhaka Medical College	2009-14
Training	Internship	2014-15
Training	Research Protocol Development (icddr,b)	2018

4.Ethics Certification:

		If Yes		
		Issuing Authority	Registration No	Valid Until
No ☐	Yes ☒	**NIH**	**2225935**	**Issued 2017**

Note: If the response is "no", please get certification from CITI or NIH before study initiation and submit a copy to the Committee Coordination Secretariat

5. List of ongoing research protocols/ activities

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time

6.Publications

Types of publications	Numbers
m. Original scientific papers in peer-review journals	
n. Peer reviewed articles and book chapters	
o. Papers in conference proceedings	1
p. Letters, editorials, annotations, and abstracts in peer-reviewed journals	
q. Working papers	
r. Monographs	

7.Five recent publications including publications relevant to the present research protocol
7.3.

Certificate of Completion

The National Institutes of Health (NIH) Office of Extramural Research certifies that **Imteaz Mahmud** successfully completed the NIH Web-based training course "Protecting Human Research Participants."

Date of Completion: 02/15/2017

Certification Number: 1005286

NIH National Institutes of Health Office of Extramural Research

Biography of the Investigators

Provide biographical data in the following format for all key personnel including the Principal Investigator. Copy the same format for each of them.
Note: Biography of the External Investigators may, however, be submitted in the format as convenient to them..

1. **Name: Dr Nurun Nahar Naila**

2. **Present Position:** Assistant scientist

3. **Educational background**: (last degree and diploma & training relevant to the present research proposal)

	Institution	Year
MPH	University of New South Wales, Australia	2012
MBBS	Bangladesh Medical College and Hospital, Bangladesh	2006
Training	Qualitative Research method, International Centre for Diarrhoeal Disease Research, Bangladesh	2015
Training	Scientific writing, James P Grant School of Public Health, Bangladesh	2015

4. **Ethics Certification:**

		If Yes		
		Issuing Authority	Registration No	Valid Until
No ☐	Yes ☒	NIH	1347016	NA

Note: If the response is "no", please get certification from CITI or NIH before study initiation and submit a copy to the Committee Coordination Secretariat

5. **List of ongoing research protocols/ activities**

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time

Co Investigator PR 16005	IRB approval, staff training and recruitment, field activities supervision, monitoring data input and data analysis	2015	2018	100%
Principal Investigator PR 17030	Protocol development, IRB approval, field supervision and monitoring, data analysis	2017'May	2017'Oct	100%
Principal Investigator PR 17105	IRB approval, field supervision and monitoring, primary and secondary data analysis	will be started from October'2018		30%
Principal Investigator	To explore available information for identifying all possible activities, resources and barriers related to calcium supplementation program	Will be started from October 2018		40%

6. Publications

Types of publications	Numbers
a. Original scientific papers in peer-review journals	5
b. Peer reviewed articles and book chapters	
c. Papers in conference proceedings	1
d. Letters, editorials, annotations, and abstracts in peer-reviewed journals	
e. Working papers	2
f. Monographs	

7. Five recent publications including publications relevant to the present research protocol

1 **Naila N**, Nahar B, Lazarus M, Ritter G, Hossain M, Mahfuz M, Ahmed T, Denno D, Walson J, Ickes S. "Those who care much, understand much." Maternal perceptions of children's appetite: Perspectives from urban and rural caregivers of diverse parenting experience in Bangladesh. Maternal & child nutrition. 2018 Jan;14(1):e12473.

2 Mostafa I, **Naila NN**, Mahfuz M, Roy M, Faruque AS, Ahmed T. Children living in the slums of Bangladesh face risks from unsafe food and water and stunted growth is common. Acta Paediatrica. 2018 Feb 16.

3 Hossain M, Ickes S, Rice L, Ritter G, **Naila NN**, Zia T, Nahar B, Mahfuz M, Denno DM, Ahmed T, Walson J. Caregiver perceptions of children's linear growth in Bangladesh: a qualitative analysis. Public health nutrition. 2018 Mar:1-0.

4 Khatun H, Islam SB, **Naila NN**, Islam SA, Nahar B, Alam NH, Ahmed T. Clinical profile, antibiotic susceptibility pattern of bacterial isolates and factors associated with complications in culture-proven typhoid patients admitted to an urban hospital in Bangladesh. Tropical Medicine & International Health. 2018 Apr;23(4):359-66.

5 Mahfuz M, Alam MA, Islam SB, **Naila NN**, Chisti MJ, Alam NH, Sarker SA, Ahmed T. Treatment outcome of children with persistent diarrhoea admitted to an urban hospital, Dhaka during 2012–2013. BMC pediatrics. 2017 Dec;17(1):142.

Certificate of Completion

The National Institutes of Health (NIH) Office of Extramural Research certifies that **Nurun Naila** successfully completed the NIH Web-based training course "Protecting Human Research Participants".

Date of completion: 12/23/2013

Certification Number: 1347016

Consent Form

Primary MAM

Protocol No.18073	Version No. 1.1	Date: 13-09-2018

Purpose of the research

Protocol Title: Community-based clinical trial with microbiota directed complementary foods (MDCF) made of locally available food ingredients for the management of children with primary moderate acute malnutrition (MAM).

Principal Investigator's name: Dr Tahmeed Ahmed

Organization: icddr,b

Purpose of the research

Bangladesh is a country with one of the highest childhood malnutrition burden in the world. According to Bangladesh Demographic Health Survey 2014, 36% under five children are shorter for their age and among them 12 % are suffering from its severe form. Acute malnutrition is associated with an impaired development of the gut microbial community (microbiota immaturity). In a previous randomized clinical trial conducted at icddr,b in children with severe acute malnutrition (SAM) microbiota immaturity was only slightly improved in children treated with one of two current therapeutic foods and the children remained shorter for their age and underweight throughout the follow up period. Through our previous and ongoing research we now know about the members of the gut microbiota that can promote growth in children and also about certain food ingredients that promote the proliferation of such beneficial microbiota.

This clinical community based trial is designed for the management of children with primary moderate acute malnutrition with potential MDCF.

Why invited to participate in the study?

We are conducting this study in children who are 12-18 months old and suffering from moderate acute malnutrition. Maternal nutritional status is associated with child nutritional status, as shown through the results of our eight country MAL-ED study. In addition, neonatal and maternal factors were early determinants of lower length-for-age, and their contribution remained important throughout the first 24 months of life. So we are inviting you and your children to participate in this study. Your child and other similar children need proper dietary intervention to treat this. The proposed ingredients of the diets are to assess whether it is acceptable and promotes growth in children. We assume that your child's malnutrition is at least in part due to microbiota immaturity, and this is why we are inviting you and your child to help us in participating in this study.

Methods and procedures

If you agree to our proposal of including you and your child in the study, you might expect the followings:

- We would collect 2 mL of urine samples at enrolment, one week after enrolment and monthly once during the intervention and post intervention phase.
- Weekly 2 gm of stool samples will be collected during the study period.

- A total of 6 mL of blood in three occasions, with 2 mL each on every occasion (equivalent to near about half tea-spoonful) venous blood would be collected prior to enrolment, at the end of first month of intervention and after the completion of the intervention.
- After 1 week of enrolment, we would ask you and your child to visit the nutrition centre twice daily for the first month, and once daily for the second month to take the nutrition supplement. There will be 2 groups who will receive the nutrition supplement and your child will be randomly assigned to any one of the groups.
- Your child will receive one type of food or the other type of food (that will be fixed based on a process like lottery) to observe how much your child can eat.
- We would ask you some questions regarding current illness and health condition of your child. We will also perform thorough physical examinations and measure length and weight weekly after enrolment in the study.
- We will also ask for information on your socioeconomic condition and family structure. Your child will be monitored everyday for any possible side effects/adverse events (e.g. rash, urticaria from food allergy or any significant changes in clinical status).
- If any side effects/adverse event are observed then it will be treated according to the standard management protocol followed at Dhaka Hospital of icddr,b.
- In order to understand the biological state of nutrition of the mother, enrolment samples of stool, 5 ml blood and urine will be asked of the mother. These samples will be analysed for gut microbiota, and proteomics, and the results correlated with those of the enrolled children.
- We will also record maternal height and weight.
- We assure you that the stool, blood samples and urine samples will not be used for any other purposes.

Risk and benefits

Anticipated potential benefits:

Your child will be directly and indirectly benefited from participating in the study. Moreover, your child would be able to contribute to our understanding of malnutrition and to develop more effective treatments. In the long term, the results of this study could benefit other children in Bangladesh and elsewhere by helping us understand the effects of providing nutrient supplements. Nutritional counselling will help you to routine your child's daily food intake. The goal is to test special food (MDCF) that repair the persistent microbiota immaturity and promote growth in children with MAM.

Anticipated potential risks:

There is no major risk involved in participation of your child in the study. Possible adverse events may be vomiting, diarrhoea, skin rash, urticaria from food. Despite taking precautions, if your child develops any symptoms due to this study procedure, we would provide appropriate treatment at the Dhaka Hospital of icddr,b.

At the time of collection of the blood sample, your child will feel a momentary pain due to the needle prick. There is also a rare chance of bluish discoloration surrounding the prick site due to mild leakage of blood in the skin, and very distant possibility of local or systemic infections or problems. However, we will take required precautions, including using of disposable syringes and needles, to prevent these problems. All blood samples will be obtained by a qualified health care professional. A total of 6 mL of blood sample (equivalent to one tea-spoonful) will be obtained from your child just before the intervention (2 mL of blood

sample), end of first month of intervention (2 mL of blood sample) and after the intervention (2 mL of blood sample) is complete.

Privacy, anonymity and confidentiality

We will keep all information collected from you/your child confidential, locked in a secure place under the responsibility of the principal investigator from icddr,b. No one other than this group of investigators, regulatory authorities and the Ethical Review Committee (a group of experts which protects the interest of study participants) of icddr,b, investigators in the Washington University School of Medicine would have access to such information. The biological samples will be sent to Washington University for further analysis. Your child's name and identity will not be disclosed while analyzing or publishing the results of this study.

Future use of information

In the case of future use of the information collected from this study, privacy, anonymity and confidentiality of information will be maintained. We will store the stool and blood sample in a way that your child's identity will not be recognised, and use the samples for performing tests that are modified in the near future for superior results, as well as new tests for studying growth and metabolism. No further consent will be requested for such studies.

Right not to participate and withdraw

Your child's participation in the study is voluntary, and you have the sole authority to decide for or against your patient's participation. You would also be able to withdraw your child's participation any time during the study, without showing any cause. Refusal to take part in or withdrawal from the study will involve no penalty or loss of care, benefits or attention.

Principle of compensation

There is no cost to you for participating in this study. You will not have to pay for the supplement or any of the tests we are doing. You will not get any money for participating in this study either. If your child has a study related injury s/he will receive standard care at the Dhaka Hospital (Cholera Hospital) in Mohakhali, Dhaka.

Answering your questions/Contact persons

We will happily provide you further information about the study, if any, now or at a later time. You may communicate with the principal investigators of the study or her/his designated person at the contact address given below. We will answer to your question related to your/your child's medical condition(s), treatment, and results of any or all tests performed on you/your child.

You may communicate with Dr. Tahmeed Ahmed, the principal investigator of this study personally at his office at icddr,b or by telephone (+88-02-98271030) Ext-2300

If you agree to our proposal of enrolling your child in our study, please indicate that by putting your signature or your left thumb impression at the specified space below.

If you have questions regarding your rights as a research participant, please call IRB Secretariat, RA, MA Salam Khan, Phone No: 9886498 or PABX 8860523-32 Extension. 3206 at the icddr,b Research Ethics office.

(NOTE: In case of representative of the PI, she/he shall put her/his full name and designation and then sign)
If you agree to our proposal of enrolling you/your patient in our study, please indicate that by putting your signature or your left thumb impression at the specified space below

Thank you for your cooperation,

_____ _____

Signature or left thumb impression of Parent/ Guardian/ Attendant — Date

_____ _____

Signature or left thumb impression of the witness — Date

_____ _____

Signature of the PI or his/her representative — Date

"কমিউনিটি-বেসড ক্লিনিকাল ট্রায়াল উইথ মাইক্রোবায়টা-ডিরেক্টেড কম্পলিমেন্টারি ফুডস (এমডিসিএফস) মেইড অফ লোকালি এভেইলেবল ফুড ইনগ্রেডিয়েন্টস ফর দা ম্যানেজম্যান্ট অফ চিল্ড্রেন উইথ প্রাইমারি মডারেট একিউট ম্যালনিউট্রিশন"।

প্রধান গবেষকের নাম: ডাঃ তাহমীদ আহমেদ

প্রতিষ্ঠান: আন্তর্জাতিক উদরাময় গবেষনা কেন্দ্র, বাংলাদেশ

গবেষনার উদ্দেশ্য

বিশ্বের যে সব দেশে শিশু অপুষ্টির হার অনেক বেশি, বাংলাদেশ তাদের অন্যতম। বাংলাদেশ স্বাস্থ্য ও জনমিতি জরিপ ২০১৪ অনুযায়ী দেশের শতকরা ৩৬ ভাগ শিশু তাদের বয়সের তুলনায় খর্বাকৃতির, এবং তাদের মধ্যে শতকরা ১২ ভাগ মারাত্নক ভাবে খর্বাকৃতির।ইতোপূর্বে আই সি ডি ডি আর, বি তে দুই ধরনের পথ্য নিয়ে একটি চিকিৎসা বিষয়ক গবেষণায় দেখা গিয়েছে যে, মারাতক তীব্র অপুষ্টিতে আক্রান্ত শিশুদের চিকিৎসার ফলে তাদের মাইক্রোবায়োটা (আন্ত্রিক অনুজীবের) অপরিপক্বতা (যা খাবার হজমের সমস্যার সাথে সম্পর্কিত) কিছু অংশে দূর হলেও পরবর্তী সময়ে তারা বয়স অনুযায়ী খর্বাকৃতির ও কম ওজনের থেকে যায়। আমাদের পূর্ববর্তী ও বর্তমান চলমান বিভিন্ন গবেষণা থেকে আমরা এখন অন্ত্রের অনুজীব সমুহের নাম জানি যারা কিনা শিশুদের বৃদ্ধির জন্য উপকারী এবং কিছু কিছু পুষ্টি উপাদান যারা উপকারী অনুজীবের সংখ্যা বৃদ্ধির জন্য সহায়কক।

এই গবেষণাটি পরিকল্পানা করা হয়েছে মাঝারি অপুষ্টিতে আক্রান্ত বাচ্চাদে র আমাদের বিশেষ উপায়ে তৈরী খাবার (এম.ডি. সি.এফ.) দিয়ে ব্যবস্থাপনার জন্য।

কেন গবেষনায় অংশগ্রহনের জন্য আপনাকে আমন্ত্রন জানানো হচ্ছে?

আমরা এই গবেষনায় ১২-১৮ মাস বয়সী সেইসব শিশুদের অঃতর্ভুক্ত করেছি যারা তাদের বয়সের তুলনায় খাটো ও চরম তীব্র অপুষ্টির শিকার।আবার ৮ টি দেশে সংঘটিত এম.এ.এল.ই.ডি গবেষণায় দেখা গেছে, বাচ্চার পুষ্টির সাথে মায়ের পুষ্টির সম্পর্ক রয়েছে। এটা প্রতিরোধ করার জন্য আপনার শিশু ও অন্যান্য অনুরূপ শিশুদের সঠিক খাদ্যাভাসের প্রয়োজন।এই প্রস্তাবিত খাদ্যের উপাদানগুলোর গ্রহণযোগ্যতা শিশু ও মায়েদের মধ্যে পরিমাপ করা হবে। আমরা মনে করছি যে, আপনার শিশু মাইক্রোবায়োটা অপরিপক্কতার কারণে সে তার বয়সের তুলনায় খাটো ও অপুষ্টি তে আক্রান্ত, এবং এই কারণে আমরা আপনাকে ও আপনার শিশুকে এই গবেষণায় অংশগ্রহণের জন্য আমন্ত্রণ জানাচ্ছি।

পদ্ধতি ও প্রক্রিয়া

আপনি যদি আমাদের প্রস্তাবে সম্মত হয়ে আপনার শিশু সহ এই গবেষনায় অংশগ্রহন করেন, তাহলে আপনি নিম্নলিখিত বিষয়গুলো আশা করতে পারেন।

- আমরা আপনার বাচ্চার মলের নমুনা (১-২ গ্রাম) সংগ্রহ করব প্রথম নিয়গের সময়, প্রথম নিয়গের ১ সপ্তাহ পর, প্রথম মাসের প্রতি সপ্তাহে এবং পরবর্তী দুই মাস এবং পদক্ষেপ পরবর্তী সময় প্রতি মাসে একবার করে। আমরা বিশেষ পদ্ধতি অবলম্বন করে মলের নমুনায় জীবাণু বের করব।
- এই সময়ে বাচ্চার উচ্চতা এবং ওজনও পরিমাপ করা হবে
- আমরা ২ মিলি লিটার পরিমান প্রস্রাব এর নমুনা সংগ্রহ করব প্রথম নিয়গের সময়, তার এক সপ্তাহ পর এবং প্রতি মাসে একবার করে ইন্টারভেনশন ও ইন্টারভেনশন পরবর্তী সময়ে।

- বাচ্চার শিরা থেকে মোট ৬ মি.লি লিটার (আধা চা চামচ পরিমান) রক্ত সংগ্রহ করা হবে প্রথম নিয়গের সময় (২ মি.লি), ইন্টারভেনশন এর প্রথম মাস পর (২ মি.লি) এবং ইন্টারভেনশন সমাপ্ত হবার পর (২ মি.লি)।
- প্রথম নিয়গের ১ মাস পর, আমরা আপনাকে এবং আপনার বাচ্চাকে প্রথম মাসে দিনে ২ বার এবং দ্বিতীয় মাসে দিনে ১ বার করে আস্তে বলব সম্পূরক পুষ্টি গ্রহন করার জন্য। এই ক্ষেত্রে দুইটি গ্রুপ থাকবে যারা সম্পূরক পুষ্টি গ্রহন করবে এবং আপনার বাচ্চা লটারির মত পদধতির মাধ্যমে যে কোন একটি গ্রপের জন্য নির্বাচিত হবে।
- আপনার বাচ্চা এম.ডি. সি.এফ অথবা আর.ইউ.এস.এফ. এর মধ্য থেকে যে কোন এক ধরনের খাবার পাবে এবং পরিক্ষা করে দেখা হবে আপনার শিশু কত টুকু খাবার খেতে পারে।
- আমরা আপনাকে আপনার শিশুর সাম্প্রতিক অসুস্থতা ও স্বাস্থ্যের অবস্থা সংক্রান্ত কিছু প্রশ্ন জিজ্ঞেস করবো। এছাড়াও প্রতি ২ সপ্তাহে একবার করে আপনার শিশুর ওজন ও উচ্চতা পরিমাপ করা হবে।
- আমরা আপনার আর্থ-সামাজিক অবস্থা ও পরিবার এর ধরন নিয়ে কিছু প্রশ্ন জিজ্ঞেস করবো। খাবার দেয়ার সময় প্রতিদিন আপনার শিশুকে সম্ভাব্য প্রতিকূল ঘটনার (যেমন খাবারে এলার্জির জন্য শরীরে চুলকানি বা চাকা চাকা দাগ কিংবা শারীরিক অবস্থার উল্লখেযোগ্য পরিবর্তন) জন্য পর্যবেক্ষন করা হবে।
- এ সময়ে কোন প্রতিকূল ঘটনা ঘটলে আমরা আপনার শিশুকে আই সি ডি ডি আর বি এর ঢাকা হাসপাতালে মানসম্পন্ন সেবা প্রদান করবো।
- মায়ের পুষ্টির অবস্থা বোঝার জন্য, বাচ্চা প্রথম নিয়গের সময় মায়ের মলের নমুনা, ৫ মিলি রক্ত, এবং প্রস্রাব এর নমুনা চাওয়া হবে। এই নমুনা সমূহ অন্ত্রের অণুজীব এবং প্রটিওমিক্স এর অবস্থা জানার জন্য পর্যালোচনা করা হবে,এবং প্রাপ্ত ফলাফলের সাথে নিয়গকৃত শিশুদের ফলাফল সম্পর্কযুক্ত করা হবে।
- আমরা আপনাকে নিশ্চিতকরতে চাই যে, রক্ত ও মলের নমুনা অন্য কোন কাজে ব্যবহার করা হবে না।

ঝুঁকি এবং সুবিধা

গবেষনা থেকে প্রাপ্ত সম্ভাব্য সুবিধাঃ

আপনার শিশু এই গবেষনায় অংশগ্রহনের মাধ্যমে প্রত্যক্ষ ও পরোক্ষভাবে উপকৃত হবে। তার অংশগ্রহন আমাদের শিশু অপুষ্টির সমস্যাকে ভালভাবে বুঝতে ও এর সম্পর্কে জ্ঞান অর্জন করতে সাহায্য করবে। শিশুরা বিনামূল্যে এই বিশেষ ধরনের খাবার গ্রহনের মাধ্যমে উপকৃত হবে। ভবিষ্যতে এই গবেষণার ফলাফল আমাদের বুঝতে সাহায্য করবে, যে কিভাবে পুষ্টিকর উপাদান খাওয়ানোর ফলে বাংলাদেশ ও বিশ্বের অন্যান্য স্থানে শিশুরা উপকৃত হয়। পুষ্টি বিষয়ক আলোচনা আপনাকে আপনার শিশুর প্রাত্যহিক পুষ্টিকর খাদ্য গ্রহন নিশ্চিত করতে সহায়তা করবে। এই গবেষনার লক্ষ্য হচ্ছে, একটি বিশেষ ধরনের খাদ্য (এম ডি সি এফ) পরীক্ষা করা যা বয়সের তুলনায় খর্বাকৃতি ও অতি তীব্র অপুষ্টিতে আক্রাঃত শিশুদের অন্ত্রে বিদ্যমান অনুজীবের অপরিপক্বতা ও তাদের
শারীরিক বৃদ্ধি তরান্বিত করবে

গবেষনা থেকে প্রাপ্ত সম্ভাব্য ঝুঁকিঃ

এই গবেষনায় অংশগ্রহনের কারনে আপনার শিশুর কোন ক্ষতির সম্ভাবনা নেই। সম্ভাব্য ক্ষতিগুলোর মধ্যে খাবার খাওয়ার পরে বমি, পাতলা পায়খানা, শরীরে কোন কোন স্থানে চুলকানি বা চাকা চাকা দাগ হতে পারে। এসব বিষয়ে যথেষ্ট সতর্কতা অবলম্বনের পরেও গবেষনার কারনে এমন ঘটনা ঘটলে আমরা আপনার শিশুকে আই সি ডি ডি আর বি এর ঢাকা হাসপাতালে মানসম্পন্ন সেবা প্রদান করবো।
এই গবেষনায় রক্তের নমুনা সংগ্রহের সময় সামান্য অসস্থি বা ব্যথা অনুভূত হতে পারে। এছাড়াও সুঁই প্রবেশ করানোর ফলে ঐ স্থান নীলচে বর্ণ ধারন করতে পারে বা ফুলে উঠতে পারে। কিছু কিছু ক্ষেত্রে এর ফলে সেখানে সংক্রমন ও হতে পারে ও কদাচিৎ সেখানে রক্ত জমাট বাঁধতে পারে। দক্ষ নমুনা সংগ্রাহকের মাধ্যমে নিরাপদ প্রক্রিয়ায় উক্ত নমুনা সংগ্রহ করা হবে এবং এর ফলে আপনার শিশুর কোন ক্ষতির সম্ভাবনা নেই। সর্বমোট চার মি.লি (প্রায় ১ চা চামচ পরিমাণ)

রক্তের নমুনা সংগ্রহ করা হবে। ২ মি.লি (অর্ধেক চা চামচ পরিমাণ) তালিকভুক্তির সময় এবং ২ মি.লি (অর্ধেক চা চামচ পরিমাণ) গবেষনার শেষে সংগ্রহ করা হবে।

গোপনীয়তা ও বিশ্বস্ততা

আমরা আপনাকে নিশ্চিত করছি যে এই গবেষণার প্রতিটি পর্যায়ে আপনার বাক্তিগত তথ্যের গোপনীয়তা সুনিশ্চিত করব। আমরা সকল রোগ, চিকিৎসা ও পরীক্ষা সম্পর্কিত তথ্য তালা-চাবি দিয়ে গোপনে সুরক্ষিত রাখব। এই গবেষণা সংশ্লিষ্ট গবেষক এবং আইসিডিডিআর,বির নৈতিকতা সমীক্ষা কমিটি (ই আর সি) ছাড়া কেউ এই তথ্য ব্যবহার করতে পারবে না। ওয়াশিংটন বিশ্ববিদ্যালয়র গবেষকদেরও এই তথ্য দেখার অধিকার থাকবে। গবেষণায় প্রাপ্ত জৈব নমুনাসমূহ পরবর্তীতে বিশ্লেষণ এর উদ্দেশ্যে ওয়াশিংটন ইউনিভার্সিটিতে পাঠানো হবে। আপনার শিশুর নাম ও পরিচয় কোনক্রমে এই গবেষণার ফলাফল প্রকাশের সময় গোপন রাখা হবে।

ভবিষ্যতে তথ্যের ব্যবহারঃ

ভবিষ্যতে এই গবেষণা প্রাপ্ত তথ্য ব্যবহারের প্রয়োজন হলে, এর বিশ্বস্ততা ও গোপনীয়তা রক্ষা করা হবে এবং গোপন রাখা হবে। এই গবেষণার ফলাফল আমাদেরকে ভবিষ্যতে আপনার রক্ত ও মলে উপস্থিত পদার্থগুলো নিয়ে আরও অধিকতর গবেষণায় সাহায্য করবে। আপনার এই রক্ত ও মলের নমুনা আই সি ডি ডি আর,বি তে সংরক্ষণ করা হবে। এই সকল নমুনা পুনরায় ব্যবহারের জন্যে আপনার কোন সম্মতি নেয়া হবে না।

গবেষণায় অংশগ্রহণ না করার অধিকারঃ

এই গবেষণায় আপনার অংশগ্রহণ পুরোপুরি আপনার স্বেচ্ছাধীন এবং অংশগ্রহণের বিপরীতে সকল সিদ্ধান্ত নেয়ার অধিকার সম্পূর্ণরূপেই আপনার। এই গবেষণার যে কোন পর্যায়ে আপনি এমনকি কোন কারণ দর্শানো ব্যাতিরেকে আপনার অংশগ্রহণ স্থগিত করতে পারেন। এর ফলে আপনার কোন ক্ষতি হবে না এবং আপনাকে কোন ক্ষতিপূরণও দিতে হবে না।

ক্ষতিপূরণের নিয়মাবলীঃ

এই গবেষনায় অংশগ্রহনের জন্য আপনার কোন খরচ হবে না। আপনাকে এই গবেষনায় ব্যবহৃত সম্পূরক খাদ্যের জন্য বা গবেষনার অন্তর্গত কোন পরীক্ষার জন্য কোন মূল্য দিতে হবে না। এই গবেষনায় অংশগ্রহনের জন্য আপনিও কোন অর্থসাহায্য পাবেন না। গবেষনা সংক্রান্ত অসুস্থতার ক্ষেত্রে আপনার শিশু ঢাকার মহাখালীতে অবস্থিত কলেরা হাসপাতা ল থেকে মানসম্পন্ন সেবা পাবে।

যে কোন প্রশ্নের উত্তর/ যোগাযোগের বিষয়েঃ

আমরা অত্যন্ত আনন্দের সাথে আপনার যে কোন প্রশ্নের উত্তর দিতে এখন অথবা ভবিষ্যতেও সর্বদা প্রস্তুত থাকব। আপনি চাইলে প্রধান গবেষক অথবা তার যে কোন কর্মীর সাথে অথবা নিচের ঠিকানায় যোগাযোগ করতে পারেন। আমরা এই গবেষণা সংক্রান্ত যে কোন তথ্য (স্বাস্থ্যগত/ জৈবরাসায়নিক পরীক্ষা সম্পর্কিত) দিতে সর্বদা প্রস্তুত। কিন্তু আমরা আপনাকে জানাতে চাই যে, কিছু কিছু জৈবরাসায়নিক পরীক্ষার তথ্য পেতে বেশকিছু সময়ের প্রয়োজন হতে পারে কেননা সেগুলো পরীক্ষার জন্যে দেশের বাহিরে পাঠানো হবে। পরবর্তী যোগাযোগের জন্যে- ডাঃ তাহমীদ আহমেদ, সিনিয়র ডিরেক্টর, নিউট্রিশন ও ক্লিনিকাল সার্ভিসেস ডিভিশন, আইসিডিডিআর, বি ৬৮, শহীদ তাজউদ্দীন আহমেদ সরণি, মহাখালী, ঢাকা ১২১২, বাংলাদেশ। ফোনঃ +৮৮-০২-৯৮২৭১০৩ অথবা পিএবিএক্স ৮৮৬০৫২৩-৩২ এক্সটেনশন- ২৩০০।

নৈতিকতা সমীক্ষা কমিটির দায়িত্বপ্রাপ্ত প্রতিনিধির নাম ও যোগাযোগের ঠিকানাঃ জনাব এম এ সালাম খান, ফোনঃ ৯৮৮৬৪৯৮ অথবা পিএবিএক্স ৮৮৬০৫২৩, এক্সটেনশন- ৩২০৬)

আপনি যদি আমাদের এই গবেষণায় অংশগ্রহণ করতে চান, তাহলে নিচের সুনির্দিষ্ট জায়গায় আপনার স্বাক্ষর অথবা টিপসই দিন।

আপনার সাহায্যের জন্যে ধন্যবাদ।

অংশগ্রহনকারীর মা/বাবা/অভিভাবক/সেবাদানকারীর স্বাক্ষর/বাম হাতের বৃদ্ধাঙ্গুলীর ছাপ
.....

তারিখ

স্বাক্ষীর স্বাক্ষর/বাম হাতের বৃদ্ধাঙ্গুলির ছাপ
.....

তারিখ

প্রধান গবেষক/তার প্রতিনিধির স্বাক্ষর
.....

তারিখ
দ্রষ্টব্য: প্রতিনিধি তার পূর্ণ নাম ও পদ লিখে তারপর স্বাক্ষর করবেন ।

Gender Analysis Tools

Gender Analysis Tool: Relation of gender to MAM	Are there sex differences in	How do biological differences between women and men influence their	How do the different roles and activities of men and women affect their	How do gender norms / values affect men and women's	How do access to, and control over resources affect men and women's

Vulnerability: Incidence ** **Prevalence ****			In Bangladesh, more than 2 million children suffer from MAM		
Health seeking behaviour	Since children are dependent on their parents for accessing health care, and we assume that morbidities of a female child is taken less seriously than that of a male child, it can be said that health seeking behaviour differs from that of a female child.	Not applicable as biological difference has no role on this	Does not have a role in health seeking behaviour of a child.	Since children are dependent on their parents for accessing health care, and we assume that morbidities of a female child is taken less seriously than that of a male child, it can be said that health seeking behaviour differs from that of a female child.	In the present context of the country, it can be assumed that, there is less money allocated for treatment of female child. So their health seeking behaviour differs from that of a male child.
Ability to access health services	Not applicable as children are dependent on their parents and unable to access health services	Not applicable as biological difference has no role on this	Not applicable as children are dependent on their parents and unable to access health services		Not applicable
Experience with health services and health providers	No difference reported for treatment of MAM	Not applicable as biological difference has no role on this	In most of the health programs male and female get equal priorities.		No data available
Preventive and Treatment options, responses to treatment or rehabilitation	No such differences have been reported that make female child more vulnerable to adverse health effect of the different methods	No data available to support any difference that occur between male and female due to treatment options.	We assume that after diarrhoea or any acute illness female child are not given adequate nutritious food compared to male. So, it puts females at risk for being MAM		In the present context of the country, less priority is given to a female child therefore less money allocated for treatment, prevention and rehabilitation

Check-List

Check-list for Submission of Research Protocol
For Consideration of the Research Review Committee (RRC)
[Please check all appropriate boxes]

1. Has the proposal been reviewed, discussed and cleared by all listed investigators?

   ☒ Yes ☐ No

   If the response is No, please clarify the reasons:

2. Has the proposal been peer-reviewed externally?

   ☒ Yes ☐ No ☐ External Review Exempted

   If the response is 'No' or "External Review Exempted", please explain the reasons:

   If the response is "Yes", please indicate if all of their comments have been addressed?

   ☒ Yes (please attach)

   ☐ No (please indicate reason(s)):

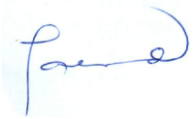
3. Has the budget been reviewed and approved by icddr,b's Finance?

   ☒ Yes ☐ No (reason): _____

4. Has the Ethics Certificate(s) been attached with the Protocol?

   ☐ Yes ☐ No

   If the answer is 'No', please explain the reasons:



_____ _12.09.2018__

Signature of the Principal Investigator Date

icddr,b

Microbiota Directed Complementary Food (MDCF) - Study
Food Frequency Questionnaire

PID: ☐☐☐ – ☐☐☐☐

Date: ☐☐/☐☐/☐☐	Staff ID: ☐☐

1	Are you breastfeeding your child? If No, Skip to question 4.	01=Yes 00=No	☐☐
2	Last night, how many times did you breastfeed your child from sunset to sunrise?		☐☐
3	Yesterday, during the day, how many times did you breastfeed your child?		☐☐
4	Do you give your child infant formula? If No, Skip to question 7.	01=Yes 00=No	☐☐
5	Last night, how many times did you feed your child infant formula from sunset to sunrise?		☐☐
6	Yesterday, during the day, how many times did you feed your child infant formula?		☐☐
7	Do you give your child other milks, such as tinned, packed, powdered or fresh animal milk? If No, Skip to question 10.	01=Yes 00=No	☐☐
8	Last night, how many times did you feed your child animal milks from sunset to sunrise?		☐☐
9	Yesterday, during the day, how many times did you feed your child animal milk?		☐☐
10	If your child eating any semi-solid, mashed, or solid foods? If No, Skip to question 15.	01=Yes 00=No	☐☐
10a	If yes, how many times?		☐☐
Yesterday, during the day and last night, did the participant have:			
11	Plain water? If No, Skip to question 12.	01=Yes 00=No	☐☐
11a	If yes, how many times?		☐☐
12	Tea, coffee, or any other warm/hot drinks? If No, Skip to question 13.	01=Yes 00=No	☐☐
12a	If yes, how many times?		☐☐
13	Fruit or vegetable juices (prepared at home)? If No, Skip to question 14.	01=Yes 00=No	☐☐
13a	If yes, how many times?		☐☐
14	Any other liquids, such as sugar water, thin soup or broth, carbonated drinks, commercially packed juices. If No, Skip to question 15.	01=Yes 00=No	☐☐

14a	If yes, how many times?		
Thinking about yesterday, during the day and at night, did the participant have any of the following foods, even if they were in combination with other foods?			
15	Rice, bread, noodles, or other foods made from grains? If No, Skip to question 16.	01=Yes 00=No	
15a	If yes, how many times?		
16	White potatoes or other foods made from roots? If No, Skip to question 17.	01=Yes 00=No	
16a	If yes, how many times?		
17	Carrots or sweet potatoes that are yellow or orange inside? If No, Skip to question 18.	01=Yes 00=No	
17a	If yes, how many times?		
18	Any dark green leafy vegetables such as spinach? If No, Skip to question 19.	01=Yes 00=No	
18a	If yes, how many times?		
19	Foods made with beans, lentils, peas, corn, ground nuts or any other legumes? If No, Skip to question 20.	01=Yes 00=No	
19a	If yes, how many times?		
20	Ripe mangoes, papayas, or other sweet yellow/orange or red fruit? If No, Skip to question 21.	01=Yes 00=No	
20a	If yes, how many times?		
21	Any other fruits or vegetables such as banana, apple, oranges, tomatoes, squash etc.? If No, Skip to question 22.	01=Yes 00=No	
21a	If yes, how many times?		
22	Liver, kidney, or other organ meats? If No, Skip to question 23.	01=Yes 00=No	
22a	If yes, how many times?		
23	Any meat, such as chicken, beef, lamb, goat, ducks (others)? If No, Skip to question 24.	01=Yes 00=No	
23a	If yes, how many times?		
24	Eggs? If No, Skip to question 25.	01=Yes 00=No	
24a	If yes, how many times?		
25	Fresh or dried fish or shellfish? If No, Skip to question 26.	01=Yes 00=No	
25a	If yes, how many times?		
26	Cheese, yogurt, or other dairy products? If No, Skip to question 27.	01=Yes 00=No	
26a	If yes, how many times?		

27	Any sugary foods such as pastries, cakes, or biscuits? If No, Skip to question 28.	01=Yes 00=No	☐☐
27a	If yes, how many times?		☐☐
28	Any commercially available foods? If No, Skip to question 29.	01=Yes 00=No	☐☐
28a	If yes, how many times?		☐☐
29	Any locally produced/vendor foods (such as rice cakes, chanachur, etc.)? If No, Skip to question 30.	01=Yes 00=No	☐☐
29a	If yes, how many times?		☐☐
30	Yesterday, counting meals and snacks, how many times did the participant ate?		☐☐
31	Yesterday during the day and at night, did the participant eat anything else other than the foods that were mentioned right now? If No, Skip to question 54.	01=Yes 00=No	☐☐
31a	If yes, how many times?		☐☐
32	Please name the foods:		
	a) ……		
	b) ……		
	c) ……		
33	Yesterday during food preparation, did oil was mixed with it? If No, Skip to question 34.	01=Yes 00=No	☐☐
33a	If yes, how many spoons?		☐☐
34	How would the responder describe participant's appetite?	01=Poor 02=Fair 03=Good 04=Very good	☐☐
35	Are there any additional comment?	01=Yes 00=No	☐☐
35a	If yes, record comment here:		

Questionnaire

A. Identification :

Sl no.	Questions	Code list	Code
1.	Name of the child	----------	
2.	Mother's/Caregiver's name	----------	
3.	Father's name	----------	

4.	Date of birth of the reference child	Day Month Year	
5.	Sex of the child	0=Boy; 1=Girl	
6.	Birth order of the reference chid in his/her family	----------	

B. Socio-demographic Information:

Sl no.	Questions	Code list	Code
7.	What is the religion of the respondent?	1=Islam, 2=Hindu, 3=Christian, 4=Others (Specify…….)	
8.	Marital status of mother	1=Married, living with husband, 2=Widowed, 3=Separated, 4=Divorced	
9.	Respondent's relationship with child	1=Mother, 2=sister, 3=Grandmother, 4=Aunt, 5=other…….. (Specify)	
10.	Mother's education qualification	1-9=Write down which class passed 10=SSC/Dakhil 11=HSC/Alim 12=Gradudate/Fazil 13=Master/Doctor/Engineer/Lawyer/Kamil 14=Diploma/vocational training 22=Only can write her name 33= Can not write her name	
11.	Father's education	Write down which class passed 10=SSC/Dakhil 11=HSC/Alim 12=Gradudate/Fazil 13=Master/Doctor/Engineer/Lawyer/Kamil 14=Diploma/vocational training 22=Only can write her name 33= Can not write her name	
12.	Who is the household's head?	0=Male, 1=Female	
13.	Household head's occupation according to respondent	1=Agriculture, 2=Fishing, 3=Household based work(weaving, handicraft), 4=Cattle, poultry rearing, 5=Skilled labor, 6=day labour, 7=Service (earn money monthly basis), 8=Small Business (milkman, fruit/vegetable seller), 9=Big Business, 10=Unemployed, 11=Housewife/Househusband, 99=DK	

C. Household characteristics

Sl No	**Questions**	**Answer**	
14.	What is the principal material used for the walls of your household?	1=Wood 2=Concrete, brick, stone 3=Tin	

		4=Bamboo, other natural materials Others _____ (specify)	
15.	What is the principal material used for the floors in your household?	1=Mud or other natural materials 2=Cement 3=Wood Others _____ (specify)	
16.	What is the principal material used for the roof of your household?	1=Bamboo or other natural materials 2=Tin 3=Cement, concrete, tile Others _____ (specify)	
17.	What is the principal type of fuel for cooking used by your household?	1=Gas 2=Kerosene 3=Wood 4=Dung 5=Crop residue Others _____ (specify)	
18.	What is the principal source of drinking water for your household?	1=Piped water in residence 2=Piped water in yard 3=Well 4=Tube well 5=Pond/ ditch/ canal/lake/tank 6=River water 7=Rainwater Others _____ (specify)	
19.	What is main source of water for cooking?	1=Piped water in residence 2=Piped water in yard 3=Well 4=Tube well 5=Pond/ ditch/ canal/lake/tank 6=River water 7=Rainwater Others _____ (specify)	
20.	What is the principal type of toilet used by the household?	1=Open pit 2=Pit 3=Ring slab (water sealed) 4=Ring slab (not water sealed) 5=Sanitary (Water seal and septic tank) 6=Hanging/anywhere/open Others..... ....(specify)	
21.	What does your household members use to wash your hands after defecation?	1=Soap 2=Ash 3=Mud 4=Water from tube-well only water (other sources) only	

		5=Does not wash hands Others _____ (specify)	
22. *	In your household, is/are there Electricity? Radio? Television? Bicycle? Telephone/mobile? Refrigerator/freeze? Almirah(wardrobe)/showcase? Table ? Chair, bench ? Watch, clock ? Cot, bed ? Sewing machine? Motorcycle, scooter ? Vehicle (animal run)? Car/truck/micro-bus? Boat/troller?	Electricity (1=Yes, 0=No) Radio (1=Yes, 0=No) Television (1=Yes, 0=No) Bicycle (1=Yes, 0=No) Telephone/mobile (1=Yes, 0=No) Refrigerator/freeze (1=Yes, 0=No) Almirah/showcase (1=Yes, 0=No) Table (1=Yes, 0=No) Chair, bench (1=Yes, 0=No) Watch, clock (1=Yes, 0=No) Cot, bed (1=Yes, 0=No) Sewing machine (1=Yes, 0=No) Motorcycle, scooter (1=Yes, 0=No) Vehicle (animal run) (1=Yes, 0=No) Car/truck/micro-bus (1=Yes, 0=No) Boat/troller (1=Yes, 0=No)	

*Multiple answer will be considered

APPETITE TEST

Study ID: |__|__|__| Type (code) of MDCF: |__|

Date	Time	Body weight (kg)	Amount offered (gm)	Amount left (gm)	Estimated amount of vomiting (gm)	Amount taken orally excluding vomiting	% consumed	Appetite test*: (pass or fail)

*If a child eats half of the amount offered of MDCF/RUSF by 45 minutes; then the child will be considered as passed the appetite test

Anthropometric measurement:

Sl no.	Questions	Code list	Code
1	Child's weight	----------kg	
2	Length	----------cm	
3	MUAC	----------cm	

Morbidity (Checklist) **Participant ID:** ☐☐☐☐
MORBIDITY

Visit Number: ☐☐ **Date:** ☐☐/☐☐/☐☐

Now I would like to ask about the health of [NAME] in the previous day

Sl no.	Questions	Code list	Code
1.	Fever	0=No. 1=Yes	☐
2.	3 or more bowel movements in 24 hour	0=No. 1=Yes	☐
3.	Watery or soft stool?	0=No. 1=Yes	☐
4.	Mucus or blood in the stool?	0=No. 1=Yes	☐
5.	Stomach pain or cramps?	0=No. 1=Yes	☐
6.	Nausea	0=No. 1=Yes	☐
7.	Vomit	0=No. 1=Yes	☐
8.	Abrasion, scrapes or bruising?	0=No. 1=Yes	☐
9.	Skin itching on the body or scalp?	0=No. 1=Yes	☐
10.	Toothache?	0=No. 1=Yes	☐
11.	Constant cough?	0=No. 1=Yes	☐
12.	Congestion / runny nose?	0=No. 1=Yes	☐
13.	Panting/wheezing / difficulty breathing?	0=No. 1=Yes	☐

Participant ID: ☐☐☐-☐☐☐☐			
A. Blood Collection Form			
Blood collection event (give a tick)		☐ Before intervention	☐ After intervention
#	**Question**	**Code**	**Response**
1	Phlebotomist ID:	☐☐	
2	Date of collection:	DD/MM/YY	☐☐/☐☐/☐☐
3	Time of collection:	(24 Hr Scale; HH:MM)	☐☐:☐☐
4	Last meal (for child):	Breast milk only = 01; Snack only = 02; Meal only = 03; Food and breast milk = 04	☐☐
5	Time since last meal	Within 30 Min = 01; Within 2 Hrs = 02; > 2 Hrs = 03	☐☐
6	Up to 2 mL blood collected?	Yes=01, No=00	☐☐
Observations/Notes:			

Participant ID: ☐☐☐-☐☐☐☐			
A. Stool Collection Form			
If no response for any question, write NA as response.			
	Stool collection event (give a tick)	☐ Before intervention	☐ After intervention
#	**Question**	**Code**	**Response**
01	Field staff ID	##	☐☐

02	Date of collection	DD/MM/YY	□□/□□/□□
03	Time stool specimen was produced	Time (24 Hr Scale; HH:MM)	□□:□□
04	Time stool specimen was picked up by field worker	Time (24 Hr Scale; HH:MM)	□□:□□
05	Time stool specimen was received at field office	Time (24 Hr Scale; HH:MM)	□□:□□
06	Time stool specimen left field office	Time (24 Hr Scale; HH:MM)	□□:□□
B. Stool Receiving and Processing Form			
01	Sample ID	□□□□□□□	
02	If sample is a recollection, what is sample ID of initial stool sample?	□□□□□□□□□□□	
03	Amount of stool received (gm)	##.##	□□:□□
04	Consistency of stool specimen	Watery=01, Liquid=02, Soft=03, Formed=04	□□
05	Was stool specimen bloody?	Yes=01, No=00	□□
06	Did stool specimen have mucus?	Yes=01, No=00	□□
07	Does stool specimen require a QNS setup?	Yes=01, No=00	□□

icddr,b

Memorandum

30 September 2019

To: Dr Tahmeed Ahmed
Principal Investigator of research protocol PR-18073
Nutrition and Clinical Services Division (NCSD)

From: Shafiqul Alam Sarker, MD, Ph.D, FRCP SA Sarker.
Chairperson
Research Review Committee (RRC)

Sub: Approval of an addendum to research protocol PR-118073

Thank you for your memo dated 24 September 2019 requesting for approval of an addendum proposal to previously approved research protocol PR-18073 titled "Community-based clinical trial with microbiota-directed complementary foods (MDCFs) made of locally available food ingredients for the management of children with primary moderate acute malnutrition" version No. 1.2, dated 22 October 2018 through expedited review process. I have the pleasure to inform you that your addendum proposal to the above protocol was **approved** through expedited review process. Accordingly, you may proceed to obtain approval of the Ethical Review Committee.

Other terms and conditions for implementation of your research protocol, as contained in our memo dated 13 September 2018 according approval of the research protocol shall, however, remain unchanged.

Thank you.

Cc: Senior Manager, Budget & Planning, Finance

icddr,b

Memorandum

01 October 2019

To: Dr Tahmeed Ahmed
Principal Investigator of Research Protocol # PR-18073
Nutrition and Clinical Services Division (NCSD)

From: Professor Ahmed Abu Saleh
Chairperson
Ethical Review Committee (ERC)

Subject: Approval of an addendum to research protocol # PR-18073

Thank you for your memo dated 30 September 2019 requesting for an addendum proposal to your previously approved research protocol # PR-18073 titled, "Community-based clinical trial with microbiota-directed complementary foods (MDCFs) made of locally available food ingredients for the management of children with primary moderate acute malnutrition", version no. 1.2, dated 22 October 2018, through expedited review process. I have the pleasure to inform you that the addendum proposal to the above research protocol is approved through expedited review mechanism.

Other terms and conditions for implementation of your research protocol, as contained in our memo dated 23 October 2018 according approval of the Research Protocol shall, however, remain unchanged.

I wish your success in conducting the study.

Cc: Senior Manager, Budget & Planning, Finance

Postal address
icddr,b
GPO Box 128
Dhaka 1000
Bangladesh

Delivery & visitors
icddr,b
Mohakhali
Dhaka 1212
Bangladesh

T (88 02) 9827001-10
F (88 02) 9827075, 9827077
E info@icddrb.org
www.icddrb.org

Final Protocol

icddr,b	**RRC APPLICATION FORM**

RESEARCH PROTOCOL **Number: PR-18073** **Version No. 1.3** **Version date: 24-09-2019**	**FOR OFFICE USE ONLY**		
	RRC Approval:	☒ Yes	☐ No	Date: 30.09.2019
	ERC Approval:	☐ Yes	☐ No	Date: 01.10.2019
	AEEC Approval:	☐ Yes	☐ No	Date:
	External IRB Approval	☐ Yes	☐ No	Date:
	Name of External IRB: _____			

Protocol Title: * (maximum 250 characters including space**)**
Community-based clinical trial with microbiota-directed complementary foods (MDCFs) made of locally available food ingredients for the management of children with primary moderate acute malnutrition

Short Title: (maximum **100** characters including space)
Microbiota-directed complementary food (MDCF) trial

Key Words: * Microbiota, complementary food

Name of the Research Division Hosting the Protocol: *

- ☐ Health Systems and Population Studies Division (HSPSD)
- ☒ Nutrition and Clinical Services Division (NCSD)
- ☐ Infectious Diseases Division (IDD)
- ☐ Maternal and Child Health Division (MCHD)
- ☐ Laboratory Sciences and Services Division (LSSD)
- ☐ Other (specify) _____

Has the Protocol been Derived from an Activity: * ☒ No ☐ Yes (please provide following information):

Activity No. :
Activity Title:
PI:
Grant No.: Budget Code: Start Date: End Date:

icddr,b Strategic Priority/ Initiative (SP 2015-8):* (check all that apply**)**

- ☐ Reducing maternal and neonatal mortality
- ☐ Controlling enteric and respiratory infections
- ☒ Preventing and treating maternal and childhood malnutrition
- ☐ Detecting and controlling emerging and re-emerging infections
- ☐ Achieving universal health coverage
- ☐ Examining the health consequences of climate change
- ☐ Preventing and treating non-communicable diseases
- ☐ Others (specify) _____

Research Phase (4 Ds): * (check all that apply**)**

- ☒ Discovery
- ☒ Development
- ☒ Delivery
- ☐ Evaluation of Delivery

Anticipated Impact of Research: * (check all that apply and please provide details below**)**

- ☒ Knowledge Production
- ☒ Capacity Building
- ☒ Informing Policy
- ☒ Health and Health Sector Benefits
- ☐ Economic Benefits

Please provie details here:

Which of the Sustainable Development Goal This Protocol Relates to?:* (check all that apply**)**

- ☐ 1. End poverty in all its forms everywhere
- ☒ 2. End hunger, achieve food security and improved nutrition and promote sustainable agriculture
- ☒ 3. Ensure healthy lives and promote well-being for all at all ages
- ☐ 4. Ensure inclusive and equitable quality education and promote lifelong learning opportunities for all
- ☐ 5. Achieve gender equality and empower all women and girls
- ☐ 6. Ensure availability and sustainable management of water and sanitation for all
- ☐ 7. Ensure access to affordable, reliable, sustainable and modern energy for all
- ☐ 8. Promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all
- ☐ 9. Build resilient infrastructure, promote inclusive and sustainable industrialization and foster innovation
- ☐ 10. Reduce inequality within and among countries
- ☐ 11. Make cities and human settlements inclusive, safe, resilient and sustainable
- ☐ 12. Ensure sustainable consumption and production patterns
- ☐ 13. Take urgent action to combat climate change and its impacts
- ☐ 14. Conserve and sustainably use the oceans, seas and marine resources for sustainable development
- ☐ 15. Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss
- ☐ 16. Promote peaceful and inclusive societies for sustainable development, provide access to justice for all and build effective, accountable and inclusive institutions at all levels
- ☒ 17. Strengthen the means of implementation and revitalize the global partnership for sustainable development

Does this Protocol Use the Gender Framework:* (Please visit: http://shetu.icddrb.org/index.php?option=com_content&view=article&id=265&Itemid=677 for Gender Alanysis Tool with instructions)	☒ Yes (please complete Gender Analysis Tool) ☐ No

If 'no' is the response, its reason(s) in brief:

Will this Research Specifically Benefit the Disadvantaged (economically, socially and/or otherwise**):**	☒ Yes ☐ No
Does this Protocol use Behaviour Change Communication:	☐ Yes ☒ No

Principal Investigator (Should be icddr,b staff):* Sex ☐ Female ☒ Male

Dr Tahmeed Ahmed
MBBS, PhD
Senior Director and Senior Scientist
Email: tahmeed@icddrb.org

Do you have ethics certification? ☐ No ☒ Yes (please attach in your CV below)

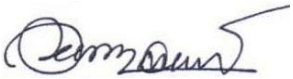
Do you have RBM training certification? ☐ No ☒ Yes (please attach the certificate with CV below)

Primary Scientific Division of the PI

Nutrition and Clinical Services Division, icddr,b

Co-Principal Investigator(s) Internal: Sex ☐ Female ☒ Male **Dr Md. Munirul Islam** MBBS, PhD Scientist 880-2-9827001, Ext-2352 Email: mislam@icddrb.org Signature or written consent of Co-PI:_____ (electronic signature or email or any sort of written consent) [if more than one, please copy and paste this row for additional Co-PIs] Do you have ethics certification? ☐ No ☒ Yes (please attach in your CV below) Do you have RBM training certification? ☐ No ☒ Yes (please attach the certificate with CV below)	Primary Scientific Division/ Programme of the Co-PI Nutrition and Clinical Services Division, icddr,b _____ Approval of the Respective Senior Director/ Programme Head (Signature)
Co-Investigator(s) - Internal: Sex ☒ Female ☐ Male **Dr Sayeeda Huq** Associate Scientist MBBS, MIPH Email: sayeeda@icddrb.org Signature or written consent of Co-I: _____ (electronic signature or email or any sort of written consent) [if more than one, please copy and paste this row for additional Co-Is Do you have ethics certification? ☐ No ☒ Yes (please attach in your CV below) Do you have RBM training certification? ☐ No ☒ Yes (please attach the certificate with CV below)	Primary Scientific Division of the Co-I Nutrition and Clinical Services Division, icddr,b
Co-Investigator(s) – Internal: Sex ☐ Female ☒ Male **Dr Mustafa Mahfuz** MBBS, MPH Associate Scientist Email: mustafa@icddrb.org Signature or written consent of Co-I: _____ (electronic signature or email or any sort of written consent) [if more than one, please copy and paste this row for additional Co-Is]	
Co-Investigator(s) – Internal: Sex ☒ Female ☐ Male **Dr Ishita Mostafa** BDS, MPH Research Investigator, Ext-2313 Email: ishita.mostafa@icddrb.org Signature or written consent of Co-I: _____ (electronic signature or email or any sort of written consent) [if more than one, please copy and paste this row for additional Co-Is]	

Co-Investigator(s) – Internal: Sex ☐ Female ☒ Male

Dr Imteaz Mahmud
MBBS, MPH
Research fellow
Email: imteaz.mahmud@icddrb.org
Signature or written consent of Co-I: 
(electronic signature or email or any sort of written consent)
[if more than one, please copy and paste this row for additional Co-Is]

Co-Investigator(s) – Internal: Sex ☒ Female ☐ Male

Dr Nurun Nahar Naila
MBBS, MPH
Assistant Scientist
Email: nurun.nahar@icddrb.org
Signature or written consent of Co-I: 
(electronic signature or email or any sort of written consent)
[if more than one, please copy and paste this row for additional Co-Is]

Collaborating Institute(s): Please provide full official address

Institution # 1

Country	USA
Contact person	**Prof Jeffrey I. Gordon**
Department (including Division, Centre, Unit)	Director, Center for Genome Sciences & Systems Biology
Institution (with official address)	Washington University School of Medicine 4444 Forest Park Expressway Campus Box 8510 St. Louis, MO 63108 Phone: +1 314-362-7243 FAX: +1314-362-7047 Email: jgordon@wustl.edu
Directorate (in case of GoB i.e. DGHS)	N/A
Ministry (in case of GoB)	N/A

Institution # 2

Country	
Contact person	
Department (including Division, Centre, Unit)	
Institution (with official address)	
Directorate (in case of GoB i.e. DGHS)	
Ministry (in case of GoB)	

Institution # 3

Country	
Contact person	
Department (including Division, Centre, Unit)	
Institution (with official address)	
Directorate (in case of GoB i.e. DGHS)	
Ministry (in case of GoB)	

Note: If less than or more than three collaborating institutions, please delete or insert blocks as needed.

Contribution by the Members of the Scientific Team:

Members' Name	Contribution								
	Research idea/ concept	Study design	Protocol writing	Respond to external reviewers' comments	Defending at IRB	Developing data collection Tool(s)	Data Collection	Data analysis/ interpretation of results	Manuscript writing
Dr Tahmeed Ahmed	☒	☒	☒	☒	☒	☒	☐	☒	☒
Prof Jeffrey I. Gordon	☒	☒	☒	☒	☐	☒	☐	☒	☒
Dr Michael Barratt	☒	☒	☒	☒	☐	☒	☐	☒	☒
Dr Md Munirul Islam	☐	☒	☒	☒	☒	☒	☐	☒	☒
Dr Sayeeda Huq	☐	☐	☐	☒	☒	☒	☐	☒	☒
Dr Mustafa Mahfuz	☐	☐	☐	☒	☒	☒	☐	☒	☒
Dr Ishita Mostafa	☐	☐	☒	☒	☒	☒	☒	☒	☒
Dr Imteaz Mahmud	☐	☐	☒	☒	☒	☒	☒	☒	☒
Dr Nurun Nahar Naila	☐	☐	☒	☒	☒	☒	☒	☒	☒
	☐	☐	☐	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐	☐	☐	☐

Study Population: Sex, Age, Special Group and Ethnicity

Research Subject:
- ☒ Human
- ☐ Animal
- ☐ Microorganism
- ☐ Other (specify): _____

Sex:
- ☒ Male
- ☒ Female
- ☐ Transgender

Age:
- ☒ 0 – 4 years
- ☐ 5 – 10 years
- ☐ 11 – 17 years
- ☐ 18 – 64 years
- ☐ 65 +

Special Group:
- ☐ Pregnant Women
- ☐ Fetuses
- ☐ Prisoners
- ☐ Destitutes
- ☐ Service Providers
- ☐ Cognitively Impaired
- ☐ CSW
- ☐ Expatriates
- ☐ Immigrants
- ☐ Refugee
- ☐ Others (specify): _____

Ethnicity:
- ☒ No ethnic selection (Bangladeshi)
- ☐ Bangalee
- ☐ Tribal group
- ☐ Other (specify): _____

NOTE: It is icddr.b's policy to include men, women, children and transgender in its research projects involving participation of humans, unless there is strong justification(s) for their exclusion.

Consent Process: (Check all that apply**)**
- ☒ Written
- ☐ Oral
- ☐ Audio
- ☐ Video
- ☐ None

Language:
- ☒ Bangla
- ☒ English
- ☐ Other (specify: _____

Project/Study Site: (Check all that apply**)**
- ☐ Chakaria
- ☐ Bandarban
- ☒ Dhaka Hospital
- ☐ Kamalapur Field Site/HDSS
- ☒ Mirpur (Dhaka)
- ☐ Matlab DSS Area
- ☐ Matlab non-DSS Area
- ☐ Matlab Hospital
- ☐ Mirzapur
- ☐ Bianibazar (Sylhet)
- ☐ Kanaighat (Sylhet)
- ☐ Jakigonj (Sylhet)
- ☐ Other community in Dhaka
  Name:
- ☒ Other sites in Bangladesh
  Name: Kurigram
- ☐ Multi-national Study
  Name of the country_____

Project/Study Type: (Check all that apply)

- ☐ Case Control Study
- ☒ Clinical Trial (Hospital/Clinic/Field)*
- ☐ Community-based Trial/Intervention
- ☐ Cross Sectional Survey
- ☐ Family Follow-up Study
- ☐ Longitudinal Study (cohort or follow-up)
- ☐ Meta-analysis
- ☐ Programme Evaluation
- ☐ Programme (Umbrella Project)
- ☐ Prophylactic Trial
- ☐ Record Review
- ☐ Secondary Data Analysis
  Protocol No. of Data Source: _____
- ☐ Surveillance/Monitoring
- ☐ Systematic Review
- ☐ Other (specify): _____

***Note**: International Committee of Medical Journal Editors (ICMJE) defines Clinical Trial as "*Any research project that prospectively assigns human participants to intervention and comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome*".

PI of the RRC- and ERC-approved Clinical Trials should provide necessary information to IRB Secretariat (Research Administration) for registration and uploading into relevant websites (usually at the https://register.clinicaltrials.gov/). They should also provide relevant information to the IRB Secretariat in the event of amendment/modification after their approval by RRC and ERC.

Biological Specimen:

a) Will the biological specimen be stored for future use?	☒ Yes ☐ No ☐ Not applicable
b) If the response is 'yes', how long the specimens will be preserved?	5 years
c) What types of tests will be carried out with the preserved specimens?	Analyzing and examining the gut bacteria and their metabolites and enteropathogens in collected fecal, urine and plasma samples
d) Will the consent be obtained from the study participants for use of the preserved specimen for other initiative(s) unrelated to this study, without their re-consent?	☒ Yes ☐ No ☐ Not applicable
e) Will the specimens be shipped to other country/ countries? If yes, name of institution(s) and country/countries.	☒ Yes ☐ No ☐ Not applicable Gordon Lab, Washington University School of Medicine, Washington University in St. Louis, USA
f) If shipped to another country, will the surplus/unused specimen be returned to icddr,b? If the response is 'no', then the surplus/unused specimen must be destroyed.	☐ Yes ☒ No ☐ Not applicable
g) Who will be the custodian of the specimen at icddr,b?	Dr Tahmeed Ahmed Senior Director and Senior Scientist Nutrition and Clinical Services Division, icddr,b
h) Who will be the custodian of the specimen when shipped outside Bangladesh?	Prof Jeffrey I. Gordon Washington University School of Medicine 4444 Forest Park Expressway Campus Box 8510 St. Louis, MO 63108, USA Phone: +1 314-362-7243 Fax: +1314-362-7047 Email: jgordon@wustl.edu
i) Who will be the owner(s) of the specimens?	Dr Tahmeed Ahmed Senior Director and Senior Scientist Nutrition and Clinical Services Division, icddr,b
j) Has a MoU been signed with regards to collection, storage, use and ownership of specimen? If the response is 'yes', please attach a copy of the MoU.. If the response is 'no', appropriate justification should be provided for not signing a MoU.	☒ Yes ☐ No ☐ Not applicable

Proposed Sample Size:
Sub-group (Name of subgroup e.g. Men, Women) and Number

Name	Number	Name	Number
Arm 1: 12-18 months old children with primary MAM (WLZ <-2 to -3)	62		
Arm 2: 12-18 months old children with primary MAM (WLZ <-2 to -3)	62		
Total sample size	124		

Determination of Risk: Does the Research Involve (Check all that apply**)**

- ☐ Human exposure to radioactive agents?
- ☐ Foetal tissue or abortus?
- ☐ Investigational new device?
  Specify: _____
- ☐ Existing data available from Co-investigator?
- ☐ Human exposure to infectious agents?
- ☐ Investigational new drug?
- ☐ Existing data available via public archives/sources?
- ☒ Pathological or diagnostic clinical specimen only?
- ☐ Observation of public behaviour?
- ☐ New treatment regime?

	Yes	No
Will the information be recorded in such a manner that study participants can be identified from the information directly or through identifiers linked to the study participants?	☐	☒
Does the research deal with sensitive aspects of the study participants' sexual behaviour, alcohol use or illegal conduct such as drug use?	☐	☒

Could information on study participants, if available to people outside of the research team:

	Yes	No
a) Place them at risk of criminal or civil liability?	☐	☒
b) Damage their financial standing, reputation or employability, or social rejection, or lead to stigma, divorce etc.?	☐	☒

Do you consider this research: (check one)

☐ Greater than minimal risk	☒ No more than minimal risk	☐ Only part of the diagnostic test

Note: Minimal Risk: The probability and the magnitude of the anticipated harm or discomfort to participants is not greater than those ordinarily encountered in daily life or during the performance of routine physical, psychological examinations or tests, e.g. the risk of drawing a small amount of blood from a healthy individual for research purposes is no greater than when the same is performed for routine management of patients.

Risk Group of Infectious Agent and Use of Recombinant DNA	
a) Will specimens containing infectious agent be collected?	☒ Yes ☐ No ☐ Not applicable
b) Will the study involve amplification by culture of infectious agents?	☒ Yes ☐ No ☐ Not applicable
c) If response to questions (a) and/or (b) is 'yes', to which Risk Group (RG) does the agent(s) belong? (Please visit http://shetu.icddrb.org/index.php?option=com_content&view=article&id=265&Itemid=677 to review list of microorganism by Risk Group)	☒ RG1 ☐ RG2 ☐ RG3 ☐ RG4
d) Does the study involve experiments with recombinant DNA?	☒ Yes ☐ No ☐ Not applicable

Does the study involve any biohazards materials/agents or microorganisms of risk group 2, 3, or 4 (GR2, GR-3 or GR4)?

☐ Yes ☒ No

[If the response is 'yes'] I, (print name of the PI) affirm that we will use the standard icddr,b laboratory procedures for biosafety of the hazardous materials/agents or microorganisms in the conduction of the study.

_____ _____

Signature of the Principal Investigator **Date**

Dissemination Plan: [please explicitly describe the plans for dissemination, including how the research findings would be shared with stakeholders, identifying them if known, and the mechanism to be used; anticipated type of publication (working papers, internal (institutional) publication, international publications, international conferences/seminars/workshops/ agencies. [Check all that are applicable]

Dissemination type	Response		Description (if the response is a yes)
Seminar for icddr,b scientists/ staff	☐ No	☒ Yes	The research findings will be presented in a seminar for icddr,b scientist/staffs along with other partners
Internal publication	☒ No	☐ Yes	
Working paper	☒ No	☐ Yes	
Sharing with GoB (e.g. DGHS/ Ministry, others)	☐ No	☒ Yes	The final results will be shared with Government of Bangladesh /Directorate General of Health Services through seminars
Sharing with national NGOs	☒ No	☐ Yes	
Presentation at national workshop/ seminar	☐ No	☒ Yes	The research findings will be presented in a seminar organized by icddr,b before GoB and other national and international agencies
Presentation at international workshop/ conference	☐ No	☒ Yes	The findings will be shared in international conferences within the region and elsewhere
Peer-reviewed publication	☐ No	☒ Yes	The research findings will be published in peer reviewed journal (s)
Sharing with international agencies	☐ No	☒ Yes	Any publications arising out of this work will be shared with international agencies
Sharing with donors	☐ No	☒ Yes	The final report will be shared with Bill and Melinda Gates Foundation
Policy brief	☒ No	☐ Yes	
Other			
Other			

Funding:

Is the protocol fully funded?	☐ Yes	☒ No
If the answer is yes, please provide sponsor(s)'s name	1.	
	2.	
Is the protocol partially funded?	☐ Yes	☒ No
If the answer is yes, please provide sponsor(s)'s name	1.	
	2.	

If fund has not been identified:

Is the proposal being submitted for funding?	☒ Yes	☐ No
If yes, name of the funding agency	1. Bill and Melinda Gates Foundation	
	2.	

Conflict of interest:
Do any of the participating investigators and/or member(s) of their immediate families have an equity relationship (e.g. stockholder) with the sponsor of the project or manufacturer and/or owner of the test product or device to be studied or serve as a consultant to any of the above?

☒ No ☐ Yes (please submit a written statement of disclosure to the Executive Director, icddr,b)

Proposed Budget:

Dates of Proposed Period of Support

(Day, Month, Year - DD/MM/YY)

Beginning Date: 1 Nov 2018

End Date: 30 June 2021

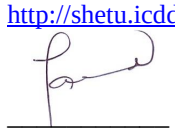
Cost Required for the Budget Period ($)

Years	Direct Cost	Indirect Cost	Total Cost
Year-1			0
Year-2			0
Year-3			0
Year-4			0
Year-5			0
Total	[redacted]5	[redacted]	[redacted]

Certification by the Principal Investigator:

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept the responsibility for the scientific conduct of the project and to provide the required progress reports including updating protocol information in the NAVISION if a grant is awarded as a result of this application.

I also certify that I have read icddr,b Data Policies and understand the PIs' responsibilities related to archival and sharing of research data, and will remain fully compliant to the Policies. (Note: The Data Policies can be found here: http://shetu.icddrb.org/index.php?option=com_content&view=article&id=273&Itemid=685)



Signature of PI

23.09.2019
Date

Approval of the Project by the Division Director of the Applicant:

The above-mentioned project has been discussed and reviewed at the Division level.

Dr Tahmeed Ahmed		23.09.2019
Name of the Division Director	Signature	Date of Approval

Table of Contents

☒ Check here if appendix is included

Project Summary

[The summary, within a word limit of 300, should be stand alone and be fully understandable.]

Principal Investigator: Dr Tahmeed Ahmed
Research Protocol Title: Community-based clinical trial with microbiota directed complementary foods (MDCFs) made of locally available food ingredients for the management of children with primary MAM
Proposed start date: 1 Nov 2018 Estimated end date: 30 June 2021
Background (brief): Burden: A total of 52 million children under 5 are suffering from acute malnutrition globally, of whom 33 million have moderate acute malnutrition (MAM). In Bangladesh, more than 2 million children suffer from MAM. According to Bangladesh Demographic Health Survey 2014 26%, 25% and 17% of children aged less than two years are stunted, underweight and wasted respectively. Knowledge gap: We have already demonstrated that children with SAM have immature gut microbiota that is partially corrected with treatment. Children with MAM have an increased risk of mortality, infections and impaired physical and cognitive development compared to well-nourished children. Although the global caseload of MAM is much greater than that of SAM, the condition has not received the same level of attention or priority. Through our previous and ongoing research we now know about the members of the gut microbiota that can promote growth in children and also about certain food ingredients that promote the proliferation of such beneficial microbiota. However, this knowledge needs to be applied on a sufficiently powered community-based clinical trial. Relevance: The rationale for this study is to assess whether long-term administration of complementary food made of locally available food ingredients can stimulate the proliferation of growth promoting members of the gut microbiota and have a positive impact on child growth. Such a food (the microbiota directed complementary food; MDCF-2) has been identified through our recently concluded Pre-proof of concept trial done on children with primary MAM. We would now like to do a clinical community-based trial of this potential MDCF-2 in the management of children with primary MAM. Hypothesis (if any): Complementary foods made of locally available food ingredients that stimulate the proliferation of growth promoting gut microbiota (MDCF-2) will improve clinical outcomes. Objectives: To investigate the efficacy of complementary food made of locally available food ingredients that can stimulate the proliferation of growth promoting gut microbiota (MDCF-2) in (i) promoting repair of microbiota immaturity (ii) promoting proliferation of beneficial members of the gut microbiota (iii) improving both ponderal and linear growth in children (iv) improving the metabolomic profile in children with MAM Methods: We will conduct a proof of concept (POC) clinical trial in 12-18 months old children with primary MAM (Weight-for-Length Z-score, WLZ between -2 and -3). This study will be conducted at Bauniabadh, Radda MCH clinic, Gabtoli of Mirpur area and possibly at the Special Nutrition Unit run by Terre des Hommes in Kurigram. We will produce MDCF-2 at the icddr,b Food Processing Laboratory or nutrition centre established at the site in sufficient quantities for clinical study. This formulation will be matched in energy

density and micronutrient content of ready-to-use supplementary foods (RUSFs) used for MAM in Bangladesh and other countries, and will meet all other requirements for a complementary/supplementary food for 12-18 months old children with MAM. We will test MDCF-2 and the current RUSF standard of care for primary MAM to see the effect on growth, proteomics and metabolomics of an intervention for 12 weeks, with a 4-week post-intervention phase.

Outcome measures will include the following:

- Ponderal growth (rate of weight gain, primary outcome variable), measured at different time points by anthropometry
- Linear growth (LAZ), measured at different time points by anthropometry
- Proteomic profile, assayed by SomaLogic scan
- Morbidity, assessed by daily records
- Change in microbiota-for-age Z score

Description of the Research Project

Hypothesis to be tested:

In a hypothesis testing research proposal, briefly mention the hypothesis to be tested and provide the scientific basis of the hypothesis, critically examining the observations leading to the formulation of the hypothesis.

Does this research proposal involve testing of hypothesis: ☐ No ☒ Yes (describe below)

Complementary foods made of locally available food ingredients that stimulate the proliferation of growth promoting gut microbiota (MDCF) will provide a new way to improve clinical outcomes, for example by improving growth of children with MAM.

Specific Objectives:

Describe the specific objectives of the proposed study. State the specific parameters, gender aspects, biological functions, rates, and processes that will be assessed by specific methods.

To investigate the efficacy of complementary food made of locally available food ingredients that can stimulate the proliferation of growth promoting gut microbiota (Microbiota-Directed Complementary Food; MDCF-2) in

(i) promoting repair of microbiota immaturity
(ii) promoting proliferation of beneficial bacteria
(iii) improving both ponderal and linear growth in children
(iv) improving the metabolomic profile with MAM

Background of the Project including Preliminary Observations:

Provide scientific validity of the hypothesis based on background information of the proposed study and discuss previous works on the research topic, including information on sex, gender and diversity (ethnicity, SES) by citing specific references. Critically analyze available knowledge and discuss the questions and gaps in the knowledge that need to be filled to achieve the proposed aims. If there is no sufficient information on the subject, indicate the need to develop new knowledge.

Moderate acute malnutrition (MAM), a major global health problem, is defined as wasting (i.e. weight-for-height between < -2 and -3 Z-scores of the WHO Child Growth Standards) and/or mid-upper-arm circumference (MUAC) greater or equal to 115 mm and less than 125 mm. According to the Global Nutrition Report 2017, 8% or 52 million under-five children were acutely malnourished globally in 2016. Stunting affected 23% or 155 million children. Approximately one in 6 children under 5 years in South Asia suffered from MAM in 2013 (i.e. 17%).[1] These children are at increased risk of severe acute malnutrition (SAM), and have a three times higher

risk of mortality from common communicable diseases than the well-nourished peers.[2] Bangladesh has one of the highest childhood malnutrition burdens in the world. According to Bangladesh Demographic Health Survey (BDHS) 2014, the prevalence of stunting among under-five children is 36%, among them 12% suffer from severe stunting (LAZ <-3).[3] Around 15% of children are wasted (WLZ <-2); more than 2 million children suffer from MAM, while 3% or 450,000 children suffer from the deadly form of SAM. Malnutrition costs Bangladesh an estimated US $1 billion a year.[4]

According to WHO recommendations, infants and children aged 6-59 months with MAM need to consume nutrient-dense foods to meet their extra needs for weight and height gain and functional recovery. Currently there are no evidence-informed recommendations on the composition of supplementary foods used to treat children with MAM. In situations of food shortage, supplementary foods have been used to treat children with moderate acute malnutrition. Interventions to address undernutrition should therefore include a strong component of MAM management. MAM prevention should be taken into consideration in food security and other development strategies as the situation becomes critical in populations where food insecurity is rampant. Food insecurity has become a worldwide concern due to the increasing number of people who remain undernourished amounting to 842 million, approximately 12% of the total world's population. From the National Micronutrient Status Survey in Bangladesh which we conducted in 2011-12, severe insecurity of food was found most commonly in slum settlements (17.2 %), compared to 12.3% at the national level, 12% in rural areas and 12.4% in urban areas of the country. Since food insecurity cannot be overcome quite readily, it is important therefore to develop interventions that depend upon locally available food ingredients and are able to harness the beneficial power of the gut microbiota on infant and child growth.[5]

One of the major factors limiting the impact of nutrition intervention is the inability of the malnourished children to increase their intake to meet increased metabolic demands. In collaborative studies between icddr,b and the Gordon Lab at Washington University in St. Louis during the Jumpstart Phase of the Breast Milk, Microbiota and Immunity (BMMI) Project, we applied Random Forests, a machine-learning-based approach, to bacterial 16S rRNA datasets generated from monthly fecal samples obtained from a birth-cohort of children living in an urban slum of Dhaka, Bangladesh. These children exhibited consistently healthy growth (WLZ -0.32+0.98). Bacterial strains were identified whose proportional representation defines a healthy gut microbiota as it assembles during the first 2-3 postnatal years. In a randomized clinical trial at icddr,b of two therapeutic foods (imported ready-to-use therapeutic food [RUTF, Plumpy'Nut] versus locally prepared rice/lentil-based Khichuri and Halwa) in Bangladeshi children with severe acute malnutrition (SAM), it was observed that the microbiota immaturity is incompletely and only transiently improved, with children remaining markedly stunted and underweight throughout the follow-up period. Bangladeshi children with MAM also exhibited significant microbiota immaturity, although less severe than children with SAM. Microbiota immaturity thus serves as a potential biomarker to identify infants at risk for undernutrition and to monitor treatment and prevention strategies.[6] Microbiota maturity indices provide a microbial measure of human postnatal development, a way of classifying malnourished states, and a parameter for judging therapeutic efficacy. SAM is associated with significant relative microbiota immaturity that is only partially ameliorated following two widely used nutritional interventions. Immaturity is also evident in less severe forms of malnutrition and correlates with anthropometric measurements. More prolonged interventions with existing or new therapeutic foods and/or addition of gut microbes required to achieve enduring repair of gut microbiota immaturity in childhood malnutrition and improve clinical outcomes.

We recently developed ready-to-use therapeutic foods using locally available food ingredients-rice, lentil, and chickpeas that are culturally relevant and acceptable. We found through a double-blind RCT that chickpea-based

and rice-lentil-based RUTF were as effective as the commercial peanut based-RUTF and well accepted by children with SAM.[7] Through a combination of the above mentioned RCT and clinical translational studies, we have identified growth promoting age-discriminatory beneficial microbiota and locally available food ingredients that support proliferation of these beneficial microbiota. Besides, results of earlier studies done on gnotobiotic animals in Washington University Centre for Genome Science has led us to suggest that a combination of food ingredients (chickpea, soy flour, peanut and green banana) will be worth studying with respect to the diet's impact on stimulating proliferation of growth-discriminatory microbiota as well as cost and sustainability. To assess the degree to which the results obtained from the gnotobiotic mouse and piglet models translate to humans, we recently performed a RRC and ERC approved study, icddr,b protocol (PR-16099) 'Pre-Proof of Concept clinical trials to optimize lead microbiota-directed complementary food (MDCF) prototypes for their ability to repair microbiota immaturity and establish their organoleptic acceptability 'and successfully completed the study. This study was designed to test the effects of three locally produced MDCF prototypes (MDCF-1, MDCF-2 and MDCF-3) and a locally produced rice-lentil-based RUSF. The objective of this pilot study was to demonstrate a Pre-proof of Concept that certain complementary foods would have a beneficial effect on young children suffering from moderate acute malnutrition by stimulating the proliferation of particular members of the gut microbiota that are known for their growth promoting effect. In that pilot study we investigated microbiota-for-age Z score as well as the impact of the proliferation of good members of the gut microbiota on certain body systems. The results of the Pre-POC pilot trial conclusively showed that one of the three microbiota directed complementary foods namely (MDCF-2; composed of a combination of chickpea, soy flour, peanut, green banana, oil, sugar and micronutrients) was associated with increased levels of certain amino acids, that have a key role in development of the long bones, development of the brain and increased production of IGF-1. This was done using the state-of-the-art DNA aptamer based SomaLogic scan. And the results suggest that this candidate MDCF-2 is effective in stimulating the growth of growth promoting members of the gut microbiota, for example *Faecalibacterium prausnitzii*. Thus, MDCF-2 promotes gut microbiota that induces the hormone insulin-like growth factor 1 (IGF-1), which promotes bone growth and remodelling.[8] In our previous study approved by the ERC (PR-09023), we compared the gut microbiota of healthy children living in slum of Mirpur with children hospitalized for the treatment of severe acute malnutrition. The microbiota was assessed by 16S Ribosomal RNA sequencing. We found that the gut microbiota of children with SAM lags chronologically behind microbiota of healthy children. For example, a two year old child with SAM may have the gut microbiota similar to that of a one year old healthy child. This is known as immaturity of the gut microbiota. We have developed two metrices to represent this gut immaturity – the relative microbiota maturity index and the microbiota-for-age Z score; these results have been published in the journal Nature in 2014. SAM is associated with significant relative microbiota immaturity that is only partially ameliorated following nutritional interventions. Immaturity is also evident in less severe forms of malnutrition and correlates with anthropometric measurements.

Based on this evidence we would now like to do a much larger clinical trial using the most promising MDCF which is MDCF-2 with the primary end point being linear growth. This trial would be on children with primary MAM.

Research Design and Methods

Describe the research design and methods and procedures to be used in achieving the specific aims of the research project. If applicable, mention the type of personal protective equipment (PPE), use of aerosol confinement, and the need for the use BSL2 or BSL3 laboratory for different part of the intended research in the methods.. Define the study population with inclusion and exclusion criteria, the sampling design, list the important outcome and exposure variables, describe the data collection methods/tools, and include any follow-up plans if applicable. Justify the scientific validity of the methodological approach (biomedical, social, gender, or environmental).

Also, discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them.

We will conduct a clinical trial among 12-18 months old children with primary MAM (WLZ <-2 to -3).

Study design: Randomized controlled intervention trial.

Study site: This study will be conducted at Bauniabadh, RADDA MCH clinic in Mirpur area and Gabtoli of Dhaka city, and possibly at the Special Nutrition Unit run by Terre des Hommes in Kurigram.

Study participants: The study participants will be 12-18 months old children of either sex with MAM (WLZ <-2 to -3).

Initial screening and enrolment: Children will be screened and enrolled through household surveys by Field Research Assistants (FRAs) following pre-specified inclusion criteria. Fulfilling the enrolment criteria and upon receiving the consent for study participation from the parents or legal guardians, the children with the respective mother/caregiver will be enrolled and randomly assigned to one of the two arms according to computer-generated random numbers. The code of assigned type of diet will be kept in closed opaque envelopes for each individual, and will be opened only when the caregiver signs the consent form.

Study design:

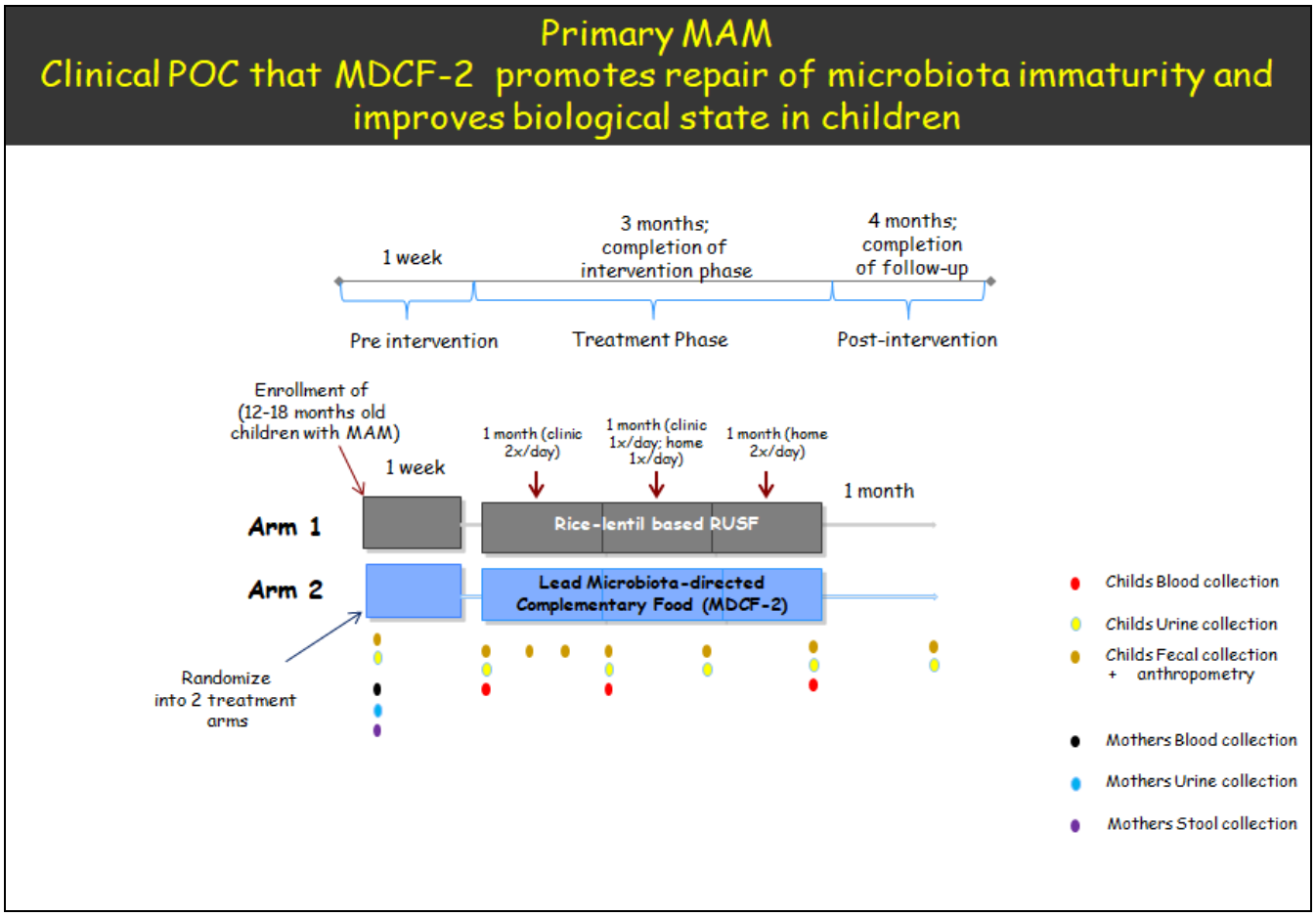


Figure: Study design for the community-based clinical trial among children with primary MAM

Arm 1 - Rice-lentil based RUSF (rationale: reference standard of care for MAM; based on knowledge of its effects on the gut microbiota or microbiota immaturity)

Arm 2 – MDCF2 with four complementary food ingredients (rationale: lead with evidence from Pre-POC clinical trials to optimize lead microbiota-directed complementary food prototypes for their ability to repair microbiota immaturity and positive effects on growth)

Fecal sample collection

Weekly fecal samples (1-2g) will be collected within 30 minutes of excretion (at home and transported back to the icddr,b) using the nitrogen dry shipper specimen collection SOP used in the recently concluded Pre-POC clinical trial (Appendex-A). Specimens will be stored at -80°C before being sent to the Gordon Lab analysis of microbiota maturity (measured before, during and after cessation of treatment with MDCF and RUSF) and PCR-based assessment of enteropathogen burden in fecal samples (measured before and after MDCF treatment).

1-2 gm of fecal samples will be collected from each child at enrollment, weekly during the 1st month of intervention, and fortnightly during the 2nd and 3rd month of intervention and post-intervention phases. Assuming 62 study participants /arm, a total of 992 fecal specimens will be collected in this study. 2 gm of faecal samples will be collected from mothers at the time of enrolment.

Urine sample collection

2 mL of urine samples will be collected from each child at enrollment, one week after enrolment and monthly once in the intervention and post-intervention phases. Assuming 62 study participants /arm, a total of 744 urine samples will be collected in this study. 5 mL of urine samples will be collected from mothers at the time of enrolment.

Blood sample collection

2 mL of blood samples each will be collected from each child prior to intervention, end of first month of intervention and just after the intervention is complete. A total of 372 plasma samples will be collected in this study. 5 mL of blood samples will be collected from mothers at the time of enrolment.

Follow up:

In order to see the sustainability of the growth-promoting microbiota, we need to follow the participants for a longer duration. We will follow this children at month 6, month 12, month 24, month 36, and month 48. During this period, we will collect additional stool (2 gm), blood (2 ml) and urine (1.5 ml) samples once at each time points.

*Maternal nutritional status is associated with child nutritional status, as shown through the results of our eight country MAL-ED study. In addition, neonatal and other maternal factors were early determinants of lower length-for-age, and their contribution remained important throughout the first 24 months of life.[9] The other maternal factors, we believe, would include maternal gut microbiota as well as maternal blood amino acid profile. Initiatives to address childhood stunting should also consider improvements to the composition of complementary foods (i.e., higher protein) and strategies to reduce gut pathogen exposure. As such, we will record maternal height and weight. In order to understand the biological state of nutrition of the mother, enrolment samples of stool, blood and urine will be asked of the mother. These samples will be analyzed for gut microbiota, and proteomics, and the results correlated with those of the enrolled children.

Table 1: Summary of the work plan for the Clinical Trial

Work Plan for the Clinical Study												
Week	0	1	2	3	4	5	6-7	8-9	10-11	12-13	14-15	16-17
		1 month (clinic 2x/day)					1 month (clinic 1x/day; home 1x/day)		1 month (home 2x/day)			
Study activity	Enrolment & randomization in treatment arms	Nutritional therapy starts	ITN	ITN	ITN	ITN	ITN	ITN	ITN	Nutritional therapy ends	Follow up	Follow up
Food Frequency questionnaire	X	X				X		X		X		X
Anthropometry	X	X		X		X	X	X	X	X	X	X
Urine sample (2 ml)	X	X				X		X		X		X
Fecal sample (1-2)g	X	X	X	X	X			X		X		X
Blood sample (2 ml)		X				X				X		

Feeding sessions

The children and mothers/caregivers will be requested to come to the nutrition centers established at the sites preferably between 9-11 am and 3-5 pm on day 1. The mothers will be requested not to give any food and breast milk in the 2 hours preceding the observed meal time. The child will be offered 25 grams of MDCF or RUSF as decided by random allocation in each of the two meals between 9-11 am and 3-5 pm. The mothers will be asked to spoon feed the pre-weighed diets to their children until s/he refuses to eat, as described below. After a two-minute pause, the same diet will be offered a second time until s/he refuses again. After a second two-minute pause, the diet will be offered a third time until refused again. After this third refusal, the feeding episode will be considered as 'terminated'. The duration of the feeding (excluding the intervening 'rest periods') will be recorded by stopwatch, and the total duration of the feeding will be noted. This feeding episode will last for maximum 60 minutes. Measured volumes of plain water will also be given and the amount of water taken during this meal period of 60 minutes will be measured. The feeding episode will take place under the direct supervision of trained study personnel. Children will be considered as refusing further intake if they move their head away from the food, cry, clamp the mouth or clinch the teeth, or become agitated, spit out the food or refuse to swallow. The amount of MDCF/RUSF actually ingested will be calculated by subtracting the left over from the offered amount. Pre-weighed napkins will be provided; any food that is regurgitated, vomited or spilled will be swabbed, weighed and subtracted from the amount offered. The amount of consumed food (g), energy (kcal) and category of acceptability will be analyzed. The enrolled children will be monitored daily by Field Research Assistants for any possible side effects/adverse events (e.g. rash, urticaria due to food allergy or any significant changes in clinical status) for a week. If any side effects/adverse events are observed, they will be treated according to standard of care. A standardized production procedure will be followed to control the quality of RUSF and MDCF following international standard protocol. RUSF and MDCF will be prepared at the food processing

laboratory. Preparation of food under different steps, that is, roasting, particle size reduction, homogeneous blending, and supplying to the nutrition centres will be monitored by icddr,b investigators. Food will be prepared everyday to ensure that no unexpected contamination and nutrient losses occur during preparation. Although raw food ingredients will be very carefully procured from the local market and stored in reasonable quantities, we will prepare, dispense, and feed the children the same day the MDCF and RUSF are prepared. Every child will be offered 25gm of the diet twice daily at the feeding center for the first 4 weeks. In the following month, the child will be offered 25gm of the diet at the feeding center and additional 25gm will be provided in a clean container to feed at home. In the third month, two separate containers containing 25gm diet will be provided every day to each enrolled child at participant's home.

In this study nutritional status will be assessed through anthropometry, comparing with WHO growth reference standards. At the beginning of the study, information will be sought on the demographic characteristics (families' wealth, standard of housing, family structure and parental characteristics etc.), and FRAs will record the children's weight using a digital scale with 2g precision (Seca, model 728, Germany), length (using infantometer, Seca, model 416, Germany), and mid upper-arm circumference to the nearest mm (using a non-stretch insertion tape). Anthropometrics will be done according to the standard procedures and all measurements will be taken thrice and the middle one will be recorded.

All interventions in each study will be administered to children at the Mirpur health clinic/RADDA clinic or in Kurigram. Mothers/primary caregivers will be advised to maintain their child's current dietary and breastfeeding practices.

We will complete enrolment within 12 months and the follow up as well as data analysis within an additional 6 months. However, this trial on primary MAM will continue simultaneously with the other clinical trial on Post SAM-MAM that will have a longer period of duration.

Inclusion criteria

All of the following criteria must be met for a child to be eligible to participate in the study:

- Parent(s) willing to sign consent form
- Child age 12-18 months and no longer exclusively breast fed
- WLZ (<-2 to -3) without bilateral pedal edema at the time of randomization
- Parent(s) willing to bring the child to the feeding center twice daily for 4 weeks for nutritional therapy, once daily for next 4 weeks and provide feeding once daily at home for 4 weeks and twice daily for next 4 weeks.
- The informed consent document will explicitly request permission to use the collected fecal samples for future studies, including but not limited to culturing component bacterial strains

Exclusion criteria

- Medical conditions: Children with tuberculosis (diagnosis based on WHO 2014 guidelines which have been incorporated in the national TB control guidelines of Bangladesh). The guidelines depend upon the following five diagnostic principles (three out of five should be positive): 1. Specific symptoms of TB, 2. Specific signs, 3. Chest X-ray, 4. Mantoux test, and 5. History of contact. [10] or any congenital/acquired disorder affecting growth i.e. known case of trisomy-21 or cerebral palsy; children on an exclusion diet for the treatment of persistent diarrhea; having known history of soy, peanut or milk protein allergy
- Antibiotic use within the last 15 days
- Receiving concurrent treatment for another condition

- Severe anemia (<8mg/dl) will be assessed by Hemocue (Model no. Hemocue Hb 301)
- Failure to obtain informed written consent from parents or caretakers

Recruitment, Screening and Consenting

Census, screening, enrolment of study participants will be done in the catchment areas of the sites in Dhaka city and in Kurigram. Parents of children who meet the MAM criteria (for Primary MAM trial) will be approached about enrolment into the study. A Field Research Assistant will explain the study in detail, answer any questions from the parent(s), and invite the parent(s) to enroll the child in the study.

At the beginning of the study, information will be sought on the demographic characteristics (families' wealth, standard of housing, family structure and parental characteristics etc.), and FRAs will record the children's weight using a digital scale with 2 g precision (Seca, model 728, Germany), length (using infantometer, Seca, model 416, Germany), and mid-upper-arm circumference to the nearest mm (using a non-stretch insertion tape). Study participants will be asked to come directly to the nutrition center for nutritional therapy. They will be provided with a cell phone number to reach the clinic staff, and a member of the staff may visit a family's household for directly observed nutritional therapy with prior arrangement if needed.

Preparation of MDCF 2 and RUSF

Based on compatible combinations of complementary food ingredients identified in the Pre-POC study described above, we will produce MDCF2 as well as Rice-lentil RUSF at the icddr,b Food Processing facility in Mirpur and in Kurigram (to be established in both places) in sufficient quantities for clinical study. The two diets will be matched in energy density and micronutrient content. The energy density of MDCF is 125 kcal/25 g (per serving), and caloric distribution is targeted to be 45-50 percent from fat and 8-10 percent from protein. Experiments in development of the MDCF prototypes and assessment of the organoleptic properties have been done during the Pre-POC clinical trial. After receiving the raw materials (rice/lentil/chickpea) we will take out the foreign materials/grains or seeds (if there is any), and then in an open pan the raw materials will be roasted. We will maintain the temperature at 120-130°C for roasting. Usually it takes 8-10 minutes for roasting 100g of each raw material. Continuous stirring is essential to ensure roasting of single seeds/grains. After completion of roasting we will keep it aside for cooling and then we will grind. We will take the powder and strain it using a strainer. After straining for 4 to 5 times, we will take the fine powder for mixing with the other ingredients (oil for both MDCF2 and RUSF, and milk powder only for RUSF). We will also grind sugar and the fine powder will be used for mixing. Finally we will add the pre-weighed premix powder. The processing of whole green banana for inclusion in MDCF2 is different from the other ingredients. Green banana with skin will be placed in a deep pan in boiling water (100°C-110°C) and boiled for about 17-20 minutes until they are cooked and tender. The skin of the green banana will be peeled off and the edible white part would be taken and grated into small pieces. Then they will be taken in a pot and allowed to cool. We will smash the small pieces of banana with spoon/hand crusher. The weights of all other ingredients will be recorded. Recipes will be produced in small batches by mixing all ingredients in an electric blender. A small amount (1 percent) of soy lecithin shall be added to the recipe in order to improve the consistency and prevent oil separation.

Anthropometry

The age of the child will be verified against documentation (birth certificate or immunization card, if available) or caregiver's report of the child's birth date. Length will be measured by a infantometer sensitive to 0.1 cm (SECA 416, Hamburg, Germany). Body weight will be measured by a balance sensitive to 2g (SECA 728, Hamburg, Germany). Length-for-age (LAZ), weight-for-length (WLZ) and Weight-for-Age (WAZ) Z-scores will

be calculated following the Multicentre Growth Reference Study (MGRS) WHO growth standards[11]. Edema will be examined by pressing the upper side of both feet for 3 seconds. Mid-upper arm circumference (MUAC) will be measured using TALC MUAC tape (UK). Regular standardization of the measuring equipments will be done using standards.

Analyses of plasma and fecal samples

Plasma samples collected from this trial will be sent to Dr Jeffrey Gordon's lab in the Center for Genome Sciences and Systems Biology at Washington University in St. Louis. Advanced mass spectroscopic- and immunoassay-based methods will be used to obtain new knowledge about the role of gut microbiota immaturity and the effects of attempting acute repair of this immaturity with lead microbiota-directed complementary food (MDCF) on biomarkers and mediators of healthy growth. Comparisons will be made with the control group (i.e., those consuming reference RUSF standard). Targeted Ultra Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS) and Gas Chromatography-Mass Spectrometry (GC-MS) will be used to profile analytes of specific interest in plasma and/or fecal samples including bile acids and short chain fatty acids (SCFAs); markers of mitochondrial function (e.g., β-hydroxybutyrate, acylcarnitines/acylCoAs, TCA cycle intermediates); amino acids in serum plus fecal samples [branch chain amino acids, tryptophan and tryptophan metabolites related to growth and inflammatory status, including those produced by bacterial tryptophan metabolism (e.g. indole acetic acid derivatives)]. Key mediators/biomarkers of linear growth (e.g., growth hormone and IGF-1), energy utilization (insulin, leptin), and bone biology [IL-6, osteoprotegerin, the C-terminal peptide of type I collagen (CTX, a marker of osteoclast activity/bone resorption), and the amino-terminal propeptide of Type 1 procollagen (P1NP, a marker of osteoblast activity/bone formation)], and systemic inflammation (CRP, AGP) will be quantified using established ELISA/Luminex assays. Proteins in blood will be identified using the SOMAlogic scan that permits identification of more than 1300 different proteins. The proteomic study done on plasma samples from children with SAM in our previous Pre-POC trial done in Dhaka has already demonstrated a number of significant and clinically relevant associations between certain proteins and clinical phenotypes using the SOMAlogic scan.

Information gathered will be used to select human fecal samples for transplantation into germ-free animals in the Gordon Lab in St Louis; these animals, who will be fed the diets of their human microbiota donors, will be used to further characterize the mechanisms that link MDCF prototypes, the gut microbiota, and host physiology/metabolism. In addition, these plasma and fecal biomarkers will be used to determine that MDCF promotes repair of microbiota immaturity and improves biological state in children.

Sample Size Calculation and Outcome (Primary and Secondary) Variable(s)

Clearly mention your assumptions. List the power and precision desired. Describe the optimal conditions to attain the sample size. Justify the sample size that is deemed sufficient to achieve the specific aims.

In the pre-POC trial of different MDCFs, the baseline weight-for-length Z score of children who received MDCF2 was -2.2 and after one month of supplementation was -1.7. If we consider the WLZ -2 at baseline and -1.7 at end line, pooled SD as 0.53 then the sample size is 49 in each arm at 80% power and 5% level of significance.

With 20% attrition 62 children will be required to be enrolled in each arm. Therefore, for the Primary MAM trial, 62 children will receive MDCF2 and 62 children will receive Rice-lentil RUSF.

Data Analysis

Describe plans for data analysis, including stratification by sex, gender and diversity. Indicate whether data will be analysed by the investigators themselves or by other professionals. Specify what statistical software packages will be used and if the study is blinded, when the code will be opened. For clinical trials, indicate if interim data analysis will be required to determine further course of the study.

The two groups of children with MAM will be compared at baseline and at different time points as shown in the illustration on trial design. The clinical outcome variables for comparison will include rate of weight gain, anthropometric indices, and morbidity. All analytes mentioned in the section 'Analyses of plasma and fecal samples' will be compared between MDCF2 and RUSF groups.

Data Safety Monitoring Plan (DSMP)

All clinical investigations (research protocols testing biomedical and/or behavioural intervention(s)) should include the Data and Safety Monitoring Plan (DSMP). The purpose of DSMP is to provide a framework for appropriate oversight and monitoring of the conduct of clinical trials to ensure the safety of participants and the validity and integrity of the data. It involves involvement of all investigators in periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of trial sites, and other factors that can affect study outcome.

Data collection tools for this study will include case report forms, laboratory worksheets and source documentation. Complete source documentation (study visits, laboratory reports, etc.) will be kept for each study participant in individual study charts. All laboratory specimens, reports, study data collection and administrative forms will be identified by coded number to maintain study participant confidentiality and to enable tracking throughout the study.

Forms, lists, logbooks, appointment books, and any other listings that link study participant ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access. All information regarding study participants will be kept in password-protected computer files or in locked file cabinets that can be accessed only by authorized study personnel. Chart information and information from study records will not be released without written permission from the study participant's parent(s). However, records may be reviewed by representatives from the Research Review Committee and Ethical Review Committee of icddr,b.

The study investigators are responsible for ensuring complete and accurate documentation for the study and for each study participant, including: medical records, records detailing each study participant's progress through the study, laboratory reports, Case Report Forms (CRFs), signed informed consent forms, correspondence with IRB(s), adverse event reports, and information regarding participant discontinuation and completion of the study. All required data will be clearly and accurately recorded in the CRFs by authorized study personnel. Only designated study-site personnel who have received appropriate training will record or change data in a CRF. The investigators are responsible for procuring the data and for quality of data recorded in the CRFs. Data entry and management will be performed at icddr,b.

Ethical Assurance for Protection of Human rights

Describe the justifications for conducting this research in human participants. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how participants' rights will be protected, and if there would be benefit or risk to each participants of the study. Discuss the ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in sick children, use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Discuss procedures safeguarding participants from injuries resulting from study procedures and/or interventions, whether physical, financial or social in nature. [Please see Guidelines]

The study will be started after obtaining IRB approval by the icddr,b Research Review Committee and Ethical Review Committee. Before enrolment in the study, informed written consent will be taken from the legal guardian of the study participants. The privacy, anonymity and confidentiality of data/information identifying the study participants will be strictly maintained. Personal identifications taken during enrolment and other study procedures will be kept under lock and key. None other than the study personnel will have access to information of personal identification and other sensitive information.

Expected risks/adverse events for this protocol are those related to blood sample collection, fecal sample collection and feeding of microbiota directed complementary food. None of these qualify as a serious adverse event (SAE). Expected Adverse Events (EAEs) related to blood draw are as follows:

- discomfort
- pain
- introduction of infection
- bleeding
- fainting or bruising

- Precaution will be taken to avoid introduction of infection by disinfecting the site of venipuncture and using sterile equipment
- The risk of bleeding and bruising will be minimized by immediate application of pressure after venipuncture
- The participant (child) will be in the sitting or supine position during blood draws to avoid injuries from fainting

All possible adverse events will be treated appropriately. These will include:

- Vomiting
- Diarrhoea
- Skin rash
- Urticaria from food allergy
- Abdominal distension
- Pain abdomen

Assessment of Adverse Events

Both serious and non-serious adverse events will be assessed for severity; relationship to study participation; actions taken; and outcomes. All SAEs will be reported to the ERC of icddr,b within 24 hours of the site's awareness of the event that will in turn be distributed to the sponsor. This will be done by direct telephone communication, fax or e-mail.

Each category for AE assessment will be coded according to the following grading systems:

Severity:

1. Mild
2. Moderate
3. Severe

Relationship to Study Participation:

1. Definitely related: Clear cut temporal association, no other possible cause
2. Possibly related: Less clear cut temporal association, other causes possible
3. Unrelated: Independent of study, evidence exists that event is definitely related to another etiology

Actions:

None

Remedial therapy (more than one dose of medicine required)
Permanently discontinued from study participation
Hospitalization
Other

Use of Animals

Describe if and the type and species of animals to be used in the study. Justify with reasons the use of particular animal species in the research and the compliance of the animal ethical guidelines for conducting the proposed procedures.

Not applicable

Collaborative Arrangements

Describe if this study involves any scientific, administrative, fiscal, or programmatic arrangements with other national or international organizations or individuals. Indicate the nature and extent of collaboration and include a letter of agreement between the applicant or his/her organization and the collaborating organization.

This project is a collaborative effort between the investigators in the Washington University School of Medicine and the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b). The clinical work, field activities will be undertaken in Dhaka, Bangladesh, under the direct supervision of the Principal Investigator whereas the laboratory analyses will be conducted at the Gordon Lab at Washington University in St. Louis. All research units and the collaborating investigators have long histories of international collaborations. All investigators have communicated during development of this project and are committed to its successful implementation.

Facilities Available

Describe the availability of physical facilities at site of conduction of the study. If applicable, describe the use of Biosafety Level 2 and/or 3 laboratory facilities. For clinical and laboratory-based studies, indicate the provision of hospital and other types of adequate patient care and laboratory support services. Identify the laboratory facilities and major equipment that will be required for the study. For field studies, describe the field area including its size, population, and means of communications plus field management plans specifying gender considerations for community and for research team members.

icddr,b has a well equipped Food Processing Lab that will facilitate preparations of diet recipes. We have ongoing studies in the Baoniabad area in Mirpur. A number of project offices are located there. More importantly, we have an excellent rapport with the community. The community elite and elders are invited every year to a dissemination meeting so that they are aware of the research being conducted.

Center for Genome Sciences, Washington University in St. Louis:
The stool samples will be aliquoted at icddr,b. Stool samples will be sent to Prof Jeffery Gordon's Lab in St Louis for the high throughput 16S ribosomal RNA gene sequencing. The presence/abundances of a broad range of enteropathogens in stool samples before and after MDCF treatment will be determined using a PCR-based assay. Stool samples may also be used to culture constituent bacterial strains for use in future studies in gnotobiotic mice in the Gordon Lab. It is pertinent to mention here that the assays and equipment to carry out those are currently available only in a few labs in the world, including the one at St Louis. The Center is home to an interdisciplinary, multi-departmental, multi-generational team of investigators from multiple schools that focus on comparative genomics, statistical genomics, and systems biology It serves as 'proving ground' for developing new strategies for educating students and faculty who wish to work at the interface of the biological, physical, computational and engineering sciences**.**

Literature Cited

Identify all cited references to published literature in the text by number in parentheses. List all cited references sequentially as they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list of Literature Cited. There is no page limit for this section, however, exercise judgment in assessing the "standard" length.

1. Development Initiatives, 2017. Global Nutrition Report 2017: Nourishing the SDGs. Bristol, UK: Development Initiatives.

2. James P, Sadler K, Wondafrash M, Argaw A, Luo H, and Geleta B et al. Children with moderate acute malnutrition with no access to supplementary feeding programmes experience high rates of deterioration and no improvement: results from a prospective cohort study in rural Ethiopia. PloS one. 2016 Apr 21;11(4):e0153530.

3. National Institute of Population Research and Training (NIPORT), Mitra and Associates, and ICF International. 2016. Bangladesh Demographic and Health Survey 2014. Dhaka, Bangladesh, and Rockville, Maryland, USA: NIPORT, Mitra and Associates, and ICF International.

4. Save the children (2015), malnutrition in Bangladesh: Harnessing social protection for the most vulnerable (2015).

5. Blanton LV, Barratt MJ, Charbonneau MR, Ahmed T, Gordon JI. Childhood undernutrition, the gut microbiota, and microbiota-directed therapeutics. Science 2016 Jun 24;352(6293):1533

6. Subramanian S, Huq S, Yatsunenko T, Haque R, Mahfuz M, Alam MA, Benezra A, DeStefano J, Meier MF, Muegge BD, Barratt MJ. Persistent gut microbiota immaturity in malnourished Bangladeshi children. Nature. 2014 Jun; 510(7505):417.

7. Choudhury N, Ahmed T, Hossain MI, Islam MM, Sarker SA, Zeilani M, Clemens JD. Ready-to-Use Therapeutic Food Made From Locally Available Food Ingredients Is Well Accepted by Children Having Severe Acute Malnutrition in Bangladesh. Food and nutrition bulletin. 2018 Mar; 39(1):116-26.

8. Yan J, Herzog JW, Tsang K, Brennan CA, Bower MA, Garrett WS, Sartor BR, Aliprantis AO, Charles JF. Gut microbiota induce IGF-1 and promote bone formation and growth. Proc Natl Acad Sci U S A. 2016 Nov 22;113(47):E7554-63.

9. MAL-ED Network Investigators. Childhood stunting in relation to the pre-and postnatal environment during the first 2 years of life: The MAL-ED longitudinal birth cohort study. PLoS medicine. 2017 Oct 25;14(10):e1002408.

10. World Health Organization. Guidance for national tuberculosis programmes on the management of tuberculosis in children. World Health Organization; 2016.

11. de Onis M, Garza C, Victora CG, Bhan MK, and Norum KR. The WHO Multicentre Growth Reference Study (MGRS): Rationale, planning, and implementation. Food and Nutrition Bulletin 2004;25(supplement 1):S3-S84.

Clinical Pathology tests (USD 7400), clinical biochemistry tests (USD 7400), and clinical microbiology tests (USD 7400) budgeted for the required laboratory investigations advised by the physicians.

Food safety tests (USD 40,000): This amount is budgeted for food safety tests of the intervention products at regular intervals.

Fuel for electric generator: USD 19,600 is budgeted for the fuel required to run the electric generators.
Sub-contracts: USD 160,000

Name	Corporate Entity Name	Mailing Address
Executive Director	Radda MCH-FP Centre	Plot 324, Road 6, Block B, Mirpur-10, Dhaka-1216, Bangladesh
Country Representative, Dhaka, Bangladesh, Terre des Hommes	Terre des Hommes, Bangladesh	House 141, Flat 5A, Road 4, Block-A, Banani, Dhaka-1213, Bangladesh

We have worked successfully with both RADDA and Terre des Hommes in our previous trial on the efficacy of local RUTFs, the samples from which have been extensively used to generate data for developing the MDCFs. These organizations have nutrition clinics where we will enroll children with Primary MAM and Post-SAM MAM in addition to icddr,b Dhaka Hospital. Contracts will be signed by the Executive Directors of icddr,b and these organizations following prescribed rules of icddr,b

Other Support

Describe sources, amount, duration, and grant number of all other research funding currently granted to PI or under consideration.

Not applicable

Reviewer Comments and Response

Reviews by the Bill and Melinda Gates Foundation

Application ID: INV-000247
Proposer: Dr. Tahmeed Ahmed

Reviewer 1

External and Internal Reviewer Feedback
Overall comment by the Bill and Melinda Gates foundation:

Response: Attached

Note: Comments of three reviewers were compiled in this single table. Multiple bullet points in a row mean that multiple reviewers have had overlapping or related comments in this area

Comments:	Response and Modifications Made:
Power & Statistical Analysis: • Two sites are important for reducing recruitment time (Mirpur and Kurigram), but this interjects heterogeneity. Has this been accounted for in the statistical analysis?	• Heterogeneity is of course there. We compared data of children from Mirpur and Kurigram. The groups were differently placed chronologically, about 3 years apart. Being urban and rural, SES and other demographics are different, income is more for the urban households.
Recruitment: • We wonder if it would be possible to condense primary MAM recruitment to 6 months, recognizing that MAM post SAM will take longer given a smaller patient population. Aim would be to have • What data will be collected regarding the past SAM and acute infection history of children? Number of previous hospitalizations (cause, length of stay) for example might be particularly important in predicting outcome.	• As mentioned in the revised proposal shared with you, we would now like to do the enrolment and most of the follow up within 12 months. Completing enrolment within 6 months would be too tight. Estimate is half the sample size. Let's start and we will devote maximum effort for expediting enrolment. • Yes, hospitalization will be uncommon but we will record all morbidities including use of antibiotics.
Intervention: • Please provide more details around food preparation in the home. How will this be accomplished and monitored? • Please clarify if the feed timings are chosen so as to minimize displacement of normally eaten meals? • How will normally consumed food diversity be measured and analyzed? • Milk powder is mentioned in the protocol. Is this part of the rice-lentil recipe, or a typo? • Could you provide a table of the breakdown of micronutrients present in MDCF2 vs. Rice Lentil intervention? • Can you clarify what is meant by "measured water"?	• MDCF will be prepared by us in bulk at the field site in Mirpur as well as Kurigram, it will never be prepared in the households by the mothers. So, there will be enough control. • Yes, spacing as done in the pilot trial will take care of the displacement. • We will serve food frequency questionnaire. • Milk powder is a constituent of RUSF. • We will provide more details. • This is drinking water, given to offset the issues related to osmolarity of the food. In retrospect, this reference to measured water intake can be deleted
Outcomes: • In the trial, please clarify why there is no planned blood collection at the end of follow-up. It may help to gauge sustainability of outcomes to have blood collection at the end.	• We can keep blood at the end. • Caregivers are always counseled on progress of the child. However, we have no answer yet to your question about ownership of microbiota. We will certainly need to think about it.

• What plans are in place to report back on the study to participants? Do participants maintain ownership of their microbiota in the event that there is commercial potential in the future? • Please clarify the rationale for recording the middle anthropometric measure out of three. • Please specify a timeline for data transfer to BMGF. Three months after collection is complete may be reasonable.	• This should be the mean. The proposal will be corrected reflecting the standard anthropometric method. • 3-4 months should be fine.
Budget: • Please clarify which budget (Washington University or icddr,b) will cover the bio specimen analysis.	• Bio specimen analysis will be covered by Washington University.

Reviewer 2

Title:
Community-based clinical trial with microbiota-directed complementary foods (MDCFs) made of locally available food ingredients for the management of children with primary moderate acute malnutrition

Summary of Referee's Opinions: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.

	Rank Score		
	High	Medium	Low
Quality of project	√		
Adequacy of project design	√		
Suitability of methodology	√		
Feasibility within time period	√		
Appropriateness of budget	Not	provided	
Potential value of field of knowledge	√		

CONCLUSIONS

I support the application:

a) without qualification ☐

b) with qualification

- on technical grounds [√]
- on level of financial [ ]

support I do not support the [ ]

application

Name of Referee: Dr Mohammad Mushtuq Husain

Signature:  Date: 29 Aug 2018

Position: Coordinator, Coordination & Support Centre (CSC)

Institution: Directorate General of Health Services (DGHS)

Detailed Comments

Title: **Community-based clinical trial with microbiota-directed complementary foods (MDCFs) made of locally available food ingredients for the management of children with primary moderate acute malnutrition**

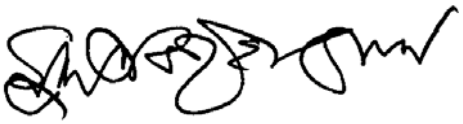
PI: **Dr Tahmeed Ahmed**

Reviewer: Dr Mohammad Mushtuq Husain

The following should be addressed before accepting the protocol:

a) Possible study site at Kurigram Terr des Hommes: Finalization of study site is needed before acceptance of protocol
b) Preparation of intervention and control foods: where will the MFCF and RUSF be produced? How quality will be controlled?
c) Method of preparing RUSF should be detailed as like MDCF
d) CONSENT FORM
    i. The term 'special food' should be avoided. Please replace it with 'one type of food' and 'other type of food', to avoid sense of discrimination
    ii. Please omit: "Children will be benefitted by receiving free special food ……."
    iii. Please write in more clear language: A total of 6 mL of blood in three occasions, with 2 mL each on every occasion (*cÖwZ ev‡i 2 wgwj/ †dvuUv K‡i †gvU 6 wgwj/‡dvuUv).* It will help to avoid apprehension of parents for bleeding too much.

Thank you



(Dr Mohammad Mushtuq Husain)

Date: 29 Aug 2018

Comments	Response
a)Possible study site at Kurigram Terr des Hommes: Finalization of study site is needed before acceptance of protocol	Collaborative agreement with Tdh will be done before commencement of the field work.
b)Preparation of intervention and control foods: where will the MDCF and RUSF be produced? How quality will be controlled?	Food processing laboratory will be established at the Mirpur area following standard procedures. We have already developed such standard laboratory during the "Pre-Proof of Concept (Pre-POC) phase of this protocol. RUSF and MDCF will be prepared at the food processing laboratory. A standardized production procedure will be followed to control the quality of RUSF and MDCF following international standard protocol. Food will be prepared everyday to ensure that no unexpected contamination and nutrient losses occur during preparation. Preparation of food under different steps, that is, roasting, particle size reduction, homogeneous blending, and supplying to feeding centre will be monitored by icddr,b investigators.
c) Method of preparing RUSF should be detailed as like MDCF	It is now incorporated
d) i. CONSENT FORM i. The term 'special food' should be avoided. Please replace it with 'one type of food' and 'other type of food', to avoid sense of discrimination	Replaced as per suggestion
ii. Please omit: "Children will be benefitted by receiving free special food ……."	Deleted as per advice
iii. Please write in more clear language: A total of 6 mL of blood in three occasions, with 2 mL each on every occasion. It will help to avoid apprehension of parents for bleeding too much.	Replaced as per advice

Biography of the Investigators

Provide biographical data in the following format for all key personnel including the Principal Investigator. Copy the same format for each of them.
Note: Biography of the External Investigators may, however, be submitted in the format as convenient to them..

1. **Name:** Dr Tahmeed Ahmed

2. **Present Position:** Senior Director, Nutrition & Clinical Services Division, icddr,b and Professor of Public Health Nutrition, James P. Grant School of Public Health, BRAC University

3. Educational background:

Degree	Institution	Year
PhD	University of Tsukuba, Japan	1996
MBBS	University of Dhaka	1983
Training	Clinical training in Pediatrics, University of Tsukuba Hospital	1990-1992
Training	Residential training in Pediatrics, Dhaka Shishu Hospital	1989-1990

4. Ethics Certification:

		Issuing Authority	Registration No	Valid Until
No ☐	Yes ☒	NIH	1933611	Issued on 12 August 2015

5. List of ongoing research protocols/ activities:

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time
MAL-ED	PI	Nov 2008	March 2017	40
PR-11005	PI	June 2011	June 2017	30
Aflatoxin	Co-I	February 2013	May 2016	5
Hypernatremia follow up	Co-I	January 2016	January 2017	-
PR-15101	Co-PI	January 2016	March 2018	10
PR-16007	PI	November 2015	November 2019	15
PR-16009	PI	July 2016	October 2018	20

6. Publications

Types of publications	Numbers
a. Original scientific papers in peer-review journals	182
b. Book chapters	18
c. Papers in conference proceedings	25
d. Letters, editorials, annotations, and abstracts in peer-reviewed journals	5
e. Working papers	10
f. Monographs	1

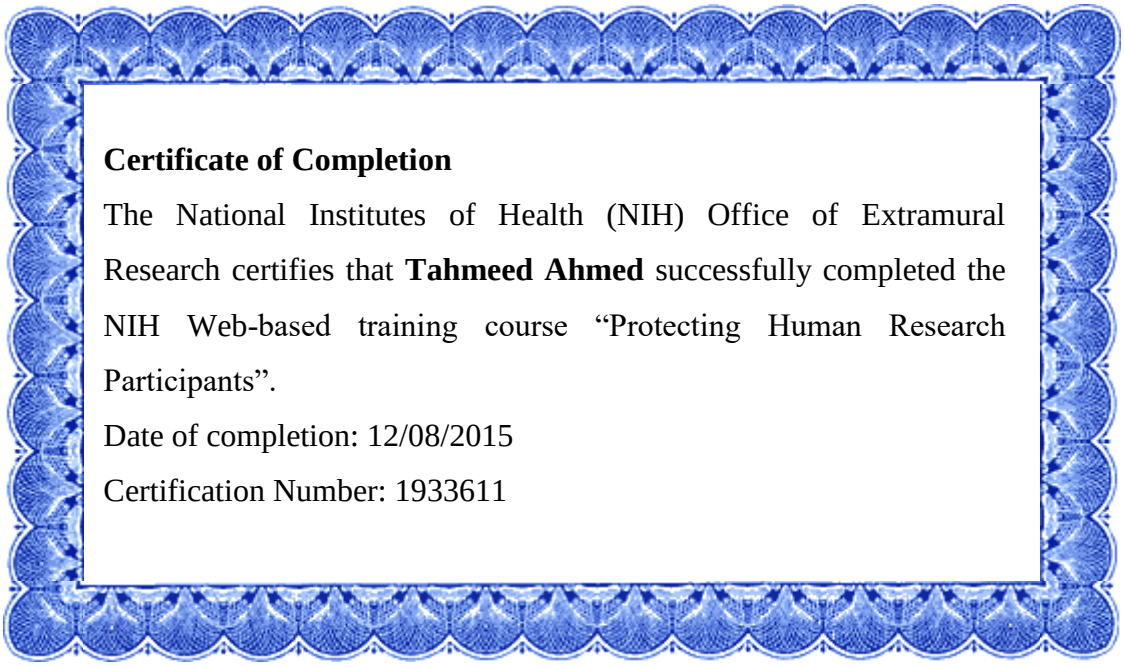
7. Five recent publications including publications relevant to the present research protocol

1. Ahmed T, Choudhury N, Hossain I, Tangsuphoom N, Islam MM, de Pee S, Steiger G, Fuli R, Sarker SA, Parveen M, West KP, Christian P. Development and acceptability testing of ready-to-use supplementary food made from locally available food ingredients in Bangladesh. BMC Pediatr 2014 Jun 27; 14:164.
2. Subramanian S, Huq S, Yatsunenko T, Haque R, Mahfuz M, Alam MA, Benezra A, DeStefano J,

Meier MF, Muegge BD, Barratt MJ, VanArendonk LG, Zhang Q, Province MA, Petri WA Jr, Ahmed T, Gordon JI. Persistent gut microbiota immaturity in malnourished Bangladeshi children. Nature 2014 doi: 10.1038/nature13421.

3. Bhutta ZA, Das JK, Rizvi A, Gaffey MF, Walker N, Horton S, Webb P, Lartey A, Black RE, The Lancet Nutrition Interventions Review Group (Bhutta ZA, Rizvi A, Das JK, Salam RA, Yousafzai A, Lassi ZS, Lenters L, McPhail C, Wazny K, Gaffey MF, Zlotkin S, Imdad A, Haider BA, Welch V, Martorell R, Black RE, Walker N, Tam Y, Ahmed T, and the Maternal and Child Nutrition Study Group (Black RE, Victora C, Walker S, Alderman H, Bhutta ZA, Gillespie S, Haddad L, Horton S, Lartey A, Mannar V, Ruel M, Webb P). Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost? Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost? Lancet 2013 Jun 6 [Epub ahead of print].
4. Ahmed T, Auble D, Berkley JA, Black R, Ahern PP, Hossain M, Hsieh A, Ireen S, Arabi M, Gordon JI. An evolving perspective about the origins of childhood undernutrition and nutritional interventions that includes the gut microbiome. Ann N Y Acad Sci 2014 Aug 12. [Epub ahead of print]
5. Chisti MJ, Salam MA, Smith JH, Ahmed T, Pietroni MAC, Shahunja KM, Shahid ASMSB, Faruque ASG, Ashraf H, Bardhan PK, Sharifuzzaman, Graham SM, Duke T. Bubble continuous positive airway pressure for children with severe pneumonia and hypoxaemia in Bangladesh: an open, randomized controlled trial. Lancet 2015 Aug 19

Ethics certificates



Certificate of Completion

The National Institutes of Health (NIH) Office of Extramural Research certifies that **Tahmeed Ahmed** successfully completed the NIH Web-based training course "Protecting Human Research Participants".

Date of completion: 12/08/2015

Certification Number: 1933611

Biography of the Investigators (1)

Provide biographical data in the following format for all key personnel including the Principal Investigator. Copy the same format for each of them.
Note: Biography of the External Investigators may, however, be submitted in the format as convenient to them..

1. **Name:** Dr Munirul Islam

2. **Present Position:** Scientist, Nutrition and Clinical Services Division, icddr,b

3. **Educational background:**

Degree	Institution	Year
PhD	PhD in Nutrition, Designated Emphasis in International Nutrition (University of California at Davis, California, USA)	2007
MBBS	Dhaka Medical College, University of Dhaka	1995

4. **Ethics Certification:**

		If Yes		
		Issuing Authority	Registration No	Valid Until
No ☐	Yes ☒	COLLABORATIVE INSTITUTIONAL TRAINING INITIATIVE (CITI)	ID:1306963	07/21/2017

5. **List of ongoing research protocols/ activities**

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time
PR-14036	Co-PI	2014	2017	30
PR-12082	Co-PI	2012	2015	25
PR-13008	PI	2015	2016	20
PR-2008-020	Co-I	2009	2015	10

6. **Publications**

Types of publications	Numbers
a. Original scientific papers in peer-review journals	44
b. Peer reviewed articles and book chapters	07
c. Papers in conference proceedings	20
d. Letters, editorials, annotations, and abstracts in peer-reviewed journals	0
e. Working papers	0
f. Monographs	1

7. **Five recent publications including publications relevant to the present research protocol**

1. Islam MM, Brown KH. Zinc transferred through breast milk does not differ between appropriate- and small-for- gestational-age, predominantly breast-fed Bangladeshi infants. J Nutr 2014; 144:771-6.
2. Baxter JA, Roth DE, Al Mahmud A, Ahmed T, Islam MM, Zlotkin SH. Tablets Are Preferred and More Acceptable Than Powdered Prenatal Calcium Supplements among Pregnant Women in Dhaka, Bangladesh. J Nutr 2014; 144: 1106-12.

3. Ahmed T, Mahfuz M, Islam MM, Mondal D, Hossain MI, Ahmed AMS, Tofail F, Gaffar SMA, Haque R, Guerrant RL, Petri WA. The MAL-ED Cohort Study in Mirpur, Bangladesh. CID 2014:59 (Suppl 4); S280-6.
4. Mahfuz M, Ahmed T, Ahmed AMS, Islam MM, Hossain MI. Weight Gain in Malnourished Children after 5 Months Food Supplementation in a Slum Setting in Bangladesh. FNS 2014, 5, 1365-1373
5. **Islam MM, Woodhouse LR, Hossain MB, Ahmed T, Huda MN, Ahmed T, Hotz C, Brown KH.** Zinc absorption from mixed diets containing either high-zinc rice or conventional rice, with or without additional exogenous zinc, among young Bangladeshi children. J Nutr. 2013;143:519-525

Ethics certificates

COLLABORATIVE INSTITUTIONAL TRAINING INITIATIVE (CITI)
BIOMEDICAL RESEARCH - BASIC/REFRESHER CURRICULUM COMPLETION REPORT
Printed on 07/22/2014

LEARNER	Md Munirul Islam (ID: 1306963)
DEPARTMENT	Centre for Nutrition and Food Security
PHONE	+880 2 9827 001-10; Ext. 2352
EMAIL	mislam@icddrb.org
INSTITUTION	University of California, Davis
EXPIRATION DATE	07/21/2017

BIOMEDICAL RESEARCHERS AND STAFF : Choose this group to satisfy CITI training requirements for Investigators and staff involved primarily in biomedical research with human subjects.

COURSE/STAGE:	Basic Course/1
PASSED ON:	07/22/2014
REFERENCE ID:	13537842

REQUIRED MODULES	DATE COMPLETED
Belmont Report and CITI Course Introduction	07/22/14
Basic Institutional Review Board (IRB) Regulations and Review Process	07/22/14
Informed Consent	07/22/14
Social and Behavioral Research (SBR) for Biomedical Researchers	07/22/14
Records-Based Research	07/22/14
Genetic Research in Human Populations	07/22/14
Research With Protected Populations - Vulnerable Subjects: An Overview	07/22/14
Vulnerable Subjects - Research Involving Children	07/22/14
Vulnerable Subjects - Research Involving Pregnant Women, Human Fetuses, and Neonates	07/22/14
FDA-Regulated Research	07/22/14
Research and HIPAA Privacy Protections	07/22/14
Conflicts of Interest in Research Involving Human Subjects	07/22/14
University of California, Davis	07/22/14

For this Completion Report to be valid, the learner listed above must be affiliated with a CITI Program participating institution or be a paid independent Learner. Falsified information and unauthorized use of the CITI Program course site is unethical, and may be considered research misconduct by your institution.

Paul Braunschweiger Ph.D.
Professor, University of Miami
Director Office of Research Education
CITI Program Course Coordinator

Biography of the Investigators

Provide biographical data in the following format for all key personnel including the Principal Investigator. Copy the same format for each of them.
Note: Biography of the External Investigators may, however, be submitted in the format as convenient to them..

1. **Name:** Dr. Mustafa Mahfuz

2. **Present Position:** Associate scientist, Nutrition and Clinical Services Division, icddr,b

3. **Educational background:**

	Institution	Year
MPH	University of Dhaka	2006
MBBS	University of Chittagong	2001

4. **Ethics Certification:**

		If Yes		
		Issuing Authority	Registration No	Valid Until
No ☐	Yes ☒	NIH	1973495	Issued 2016

Note: If the response is "no", please get certification from CITI or NIH before study initiation and submit a copy to the Committee Coordination Secretariat

2. **List of ongoing research protocols/ activities**

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time
PR-16007	Co-PI	15.12.2015	31.11.2019	60
PR-12096	PI	28.01.2013	30.06.2017	20
2008-20	Co-I	01.10.2008	31.08.2017	10
PR- 11005	Co-I	19.08.2011		10

3. **Publications**

Types of publications	Numbers
Original scientific papers in peer-review journals	50
Peer reviewed articles and book chapters	7
Papers in conference proceedings	4
Letters, editorials, annotations, and abstracts in peer-reviewed journals	7
Working papers	0
Monographs	0

4. **Five recent publications including publications relevant to the present research protocol**

1. **Mahfuz M**, Alam MA, Islam SB, Naila N, Chisti MJ, Alam NH, Sarker SA, Ahmed T. Treatment outcome of children with Persistent Diarrhoea admitted to an Urban Hospital, Dhaka during 2012-2013. BMC Pediatrics 2017; 17:142.
2. **Mahfuz M**, Das S, Mazumder RN, Rahman M, Haque R, Gordon JI, Ahmed T et al. Bangladesh Environmental Enteric Dysfunction (BEED) study: Protocol for a community-based intervention study to

validate non-invasive biomarkers of Environmental Enteric Dysfunction. BMJ Open 2017-017768 (accepted).

3. **Mahfuz M**, Alam MA, Islam MM, Mondal D, Hossain MI, Ahmed AMS, Choudhury N, Raihan MJ, Haque R, and Ahmed T. Effect of micronutrient powder supplementation for two and four months on hemoglobin level of children 6–23 months old in a slum in Dhaka: a community based observational study. BMC Nutrition 2016, DOI: 10.1186/s40795-016-0061-y. URL: http://www.biomedcentral.com/2055-0928/2/21
4. Subramanian S, Huq S, Yatsunenko T, Haque R, **Mahfuz M**, Alam MA, Benezra A, DeStefano J, Meier MF, Muegge BD, Barratt MJ, VanArendonk LG, Zhang Q, Province MA,. Petri WA, Ahmed T, Gordon JI. Persistent gut microbiota immaturity in malnourished Bangladeshi children (research letter). Nature 2014 Jun 19;510(7505):417-21.
5. Ahmed T, **Mahfuz M**, Islam MM, Mondal D, Hossain MI, and Ahmed AMS, et al. The MAL-ED Cohort Study in Mirpur, Bangladesh. Clin Infect Dis 2014 Nov;59 (Suppl 4):S280-6.


RBM/RA/2015-074

icddr,b

Awards this certificate to

Dr. Mustafa Mahfuz

for attending the Certificate Program on

Results Based Management (RBM)

held during 06 & 07 September 2015

Director, RAS

Director, HR

Executive Director

Research Administration, icddr,b
68, Shahid Tajuddin Ahmed Sharani
Mohakhali, Dhaka 1212, Bangladesh


Certificate of Completion

The National Institutes of Health (NIH) Office of Extramural Research certifies that **Mustafa Mahfuz** successfully completed the NIH Web-based training course "Protecting Human Research Participants".

Date of completion: 01/27/2016.

Certification Number: 1973495.

Biography of the Investigators

Provide biographical data in the following format for all key personnel including the Principal Investigator. Copy the same format for each of them.
Note: Biography of the External Investigators may, however, be submitted in the format as convenient to them..

1. Name: Dr. Sayeeda Huq

2. Present Position: Associate Scientist, Consultant Physician, Nutrition Ward, Nutrition & Clinical Services Division, icddr,b

3. Educational background:

	Institution	Year
MPH	University of Sydney, Australia	2007
MBBS	Bangladesh Medical College, Dhaka, Bangladesh	1996

4. Ethics Certification:

		If Yes		
		Issuing Authority	Registration No	Valid Until
No ☐	Yes ☒	NIH	1932845	Issued 12/07/2015

5. List of ongoing research protocols/ activities

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time
PR#15113	PI	25-03-16	31-05-17	6%
PR# 09023	PI	1-10-09	30-06-13	25%
PR#09038	Co-I	01-08-12	31-12-14	10%
PR #10039	Co-I	20-01-12	19-01-15	5%
PR#11005	Co-I	18-08-11	31-12-15	5%
PR#12082	Co-I	1-08-13	30-11-15	25%

6.. Publications

Types of publications	Numbers
Original scientific papers in peer-review journals	27
Peer reviewed articles and book chapters	2
Papers in conference proceedings	12
Letters, editorials, annotations, and abstracts in peer-reviewed journals	3
Working papers	
Monographs	1

7. Five recent publications including publications relevant to the present research protocol

7.1**. S Huq,** , M I Hossain, MA Malek, ASG Faruque and M A Salam. Hypoglycaemia in under five children with diarrhoea. Journal of Tropical Pediatrics; 2007 Jun;53(3):197-201.

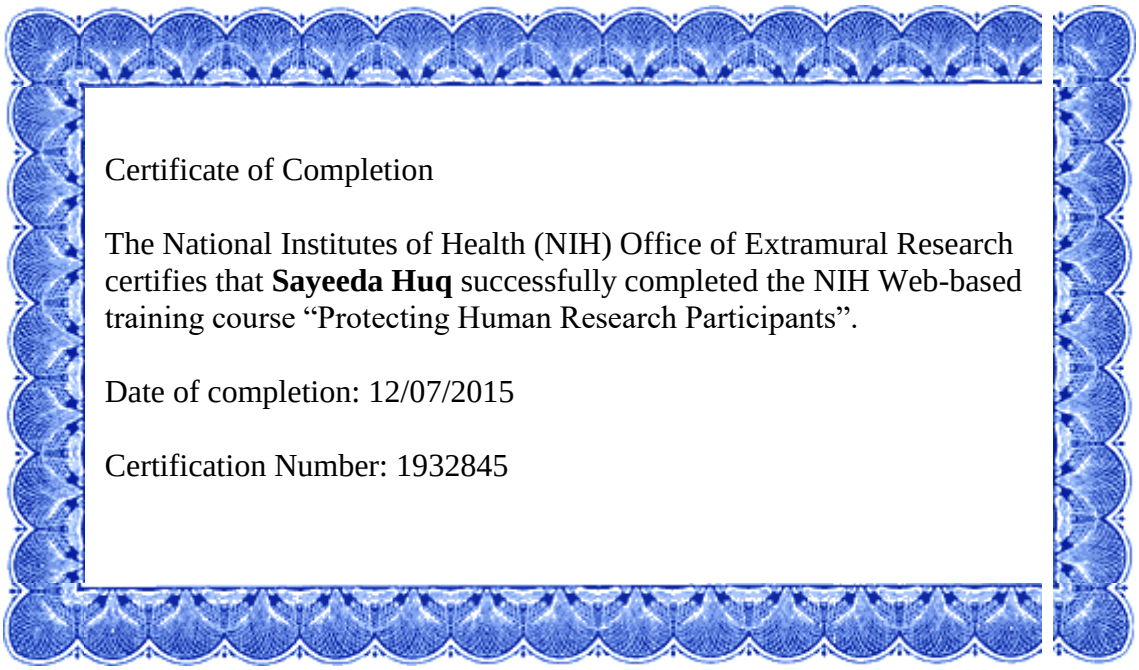
7.2. Sathish Subramanian, **Sayeeda Huq**,Tanya Yatsunenko,Rashidul Haque,Mustafa Mahfuz, Mohammed A.Alam,Amber Benezra,Joseph DeStefano,Martin F. Meier,Brian D. Muegge,Michael J. Barratt,Laura G. VanArendonk, Qunyuan Zhang, Michael A. Province,William A. Petri Jr,Tahmeed Ahmed,Jeffrey I. Gordon.

Persistent gut microbiota immaturity in malnourished Bangladeshi children. Nature http://dx.doi.org/10.1038/nature13421 (2014).

7.3. **S Huq**, Mark A.C. Pietroni, Hafizur Rahman, Mohammad Tariqul MA. Hereditary Spherocytosis. Journal of Health, Population and Nutrition , 2010 Feb; 28(1), 107-109.

7.4. Ahmed T, Islam M, Choudhury N, Hossain I, **Huq S**, Mahfuz M, Sarker SA.Results with complementary food using local food ingredients.Nestle Nutr Inst Workshop Ser. 2017;87:103-113. doi: 10.1159/000448960.2017 Mar 17.

7.5.Sumon Das, Jobayer Chisti, **Sayeeda Huq**, Mohammad Abdul Malek, Lana Vanderlee, Mohammed Abdus Salam, Tahmeed Ahmed, Abu Syed Golam Faruque, Abdullah Al Mamun. Changing trend of overweight and obesity and their associated factors in urban population of Bangladesh. Food and Nutrition Sciences, 2013,4,678-689.



Certificate of Completion

The National Institutes of Health (NIH) Office of Extramural Research certifies that **Sayeeda Huq** successfully completed the NIH Web-based training course "Protecting Human Research Participants".

Date of completion: 12/07/2015

Certification Number: 1932845

Biography of the Investigators

Provide biographical data in the following format for all key personnel including the Principal Investigator. Copy the same format for each of them.
Note: Biography of the External Investigators may, however, be submitted in the format as convenient to them..

1. **Name:** Dr. Ishita Mostafa

2. **Present Position:** Research Investigator, Nutrition & Clinical Services Division, icddr,b

3. **Educational background**: (last degree and diploma & training relevant to the present research proposal)

	Institution	Year
MPH	North South University	2011-13
BDS	University of Dhaka	2005-09
Training	Research Methodology and SPSS (icddr,b)	2013
Training	Post graduation training (ShSMC)	2011
Training	Internship	2009-10

4. **Ethics Certification:**

		If Yes		
		Issuing Authority	Registration No	Valid Until
No ☐	Yes ☒	**NIH**	**2246679**	**Issued 2016**

Note: If the response is "no", please get certification from CITI or NIH before study initiation and submit a copy to the Committee Coordination Secretariat

5. **List of ongoing research protocols/ activities**

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time
14003	PI	15.4.2014	14.4.2015	100%
16099	C0-I	4.1.2017	31.10.2018	100%

6. **Publications**

Types of publications	Numbers
g. Original scientific papers in peer-review journals	2
h. Peer reviewed articles and book chapters	
i. Papers in conference proceedings	1
j. Letters, editorials, annotations, and abstracts in peer-reviewed journals	
k. Working papers	2
l. Monographs	

7. **Five recent publications including publications relevant to the present research protocol**

7.1. Children living in the slums of Bangladesh face risks from unsafe food and water and stunted growth is common

7.2. The management of persistent diarrhoea at Dhaka Hospital of the International Centre for Diarrhoeal Disease and Research: a clinical chart review

Certificate of Completion

The National Institutes of Health (NIH) Office of Extramural Research certifies that **Ishita Mostafa** successfully completed the NIH Web-based training course "Protecting Human Research Participants".

Date of completion: 11/29/2016.

Certification Number: 2246679.

Biography of the Investigators

Provide biographical data in the following format for all key personnel including the Principal Investigator. Copy the same format for each of them.
Note: Biography of the External Investigators may, however, be submitted in the format as convenient to them..

1.Name: Dr. Imteaz Mahmud

2.Present Position: Research Fellow, Nutrition & Clinical Services Division, icddr,b

3.Educational background: (last degree and diploma & training relevant to the present research proposal)

	Institution	Year
MBBS	Dhaka Medical College	2009-14
Training	Internship	2014-15
Training	Research Protocol Development (icddr,b)	2018

4.Ethics Certification:

		If Yes		
		Issuing Authority	Registration No	Valid Until
No ☐	Yes ☒	**NIH**	**2225935**	**Issued 2017**

Note: If the response is "no", please get certification from CITI or NIH before study initiation and submit a copy to the Committee Coordination Secretariat

5. List of ongoing research protocols/ activities

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time

6.Publications

Types of publications	Numbers
m. Original scientific papers in peer-review journals	
n. Peer reviewed articles and book chapters	
o. Papers in conference proceedings	1
p. Letters, editorials, annotations, and abstracts in peer-reviewed journals	
q. Working papers	
r. Monographs	

7.Five recent publications including publications relevant to the present research protocol
7.3.

Certificate of Completion

The National Institutes of Health (NIH) Office of Extramural Research certifies that **Imteaz Mahmud** successfully completed the NIH Web-based training course "Protecting Human Research Participants."

Date of Completion: 02/15/2017

Certification Number: 1005286

NIH National Institutes of Health Office of Extramural Research

Biography of the Investigators

Provide biographical data in the following format for all key personnel including the Principal Investigator. Copy the same format for each of them.
Note: Biography of the External Investigators may, however, be submitted in the format as convenient to them..

1. **Name: Dr Nurun Nahar Naila**

2. **Present Position:** Assistant scientist

3. **Educational background**: (last degree and diploma & training relevant to the present research proposal)

	Institution	Year
MPH	University of New South Wales, Australia	2012
MBBS	Bangladesh Medical College and Hospital, Bangladesh	2006
Training	Qualitative Research method, International Centre for Diarrhoeal Disease Research, Bangladesh	2015
Training	Scientific writing, James P Grant School of Public Health, Bangladesh	2015

4. **Ethics Certification:**

		If Yes		
		Issuing Authority	Registration No	Valid Until
No ☐	Yes ☒	NIH	1347016	NA

Note: If the response is "no", please get certification from CITI or NIH before study initiation and submit a copy to the Committee Coordination Secretariat

5. **List of ongoing research protocols/ activities**

Protocol/ Activity Number	Role in the protocol/ activity (PI, Co-PI, Co-I)	Starting date	End date	Percentage of time

Co Investigator PR 16005	IRB approval, staff training and recruitment, field activities supervision, monitoring data input and data analysis	2015	2018	100%
Principal Investigator PR 17030	Protocol development, IRB approval, field supervision and monitoring, data analysis	2017'May	2017'Oct	100%
Principal Investigator PR 17105	IRB approval, field supervision and monitoring, primary and secondary data analysis	will be started from October'2018		30%
Principal Investigator	To explore available information for identifying all possible activities, resources and barriers related to calcium supplementation program	Will be started from October 2018		40%

6. Publications

Types of publications	Numbers
a. Original scientific papers in peer-review journals	5
b. Peer reviewed articles and book chapters	
c. Papers in conference proceedings	1
d. Letters, editorials, annotations, and abstracts in peer-reviewed journals	
e. Working papers	2
f. Monographs	

7. Five recent publications including publications relevant to the present research protocol

1 **Naila N**, Nahar B, Lazarus M, Ritter G, Hossain M, Mahfuz M, Ahmed T, Denno D, Walson J, Ickes S. "Those who care much, understand much." Maternal perceptions of children's appetite: Perspectives from urban and rural caregivers of diverse parenting experience in Bangladesh. Maternal & child nutrition. 2018 Jan;14(1):e12473.

2 Mostafa I, **Naila NN**, Mahfuz M, Roy M, Faruque AS, Ahmed T. Children living in the slums of Bangladesh face risks from unsafe food and water and stunted growth is common. Acta Paediatrica. 2018 Feb 16.

3 Hossain M, Ickes S, Rice L, Ritter G, **Naila NN**, Zia T, Nahar B, Mahfuz M, Denno DM, Ahmed T, Walson J. Caregiver perceptions of children's linear growth in Bangladesh: a qualitative analysis. Public health nutrition. 2018 Mar:1-0.

4 Khatun H, Islam SB, **Naila NN**, Islam SA, Nahar B, Alam NH, Ahmed T. Clinical profile, antibiotic susceptibility pattern of bacterial isolates and factors associated with complications in culture-proven typhoid patients admitted to an urban hospital in Bangladesh. Tropical Medicine & International Health. 2018 Apr;23(4):359-66.

5 Mahfuz M, Alam MA, Islam SB, **Naila NN**, Chisti MJ, Alam NH, Sarker SA, Ahmed T. Treatment outcome of children with persistent diarrhoea admitted to an urban hospital, Dhaka during 2012–2013. BMC pediatrics. 2017 Dec;17(1):142.

Certificate of Completion

The National Institutes of Health (NIH) Office of Extramural Research certifies that **Nurun Naila** successfully completed the NIH Web-based training course "Protecting Human Research Participants".

Date of completion: 12/23/2013

Certification Number: 1347016

Consent Form
Primary MAM

Protocol No.18073	Version No. 1.1	Date: 13-09-2018

Purpose of the research

Protocol Title: Community-based clinical trial with microbiota directed complementary foods (MDCF) made of locally available food ingredients for the management of children with primary moderate acute malnutrition (MAM).

Principal Investigator's name: Dr Tahmeed Ahmed

Organization: icddr,b

Purpose of the research

Bangladesh is a country with one of the highest childhood malnutrition burden in the world. According to Bangladesh Demographic Health Survey 2014, 36% under five children are shorter for their age and among them 12 % are suffering from its severe form. Acute malnutrition is associated with an impaired development of the gut microbial community (microbiota immaturity). In a previous randomized clinical trial conducted at icddr,b in children with severe acute malnutrition (SAM) microbiota immaturity was only slightly improved in children treated with one of two current therapeutic foods and the children remained shorter for their age and underweight throughout the follow up period. Through our previous and ongoing research we now know about the members of the gut microbiota that can promote growth in children and also about certain food ingredients that promote the proliferation of such beneficial microbiota.

This clinical community based trial is designed for the management of children with primary moderate acute malnutrition with potential MDCF.

Why invited to participate in the study?

We are conducting this study in children who are 12-18 months old and suffering from moderate acute malnutrition. Maternal nutritional status is associated with child nutritional status, as shown through the results of our eight country MAL-ED study. In addition, neonatal and maternal factors were early determinants of lower length-for-age, and their contribution remained important throughout the first 24 months of life. So we are inviting you and your children to participate in this study. Your child and other similar children need proper dietary intervention to treat this. The proposed ingredients of the diets are to assess whether it is acceptable and promotes growth in children. We assume that your child's malnutrition is at least in part due to microbiota immaturity, and this is why we are inviting you and your child to help us in participating in this study.

Methods and procedures

If you agree to our proposal of including you and your child in the study, you might expect the followings:

- We would collect 2 mL of urine samples at enrolment, one week after enrolment and monthly once during the intervention and post intervention phase.
- Weekly 2 gm of stool samples will be collected during the study period.

- A total of 6 mL of blood in three occasions, with 2 mL each on every occasion (equivalent to near about half tea-spoonful) venous blood would be collected prior to enrolment, at the end of first month of intervention and after the completion of the intervention.
- After 1 week of enrolment, we would ask you and your child to visit the nutrition centre twice daily for the first month, and once daily for the second month to take the nutrition supplement. There will be 2 groups who will receive the nutrition supplement and your child will be randomly assigned to any one of the groups.
- Your child will receive one type of food or the other type of food (that will be fixed based on a process like lottery) to observe how much your child can eat.
- We would ask you some questions regarding current illness and health condition of your child. We will also perform thorough physical examinations and measure length and weight weekly after enrolment in the study.
- We will also ask for information on your socioeconomic condition and family structure. Your child will be monitored everyday for any possible side effects/adverse events (e.g. rash, urticaria from food allergy or any significant changes in clinical status).
- If any side effects/adverse event are observed then it will be treated according to the standard management protocol followed at Dhaka Hospital of icddr,b.
- In order to understand the biological state of nutrition of the mother, enrolment samples of stool, 5 ml blood and urine will be asked of the mother. These samples will be analysed for gut microbiota, and proteomics, and the results correlated with those of the enrolled children.
- We will also record maternal height and weight.
- We assure you that the stool, blood samples and urine samples will not be used for any other purposes.

Risk and benefits

Anticipated potential benefits:

Your child will be directly and indirectly benefited from participating in the study. Moreover, your child would be able to contribute to our understanding of malnutrition and to develop more effective treatments. In the long term, the results of this study could benefit other children in Bangladesh and elsewhere by helping us understand the effects of providing nutrient supplements. Nutritional counselling will help you to routine your child's daily food intake. The goal is to test special food (MDCF) that repair the persistent microbiota immaturity and promote growth in children with MAM.

Anticipated potential risks:

There is no major risk involved in participation of your child in the study. Possible adverse events may be vomiting, diarrhoea, skin rash, urticaria from food. Despite taking precautions, if your child develops any symptoms due to this study procedure, we would provide appropriate treatment at the Dhaka Hospital of icddr,b.

At the time of collection of the blood sample, your child will feel a momentary pain due to the needle prick. There is also a rare chance of bluish discoloration surrounding the prick site due to mild leakage of blood in the skin, and very distant possibility of local or systemic infections or problems. However, we will take required precautions, including using of disposable syringes and needles, to prevent these problems. All blood samples will be obtained by a qualified health care professional. A total of 6 mL of blood sample (equivalent to one tea-spoonful) will be obtained from your child just before the intervention (2 mL of blood

sample), end of first month of intervention (2 mL of blood sample) and after the intervention (2 mL of blood sample) is complete.

Privacy, anonymity and confidentiality

We will keep all information collected from you/your child confidential, locked in a secure place under the responsibility of the principal investigator from icddr,b. No one other than this group of investigators, regulatory authorities and the Ethical Review Committee (a group of experts which protects the interest of study participants) of icddr,b, investigators in the Washington University School of Medicine would have access to such information. The biological samples will be sent to Washington University for further analysis. Your child's name and identity will not be disclosed while analyzing or publishing the results of this study.

Future use of information

In the case of future use of the information collected from this study, privacy, anonymity and confidentiality of information will be maintained. We will store the stool and blood sample in a way that your child's identity will not be recognised, and use the samples for performing tests that are modified in the near future for superior results, as well as new tests for studying growth and metabolism. No further consent will be requested for such studies.

Right not to participate and withdraw

Your child's participation in the study is voluntary, and you have the sole authority to decide for or against your patient's participation. You would also be able to withdraw your child's participation any time during the study, without showing any cause. Refusal to take part in or withdrawal from the study will involve no penalty or loss of care, benefits or attention.

Principle of compensation

There is no cost to you for participating in this study. You will not have to pay for the supplement or any of the tests we are doing. You will not get any money for participating in this study either. If your child has a study related injury s/he will receive standard care at the Dhaka Hospital (Cholera Hospital) in Mohakhali, Dhaka.

Answering your questions/Contact persons

We will happily provide you further information about the study, if any, now or at a later time. You may communicate with the principal investigators of the study or her/his designated person at the contact address given below. We will answer to your question related to your/your child's medical condition(s), treatment, and results of any or all tests performed on you/your child.

You may communicate with Dr. Tahmeed Ahmed, the principal investigator of this study personally at his office at icddr,b or by telephone (+88-02-98271030) Ext-2300

If you agree to our proposal of enrolling your child in our study, please indicate that by putting your signature or your left thumb impression at the specified space below.

If you have questions regarding your rights as a research participant, please call IRB Secretariat, RA, MA Salam Khan, Phone No: 9886498 or PABX 8860523-32 Extension. 3206 at the icddr,b Research Ethics office.

(NOTE: In case of representative of the PI, she/he shall put her/his full name and designation and then sign)
If you agree to our proposal of enrolling you/your patient in our study, please indicate that by putting your signature or your left thumb impression at the specified space below

Thank you for your cooperation,

_____ _____

Signature or left thumb impression of Parent/ Guardian/ Attendant — Date

_____ _____

Signature or left thumb impression of the witness — Date

_____ _____

Signature of the PI or his/her representative — Date

"কমিউনিটি-বেসড ক্লিনিকাল ট্রায়াল উইথ মাইক্রোবায়টা-ডিরেক্টেড কম্পলিমেন্টারি ফুডস (এমডিসিএফস) মেইড অফ লোকালি এভেইলেবল ফুড ইনগ্রেডিয়েন্টস ফর দা ম্যানেজম্যান্ট অফ চিল্ড্রেন উইথ প্রাইমারি মডারেট একিউট ম্যালনিউট্রিশন"।

প্রধান গবেষকের নাম: ডাঃ তাহমীদ আহমেদ

প্রতিষ্ঠান: আন্তর্জাতিক উদরাময় গবেষনা কেন্দ্র, বাংলাদেশ

গবেষনার উদ্দেশ্য

বিশ্বের যে সব দেশে শিশু অপুষ্টির হার অনেক বেশি, বাংলাদেশ তাদের অন্যতম। বাংলাদেশ স্বাস্থ্য ও জনমিতি জরিপ ২০১৪ অনুযায়ী দেশের শতকরা ৩৬ ভাগ শিশু তাদের বয়সের তুলনায় খর্বাকৃতির, এবং তাদের মধ্যে শতকরা ১২ ভাগ মারাত্নক ভাবে খর্বাকৃতির।ইতোপূর্বে আই সি ডি ডি আর, বি তে দুই ধরনের পথ্য নিয়ে একটি চিকিৎসা বিষয়ক গবেষণায় দেখা গিয়েছে যে, মারাতক তীব্র অপুষ্টিতে আক্রান্ত শিশুদের চিকিৎসার ফলে তাদের মাইক্রোবায়োটা (আন্ত্রিক অনুজীবের) অপরিপক্বতা (যা খাবার হজমের সমস্যার সাথে সম্পর্কিত) কিছু অংশে দূর হলেও পরবর্তী সময়ে তারা বয়স অনুযায়ী খর্বাকৃতির ও কম ওজনের থেকে যায়। আমাদের পূর্ববর্তী ও বর্তমান চলমান বিভিন্ন গবেষণা থেকে আমরা এখন অন্ত্রের অনুজীব সমুহের নাম জানি যারা কিনা শিশুদের বৃদ্ধির জন্য উপকারী এবং কিছু কিছু পুষ্টি উপাদান যারা উপকারী অনুজীবের সংখ্যা বৃদ্ধির জন্য সহায়কক।

এই গবেষণাটি পরিকল্পানা করা হয়েছে মাঝারি অপুষ্টিতে আক্রান্ত বাচ্চাদে র আমাদের বিশেষ উপায়ে তৈরী খাবার (এম.ডি. সি.এফ.) দিয়ে ব্যবস্থাপনার জন্য।

কেন গবেষনায় অংশগ্রহনের জন্য আপনাকে আমন্ত্রন জানানো হচ্ছে?

আমরা এই গবেষনায় ১২-১৮ মাস বয়সী সেইসব শিশুদের অঃতর্ভুক্ত করেছি যারা তাদের বয়সের তুলনায় খাটো ও চরম তীব্র অপুষ্টির শিকার।আবার ৮ টি দেশে সংঘটিত এম.এ.এল.ই.ডি গবেষণায় দেখা গেছে, বাচ্চার পুষ্টির সাথে মায়ের পুষ্টির সম্পর্ক রয়েছে। এটা প্রতিরোধ করার জন্য আপনার শিশু ও অন্যান্য অনুরূপ শিশুদের সঠিক খাদ্যাভাসের প্রয়োজন।এই প্রস্তাবিত খাদ্যের উপাদানগুলোর গ্রহণযোগ্যতা শিশু ও মায়েদের মধ্যে পরিমাপ করা হবে। আমরা মনে করছি যে, আপনার শিশু মাইক্রোবায়োটা অপরিপক্কতার কারণে সে তার বয়সের তুলনায় খাটো ও অপুষ্টি তে আক্রান্ত, এবং এই কারণে আমরা আপনাকে ও আপনার শিশুকে এই গবেষণায় অংশগ্রহণের জন্য আমন্ত্রণ জানাচ্ছি।

পদ্ধতি ও প্রক্রিয়া

আপনি যদি আমাদের প্রস্তাবে সম্মত হয়ে আপনার শিশু সহ এই গবেষনায় অংশগ্রহন করেন, তাহলে আপনি নিম্নলিখিত বিষয়গুলো আশা করতে পারেন।

- আমরা আপনার বাচ্চার মলের নমুনা (১-২ গ্রাম) সংগ্রহ করব প্রথম নিয়গের সময়, প্রথম নিয়গের ১ সপ্তাহ পর, প্রথম মাসের প্রতি সপ্তাহে এবং পরবর্তী দুই মাস এবং পদক্ষেপ পরবর্তী সময় প্রতি মাসে একবার করে। আমরা বিশেষ পদ্ধতি অবলম্বন করে মলের নমুনায় জীবাণু বের করব।
- এই সময়ে বাচ্চার উচ্চতা এবং ওজনও পরিমাপ করা হবে
- আমরা ২ মিলি লিটার পরিমান প্রস্রাব এর নমুনা সংগ্রহ করব প্রথম নিয়গের সময়, তার এক সপ্তাহ পর এবং প্রতি মাসে একবার করে ইন্টারভেনশন ও ইন্টারভেনশন পরবর্তী সময়ে।

- বাচ্চার শিরা থেকে মোট ৬ মি.লি লিটার (আধা চা চামচ পরিমান) রক্ত সংগ্রহ করা হবে প্রথম নিয়গের সময় (২ মি.লি), ইন্টারভেনশন এর প্রথম মাস পর (২ মি.লি) এবং ইন্টারভেনশন সমাপ্ত হবার পর (২ মি.লি)।
- প্রথম নিয়গের ১ মাস পর, আমরা আপনাকে এবং আপনার বাচ্চাকে প্রথম মাসে দিনে ২ বার এবং দ্বিতীয় মাসে দিনে ১ বার করে আস্তে বলব সম্পূরক পুষ্টি গ্রহন করার জন্য। এই ক্ষেত্রে দুইটি গ্রুপ থাকবে যারা সম্পূরক পুষ্টি গ্রহন করবে এবং আপনার বাচ্চা লটারির মত পদধতির মাধ্যমে যে কোন একটি গ্রপের জন্য নির্বাচিত হবে।
- আপনার বাচ্চা এম.ডি. সি.এফ অথবা আর.ইউ.এস.এফ. এর মধ্য থেকে যে কোন এক ধরনের খাবার পাবে এবং পরিক্ষা করে দেখা হবে আপনার শিশু কত টুকু খাবার খেতে পারে।
- আমরা আপনাকে আপনার শিশুর সাম্প্রতিক অসুস্থতা ও স্বাস্থ্যের অবস্থা সংক্রান্ত কিছু প্রশ্ন জিজ্ঞেস করবো। এছাড়াও প্রতি ২ সপ্তাহে একবার করে আপনার শিশুর ওজন ও উচ্চতা পরিমাপ করা হবে।
- আমরা আপনার আর্থ-সামাজিক অবস্থা ও পরিবার এর ধরন নিয়ে কিছু প্রশ্ন জিজ্ঞেস করবো। খাবার দেয়ার সময় প্রতিদিন আপনার শিশুকে সম্ভাব্য প্রতিকূল ঘটনার (যেমন খাবারে এলার্জির জন্য শরীরে চুলকানি বা চাকা চাকা দাগ কিংবা শারীরিক অবস্থার উল্লখেযোগ্য পরিবর্তন) জন্য পর্যবেক্ষন করা হবে।
- এ সময়ে কোন প্রতিকূল ঘটনা ঘটলে আমরা আপনার শিশুকে আই সি ডি ডি আর বি এর ঢাকা হাসপাতালে মানসম্পন্ন সেবা প্রদান করবো।
- মায়ের পুষ্টির অবস্থা বোঝার জন্য, বাচ্চা প্রথম নিয়গের সময় মায়ের মলের নমুনা, ৫ মিলি রক্ত, এবং প্রস্রাব এর নমুনা চাওয়া হবে। এই নমুনা সমূহ অন্ত্রের অণুজীব এবং প্রটিওমিক্স এর অবস্থা জানার জন্য পর্যালোচনা করা হবে,এবং প্রাপ্ত ফলাফলের সাথে নিয়গকৃত শিশুদের ফলাফল সম্পর্কযুক্ত করা হবে।
- আমরা আপনাকে নিশ্চিতকরতে চাই যে, রক্ত ও মলের নমুনা অন্য কোন কাজে ব্যবহার করা হবে না।

ঝুঁকি এবং সুবিধা

গবেষনা থেকে প্রাপ্ত সম্ভাব্য সুবিধাঃ

আপনার শিশু এই গবেষনায় অংশগ্রহনের মাধ্যমে প্রত্যক্ষ ও পরোক্ষভাবে উপকৃত হবে। তার অংশগ্রহন আমাদের শিশু অপুষ্টির সমস্যাকে ভালভাবে বুঝতে ও এর সম্পর্কে জ্ঞান অর্জন করতে সাহায্য করবে। শিশুরা বিনামূল্যে এই বিশেষ ধরনের খাবার গ্রহনের মাধ্যমে উপকৃত হবে। ভবিষ্যতে এই গবেষণার ফলাফল আমাদের বুঝতে সাহায্য করবে, যে কিভাবে পুষ্টিকর উপাদান খাওয়ানোর ফলে বাংলাদেশ ও বিশ্বের অন্যান্য স্থানে শিশুরা উপকৃত হয়। পুষ্টি বিষয়ক আলোচনা আপনাকে আপনার শিশুর প্রাত্যহিক পুষ্টিকর খাদ্য গ্রহন নিশ্চিত করতে সহায়তা করবে। এই গবেষনার লক্ষ্য হচ্ছে, একটি বিশেষ ধরনের খাদ্য (এম ডি সি এফ) পরীক্ষা করা যা বয়সের তুলনায় খর্বাকৃতি ও অতি তীব্র অপুষ্টিতে আক্রাঃত শিশুদের অন্ত্রে বিদ্যমান অনুজীবের অপরিপক্বতা ও তাদের
শারীরিক বৃদ্ধি তরান্বিত করবে

গবেষনা থেকে প্রাপ্ত সম্ভাব্য ঝুঁকিঃ

এই গবেষনায় অংশগ্রহনের কারনে আপনার শিশুর কোন ক্ষতির সম্ভাবনা নেই। সম্ভাব্য ক্ষতিগুলোর মধ্যে খাবার খাওয়ার পরে বমি, পাতলা পায়খানা, শরীরে কোন কোন স্থানে চুলকানি বা চাকা চাকা দাগ হতে পারে। এসব বিষয়ে যথেষ্ট সতর্কতা অবলম্বনের পরেও গবেষনার কারনে এমন ঘটনা ঘটলে আমরা আপনার শিশুকে আই সি ডি ডি আর বি এর ঢাকা হাসপাতালে মানসম্পন্ন সেবা প্রদান করবো।
এই গবেষনায় রক্তের নমুনা সংগ্রহের সময় সামান্য অসস্থি বা ব্যথা অনুভূত হতে পারে। এছাড়াও সুঁই প্রবেশ করানোর ফলে ঐ স্থান নীলচে বর্ণ ধারন করতে পারে বা ফুলে উঠতে পারে। কিছু কিছু ক্ষেত্রে এর ফলে সেখানে সংক্রমন ও হতে পারে ও কদাচিৎ সেখানে রক্ত জমাট বাঁধতে পারে। দক্ষ নমুনা সংগ্রাহকের মাধ্যমে নিরাপদ প্রক্রিয়ায় উক্ত নমুনা সংগ্রহ করা হবে এবং এর ফলে আপনার শিশুর কোন ক্ষতির সম্ভাবনা নেই। সর্বমোট চার মি.লি (প্রায় ১ চা চামচ পরিমাণ)

রক্তের নমুনা সংগ্রহ করা হবে। ২ মি.লি (অর্ধেক চা চামচ পরিমাণ) তালিকভুক্তির সময় এবং ২ মি.লি (অর্ধেক চা চামচ পরিমাণ) গবেষনার শেষে সংগ্রহ করা হবে।

গোপনীয়তা ও বিশ্বস্ততা

আমরা আপনাকে নিশ্চিত করছি যে এই গবেষণার প্রতিটি পর্যায়ে আপনার বাক্তিগত তথ্যের গোপনীয়তা সুনিশ্চিত করব। আমরা সকল রোগ, চিকিৎসা ও পরীক্ষা সম্পর্কিত তথ্য তালা-চাবি দিয়ে গোপনে সুরক্ষিত রাখব। এই গবেষণা সংশ্লিষ্ট গবেষক এবং আইসিডিডিআর,বির নৈতিকতা সমীক্ষা কমিটি (ই আর সি) ছাড়া কেউ এই তথ্য ব্যবহার করতে পারবে না। ওয়াশিংটন বিশ্ববিদ্যালয়র গবেষকদেরও এই তথ্য দেখার অধিকার থাকবে। গবেষণায় প্রাপ্ত জৈব নমুনাসমূহ পরবর্তীতে বিশ্লেষণ এর উদ্দেশ্যে ওয়াশিংটন ইউনিভার্সিটিতে পাঠানো হবে। আপনার শিশুর নাম ও পরিচয় কোনক্রমে এই গবেষণার ফলাফল প্রকাশের সময় গোপন রাখা হবে।

ভবিষ্যতে তথ্যের ব্যবহারঃ

ভবিষ্যতে এই গবেষণা প্রাপ্ত তথ্য ব্যবহারের প্রয়োজন হলে, এর বিশ্বস্ততা ও গোপনীয়তা রক্ষা করা হবে এবং গোপন রাখা হবে। এই গবেষণার ফলাফল আমাদেরকে ভবিষ্যতে আপনার রক্ত ও মলে উপস্থিত পদার্থগুলো নিয়ে আরও অধিকতর গবেষণায় সাহায্য করবে। আপনার এই রক্ত ও মলের নমুনা আই সি ডি ডি আর,বি তে সংরক্ষণ করা হবে। এই সকল নমুনা পুনরায় ব্যবহারের জন্যে আপনার কোন সম্মতি নেয়া হবে না।

গবেষণায় অংশগ্রহণ না করার অধিকারঃ

এই গবেষণায় আপনার অংশগ্রহণ পুরোপুরি আপনার স্বেচ্ছাধীন এবং অংশগ্রহণের বিপরীতে সকল সিদ্ধান্ত নেয়ার অধিকার সম্পূর্ণরূপেই আপনার। এই গবেষণার যে কোন পর্যায়ে আপনি এমনকি কোন কারণ দর্শানো ব্যাতিরেকে আপনার অংশগ্রহণ স্থগিত করতে পারেন। এর ফলে আপনার কোন ক্ষতি হবে না এবং আপনাকে কোন ক্ষতিপূরণও দিতে হবে না।

ক্ষতিপূরণের নিয়মাবলীঃ

এই গবেষনায় অংশগ্রহনের জন্য আপনার কোন খরচ হবে না। আপনাকে এই গবেষনায় ব্যবহৃত সম্পূরক খাদ্যের জন্য বা গবেষনার অন্তর্গত কোন পরীক্ষার জন্য কোন মূল্য দিতে হবে না। এই গবেষনায় অংশগ্রহনের জন্য আপনিও কোন অর্থসাহায্য পাবেন না। গবেষনা সংক্রান্ত অসুস্থতার ক্ষেত্রে আপনার শিশু ঢাকার মহাখালীতে অবস্থিত কলেরা হাসপাতা ল থেকে মানসম্পন্ন সেবা পাবে।

যে কোন প্রশ্নের উত্তর/ যোগাযোগের বিষয়েঃ

আমরা অত্যন্ত আনন্দের সাথে আপনার যে কোন প্রশ্নের উত্তর দিতে এখন অথবা ভবিষ্যতেও সর্বদা প্রস্তুত থাকব। আপনি চাইলে প্রধান গবেষক অথবা তার যে কোন কর্মীর সাথে অথবা নিচের ঠিকানায় যোগাযোগ করতে পারেন। আমরা এই গবেষণা সংক্রান্ত যে কোন তথ্য (স্বাস্থ্যগত/ জৈবরাসায়নিক পরীক্ষা সম্পর্কিত) দিতে সর্বদা প্রস্তুত। কিন্তু আমরা আপনাকে জানাতে চাই যে, কিছু কিছু জৈবরাসায়নিক পরীক্ষার তথ্য পেতে বেশকিছু সময়ের প্রয়োজন হতে পারে কেননা সেগুলো পরীক্ষার জন্যে দেশের বাহিরে পাঠানো হবে। পরবর্তী যোগাযোগের জন্যে- ডাঃ তাহমীদ আহমেদ, সিনিয়র ডিরেক্টর, নিউট্রিশন ও ক্লিনিকাল সার্ভিসেস ডিভিশন, আইসিডিডিআর, বি ৬৮, শহীদ তাজউদ্দীন আহমেদ সরণি, মহাখালী, ঢাকা ১২১২, বাংলাদেশ। ফোনঃ +৮৮-০২-৯৮২৭১০৩ অথবা পিএবিএক্স ৮৮৬০৫২৩-৩২ এক্সটেনশন- ২৩০০।

নৈতিকতা সমীক্ষা কমিটির দায়িত্বপ্রাপ্ত প্রতিনিধির নাম ও যোগাযোগের ঠিকানাঃ জনাব এম এ সালাম খান, ফোনঃ ৯৮৮৬৪৯৮ অথবা পিএবিএক্স ৮৮৬০৫২৩, এক্সটেনশন- ৩২০৬)

আপনি যদি আমাদের এই গবেষণায় অংশগ্রহণ করতে চান, তাহলে নিচের সুনির্দিষ্ট জায়গায় আপনার স্বাক্ষর অথবা টিপসই দিন।

আপনার সাহায্যের জন্যে ধন্যবাদ।

অংশগ্রহনকারীর মা/বাবা/অভিভাবক/সেবাদানকারীর স্বাক্ষর/বাম হাতের বৃদ্ধাঙ্গুলীর ছাপ
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তারিখ

স্বাক্ষীর স্বাক্ষর/বাম হাতের বৃদ্ধাঙ্গুলির ছাপ
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তারিখ

প্রধান গবেষক/তার প্রতিনিধির স্বাক্ষর
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তারিখ
দ্রষ্টব্য: প্রতিনিধি তার পূর্ণ নাম ও পদ লিখে তারপর স্বাক্ষর করবেন ।

Gender Analysis Tools

Gender Analysis Tool: Relation of gender to MAM	Are there sex differences in	How do biological differences between women and men influence their	How do the different roles and activities of men and women affect their	How do gender norms / values affect men and women's	How do access to, and control over resources affect men and women's

Vulnerability: Incidence ** **Prevalence ****			In Bangladesh, more than 2 million children suffer from MAM		
Health seeking behaviour	Since children are dependent on their parents for accessing health care, and we assume that morbidities of a female child is taken less seriously than that of a male child, it can be said that health seeking behaviour differs from that of a female child.	Not applicable as biological difference has no role on this	Does not have a role in health seeking behaviour of a child.	Since children are dependent on their parents for accessing health care, and we assume that morbidities of a female child is taken less seriously than that of a male child, it can be said that health seeking behaviour differs from that of a female child.	In the present context of the country, it can be assumed that, there is less money allocated for treatment of female child. So their health seeking behaviour differs from that of a male child.
Ability to access health services	Not applicable as children are dependent on their parents and unable to access health services	Not applicable as biological difference has no role on this	Not applicable as children are dependent on their parents and unable to access health services		Not applicable
Experience with health services and health providers	No difference reported for treatment of MAM	Not applicable as biological difference has no role on this	In most of the health programs male and female get equal priorities.		No data available
Preventive and Treatment options, responses to treatment or rehabilitation	No such differences have been reported that make female child more vulnerable to adverse health effect of the different methods	No data available to support any difference that occur between male and female due to treatment options.	We assume that after diarrhoea or any acute illness female child are not given adequate nutritious food compared to male. So, it puts females at risk for being MAM		In the present context of the country, less priority is given to a female child therefore less money allocated for treatment, prevention and rehabilitation

Check-List

Check-list for Submission of Research Protocol
For Consideration of the Research Review Committee (RRC)
[Please check all appropriate boxes]

1. Has the proposal been reviewed, discussed and cleared by all listed investigators?

   ☒ Yes ☐ No

   If the response is No, please clarify the reasons:

2. Has the proposal been peer-reviewed externally?

   ☒ Yes ☐ No ☐ External Review Exempted

   If the response is 'No' or "External Review Exempted", please explain the reasons:

   If the response is "Yes", please indicate if all of their comments have been addressed?

   ☒ Yes (please attach)

   ☐ No (please indicate reason(s)):

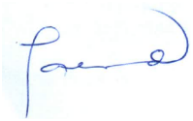
3. Has the budget been reviewed and approved by icddr,b's Finance?

   ☒ Yes ☐ No (reason): _____

4. Has the Ethics Certificate(s) been attached with the Protocol?

   ☐ Yes ☐ No

   If the answer is 'No', please explain the reasons:



_____ 23.09.2019

Signature of the Principal Investigator Date

Microbiota Directed Complementary Food (MDCF) - Study
Food Frequency Questionnaire

PID: ☐☐☐ – ☐☐☐☐

Date: ☐☐/☐☐/☐☐	Staff ID: ☐☐

1	Are you breastfeeding your child? If No, Skip to question 4.	01=Yes 00=No	☐☐
2	Last night, how many times did you breastfeed your child from sunset to sunrise?		☐☐
3	Yesterday, during the day, how many times did you breastfeed your child?		☐☐
4	Do you give your child infant formula? If No, Skip to question 7.	01=Yes 00=No	☐☐
5	Last night, how many times did you feed your child infant formula from sunset to sunrise?		☐☐
6	Yesterday, during the day, how many times did you feed your child infant formula?		☐☐
7	Do you give your child other milks, such as tinned, packed, powdered or fresh animal milk? If No, Skip to question 10.	01=Yes 00=No	☐☐
8	Last night, how many times did you feed your child animal milks from sunset to sunrise?		☐☐
9	Yesterday, during the day, how many times did you feed your child animal milk?		☐☐
10	If your child eating any semi-solid, mashed, or solid foods? If No, Skip to question 15.	01=Yes 00=No	☐☐
10a	If yes, how many times?		☐☐
Yesterday, during the day and last night, did the participant have:			
11	Plain water? If No, Skip to question 12.	01=Yes 00=No	☐☐
11a	If yes, how many times?		☐☐
12	Tea, coffee, or any other warm/hot drinks? If No, Skip to question 13.	01=Yes 00=No	☐☐
12a	If yes, how many times?		☐☐
13	Fruit or vegetable juices (prepared at home)? If No, Skip to question 14.	01=Yes 00=No	☐☐
13a	If yes, how many times?		☐☐
14	Any other liquids, such as sugar water, thin soup or broth, carbonated drinks, commercially packed juices. If No, Skip to question 15.	01=Yes 00=No	☐☐

14a	If yes, how many times?		
Thinking about yesterday, during the day and at night, did the participant have any of the following foods, even if they were in combination with other foods?			
15	Rice, bread, noodles, or other foods made from grains? If No, Skip to question 16.	01=Yes 00=No	
15a	If yes, how many times?		
16	White potatoes or other foods made from roots? If No, Skip to question 17.	01=Yes 00=No	
16a	If yes, how many times?		
17	Carrots or sweet potatoes that are yellow or orange inside? If No, Skip to question 18.	01=Yes 00=No	
17a	If yes, how many times?		
18	Any dark green leafy vegetables such as spinach? If No, Skip to question 19.	01=Yes 00=No	
18a	If yes, how many times?		
19	Foods made with beans, lentils, peas, corn, ground nuts or any other legumes? If No, Skip to question 20.	01=Yes 00=No	
19a	If yes, how many times?		
20	Ripe mangoes, papayas, or other sweet yellow/orange or red fruit? If No, Skip to question 21.	01=Yes 00=No	
20a	If yes, how many times?		
21	Any other fruits or vegetables such as banana, apple, oranges, tomatoes, squash etc.? If No, Skip to question 22.	01=Yes 00=No	
21a	If yes, how many times?		
22	Liver, kidney, or other organ meats? If No, Skip to question 23.	01=Yes 00=No	
22a	If yes, how many times?		
23	Any meat, such as chicken, beef, lamb, goat, ducks (others)? If No, Skip to question 24.	01=Yes 00=No	
23a	If yes, how many times?		
24	Eggs? If No, Skip to question 25.	01=Yes 00=No	
24a	If yes, how many times?		
25	Fresh or dried fish or shellfish? If No, Skip to question 26.	01=Yes 00=No	
25a	If yes, how many times?		
26	Cheese, yogurt, or other dairy products? If No, Skip to question 27.	01=Yes 00=No	
26a	If yes, how many times?		

27	Any sugary foods such as pastries, cakes, or biscuits? If No, Skip to question 28.	01=Yes 00=No	☐☐
27a	If yes, how many times?		☐☐
28	Any commercially available foods? If No, Skip to question 29.	01=Yes 00=No	☐☐
28a	If yes, how many times?		☐☐
29	Any locally produced/vendor foods (such as rice cakes, chanachur, etc.)? If No, Skip to question 30.	01=Yes 00=No	☐☐
29a	If yes, how many times?		☐☐
30	Yesterday, counting meals and snacks, how many times did the participant ate?		☐☐
31	Yesterday during the day and at night, did the participant eat anything else other than the foods that were mentioned right now? If No, Skip to question 54.	01=Yes 00=No	☐☐
31a	If yes, how many times?		☐☐
32	Please name the foods:		
	a) ……		
	b) ……		
	c) ……		
33	Yesterday during food preparation, did oil was mixed with it? If No, Skip to question 34.	01=Yes 00=No	☐☐
33a	If yes, how many spoons?		☐☐
34	How would the responder describe participant's appetite?	01=Poor 02=Fair 03=Good 04=Very good	☐☐
35	Are there any additional comment?	01=Yes 00=No	☐☐
35a	If yes, record comment here:		

Questionnaire

A. Identification :

Sl no.	Questions	Code list	Code
1.	Name of the child	----------	
2.	Mother's/Caregiver's name	----------	
3.	Father's name	----------	

4.	Date of birth of the reference child	Day Month Year	
5.	Sex of the child	0=Boy; 1=Girl	
6.	Birth order of the reference chid in his/her family	----------	

B. Socio-demographic Information:

Sl no.	Questions	Code list	Code
7.	What is the religion of the respondent?	1=Islam, 2=Hindu, 3=Christian, 4=Others (Specify…….)	
8.	Marital status of mother	1=Married, living with husband, 2=Widowed, 3=Separated, 4=Divorced	
9.	Respondent's relationship with child	1=Mother, 2=sister, 3=Grandmother, 4=Aunt, 5=other…….. (Specify)	
10.	Mother's education qualification	1-9=Write down which class passed 10=SSC/Dakhil 11=HSC/Alim 12=Gradudate/Fazil 13=Master/Doctor/Engineer/Lawyer/Kamil 14=Diploma/vocational training 22=Only can write her name 33= Can not write her name	
11.	Father's education	Write down which class passed 10=SSC/Dakhil 11=HSC/Alim 12=Gradudate/Fazil 13=Master/Doctor/Engineer/Lawyer/Kamil 14=Diploma/vocational training 22=Only can write her name 33= Can not write her name	
12.	Who is the household's head?	0=Male, 1=Female	
13.	Household head's occupation according to respondent	1=Agriculture, 2=Fishing, 3=Household based work(weaving, handicraft), 4=Cattle, poultry rearing, 5=Skilled labor, 6=day labour, 7=Service (earn money monthly basis), 8=Small Business (milkman, fruit/vegetable seller), 9=Big Business, 10=Unemployed, 11=Housewife/Househusband, 99=DK	

C. Household characteristics

Sl No	**Questions**	**Answer**	
14.	What is the principal material used for the walls of your household?	1=Wood 2=Concrete, brick, stone 3=Tin	

		4=Bamboo, other natural materials Others _____ (specify)	
15.	What is the principal material used for the floors in your household?	1=Mud or other natural materials 2=Cement 3=Wood Others _____ (specify)	
16.	What is the principal material used for the roof of your household?	1=Bamboo or other natural materials 2=Tin 3=Cement, concrete, tile Others _____ (specify)	
17.	What is the principal type of fuel for cooking used by your household?	1=Gas 2=Kerosene 3=Wood 4=Dung 5=Crop residue Others _____ (specify)	
18.	What is the principal source of drinking water for your household?	1=Piped water in residence 2=Piped water in yard 3=Well 4=Tube well 5=Pond/ ditch/ canal/lake/tank 6=River water 7=Rainwater Others _____ (specify)	
19.	What is main source of water for cooking?	1=Piped water in residence 2=Piped water in yard 3=Well 4=Tube well 5=Pond/ ditch/ canal/lake/tank 6=River water 7=Rainwater Others _____ (specify)	
20.	What is the principal type of toilet used by the household?	1=Open pit 2=Pit 3=Ring slab (water sealed) 4=Ring slab (not water sealed) 5=Sanitary (Water seal and septic tank) 6=Hanging/anywhere/open Others..... ....(specify)	
21.	What does your household members use to wash your hands after defecation?	1=Soap 2=Ash 3=Mud 4=Water from tube-well only water (other sources) only	

		5=Does not wash hands Others _____ (specify)	
22. *	In your household, is/are there Electricity? Radio? Television? Bicycle? Telephone/mobile? Refrigerator/freeze? Almirah(wardrobe)/showcase? Table ? Chair, bench ? Watch, clock ? Cot, bed ? Sewing machine? Motorcycle, scooter ? Vehicle (animal run)? Car/truck/micro-bus? Boat/troller?	Electricity (1=Yes, 0=No) Radio (1=Yes, 0=No) Television (1=Yes, 0=No) Bicycle (1=Yes, 0=No) Telephone/mobile (1=Yes, 0=No) Refrigerator/freeze (1=Yes, 0=No) Almirah/showcase (1=Yes, 0=No) Table (1=Yes, 0=No) Chair, bench (1=Yes, 0=No) Watch, clock (1=Yes, 0=No) Cot, bed (1=Yes, 0=No) Sewing machine (1=Yes, 0=No) Motorcycle, scooter (1=Yes, 0=No) Vehicle (animal run) (1=Yes, 0=No) Car/truck/micro-bus (1=Yes, 0=No) Boat/troller (1=Yes, 0=No)	

*Multiple answer will be considered

APPETITE TEST

Study ID: |__|__|__| Type (code) of MDCF: |__|

Date	Time	Body weight (kg)	Amount offered (gm)	Amount left (gm)	Estimated amount of vomiting (gm)	Amount taken orally excluding vomiting	% consumed	Appetite test*: (pass or fail)

*If a child eats half of the amount offered of MDCF/RUSF by 45 minutes; then the child will be considered as passed the appetite test

Anthropometric measurement:

Sl no.	Questions	Code list	Code
1	Child's weight	----------kg	
2	Length	----------cm	
3	MUAC	----------cm	

Morbidity (Checklist) **Participant ID:** ☐☐☐☐
MORBIDITY

Visit Number: ☐☐ **Date:** ☐☐/☐☐/☐☐

Now I would like to ask about the health of [NAME] in the previous day

Sl no.	Questions	Code list	Code
1.	Fever	0=No. 1=Yes	☐
2.	3 or more bowel movements in 24 hour	0=No. 1=Yes	☐
3.	Watery or soft stool?	0=No. 1=Yes	☐
4.	Mucus or blood in the stool?	0=No. 1=Yes	☐
5.	Stomach pain or cramps?	0=No. 1=Yes	☐
6.	Nausea	0=No. 1=Yes	☐
7.	Vomit	0=No. 1=Yes	☐
8.	Abrasion, scrapes or bruising?	0=No. 1=Yes	☐
9.	Skin itching on the body or scalp?	0=No. 1=Yes	☐
10.	Toothache?	0=No. 1=Yes	☐
11.	Constant cough?	0=No. 1=Yes	☐
12.	Congestion / runny nose?	0=No. 1=Yes	☐
13.	Panting/wheezing / difficulty breathing?	0=No. 1=Yes	☐

Participant ID: ☐☐☐-☐☐☐☐			
A. Blood Collection Form			
Blood collection event (give a tick)		☐ Before intervention	☐ After intervention
#	**Question**	**Code**	**Response**
1	Phlebotomist ID:	☐☐	
2	Date of collection:	DD/MM/YY	☐☐/☐☐/☐☐
3	Time of collection:	(24 Hr Scale; HH:MM)	☐☐:☐☐
4	Last meal (for child):	Breast milk only = 01; Snack only = 02; Meal only = 03; Food and breast milk = 04	☐☐
5	Time since last meal	Within 30 Min = 01; Within 2 Hrs = 02; > 2 Hrs = 03	☐☐
6	Up to 2 mL blood collected?	Yes=01, No=00	☐☐
Observations/Notes:			

Participant ID: ☐☐☐-☐☐☐☐			
A. Stool Collection Form			
If no response for any question, write NA as response.			
	Stool collection event (give a tick)	☐ Before intervention	☐ After intervention
#	**Question**	**Code**	**Response**
01	Field staff ID	##	☐☐

02	Date of collection	DD/MM/YY	□□/□□/□□
03	Time stool specimen was produced	Time (24 Hr Scale; HH:MM)	□□:□□
04	Time stool specimen was picked up by field worker	Time (24 Hr Scale; HH:MM)	□□:□□
05	Time stool specimen was received at field office	Time (24 Hr Scale; HH:MM)	□□:□□
06	Time stool specimen left field office	Time (24 Hr Scale; HH:MM)	□□:□□
B. Stool Receiving and Processing Form			
01	Sample ID	□□□□□□□	
02	If sample is a recollection, what is sample ID of initial stool sample?	□□□□□□□□□□□	
03	Amount of stool received (gm)	##.##	□□:□□
04	Consistency of stool specimen	Watery=01, Liquid=02, Soft=03, Formed=04	□□
05	Was stool specimen bloody?	Yes=01, No=00	□□
06	Did stool specimen have mucus?	Yes=01, No=00	□□
07	Does stool specimen require a QNS setup?	Yes=01, No=00	□□

Summary of Changes-Protocol

Summary of changes:

This trial was designed to follow the participants for one month after the intervention. In order to see the sustainability of the growth-promoting microbiota, we need to follow the participants for a longer duration. Hence, we proposed for an extended period of follow up at month 6, month 12, month 24, month 36, and month 48. During this period, we plan to collect additional stool (2 gm), blood (2 ml) and urine (1.5 ml) samples once at each time points.

We want to see, the long-term effect of MDCF on the growth and morbidity of the participants. In this regard, we need to perform anthropometry and collect morbidity data at month 6, month 12, month 24, month 36, and month 48 of follow up.

2. Original Statistical Analysis Plan. No amendments made.

Statistical Analysis Plan

Aim: To determine the MDCF intervention effects with child rate of weight gain, LAZ, WLZ, WAZ and MUAC compare to RUSF.

Analysis plan: All variables will be presented using descriptive statistics; normally distributed data by the mean and standard deviation (SD) and skewed distributions by the median and interquartile range (IQR). Binary and categorical variables will be presented using counts and percentages.

We will compare baseline characteristics between MDCF and RUSF groups using Student's t tests, Pearson chi-square tests and Mann-Whitney test. The effect of MDCF (IE) on LAZ, WLZ, WAZ and MUAC will be calculated as IE= ((B-A)-(D-C)); where, A=baseline mean value for the MDCF group; B= endline mean value for the MDCF group; C= baseline mean value for the RUSF group; D= endline mean value for the RUSF group.

Generalized estimating equation (GEE) models will be used to adjust within-participant correlation of outcome measures due to repeated measurement. To assess the effect of MDCF we will use difference-in-differences technique which will be $Y_{it} = \beta_0 + \beta_1$ Time $+ \beta_2$ Group $+ \delta$ (Time× Group) $+ \beta_3 X +$ error. Where, Y_{it} = outcome variable of interest for individual i at time t, Time = (1) for endline and (0) for baseline, Group = (1) for MDCF and (0) for RUSF, δ = MDCF effect, X = other covariates (Baseline characteristics etc).