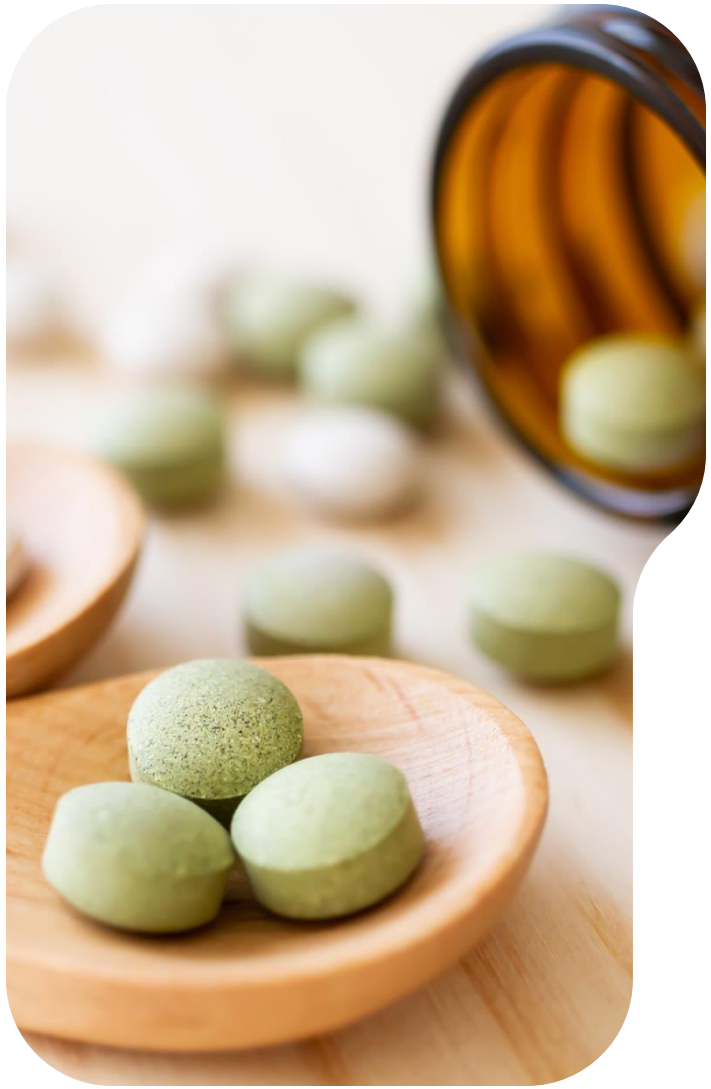
Prebiotic Researchers: How to Design Your Microbiota Clinical Study to Maximize Future Results

Stephanie-Anne Girard, PhD
Director, Scientific Affairs
SGS Nutrasource

GLOBAL PREBIOTIC ASSOCIATION
PROUD MEMBER



Speaker

Stephanie-Anne Girard, PhD
Director, Scientific Affairs
SGS Nutrasource

- 15+ years of natural health product/dietary supplement and pharmaceutical clinical research experience
- Education
  - PhD in Nutritional Sciences from the University of Florida
  - Post–Doctoral degree through an Industrial R&D Fellowship Program from the Natural Sciences and Engineering Research Council of Canada (NSERC) in collaboration with Lallemand Health Solutions.
- Director, Scientific Affairs at SGS Nutrasource
  - Lead the development of high-quality scientific documents, data interpretation, and clinical trial design, working closely with teams across biostatistics, medical writing, and regulatory affairs.
- 15+ published scientific articles in peer-reviewed journals
- WIN
  - Scientific Committee Member
  - Webinar Committee Co-Chair

Agenda

- Microbiota
  - Microbiota vs. Microbiome
  - Impact on Health and Wellbeing
  - Modulation
- Study Design Considerations
  - Understanding the “why?”
  - Prebiotic Claims
  - Responder vs. Non-Responder
  - Screening Tool
  - Primary vs. Secondary Outcomes
  - Placebo and Microbiota
- Sampling
  - Timepoints and Timing
  - Methods of Collection
- Storage
  - Preservation and Conditions
- Analysis
  - Bacteria and Metabolites
- Reporting
  - S.T.O.R.M.S. Checklist

Microbiota Matters

Microbiota vs. Microbiome

- Microbiota

  Emphasizes the **organisms themselves** (bacteria, viruses, fungi, archaea, etc.) — the living microbes present in or on a specific environment or host (e.g., gut, skin, or mouth)

- Microbiome

  Collective **genetic material of the microbiota**, meaning all the genomes of the microorganisms living in a particular environment

  Highlights the **genes and genetic interactions** and their **interactions with the host and the environment**

Microbiota Matters

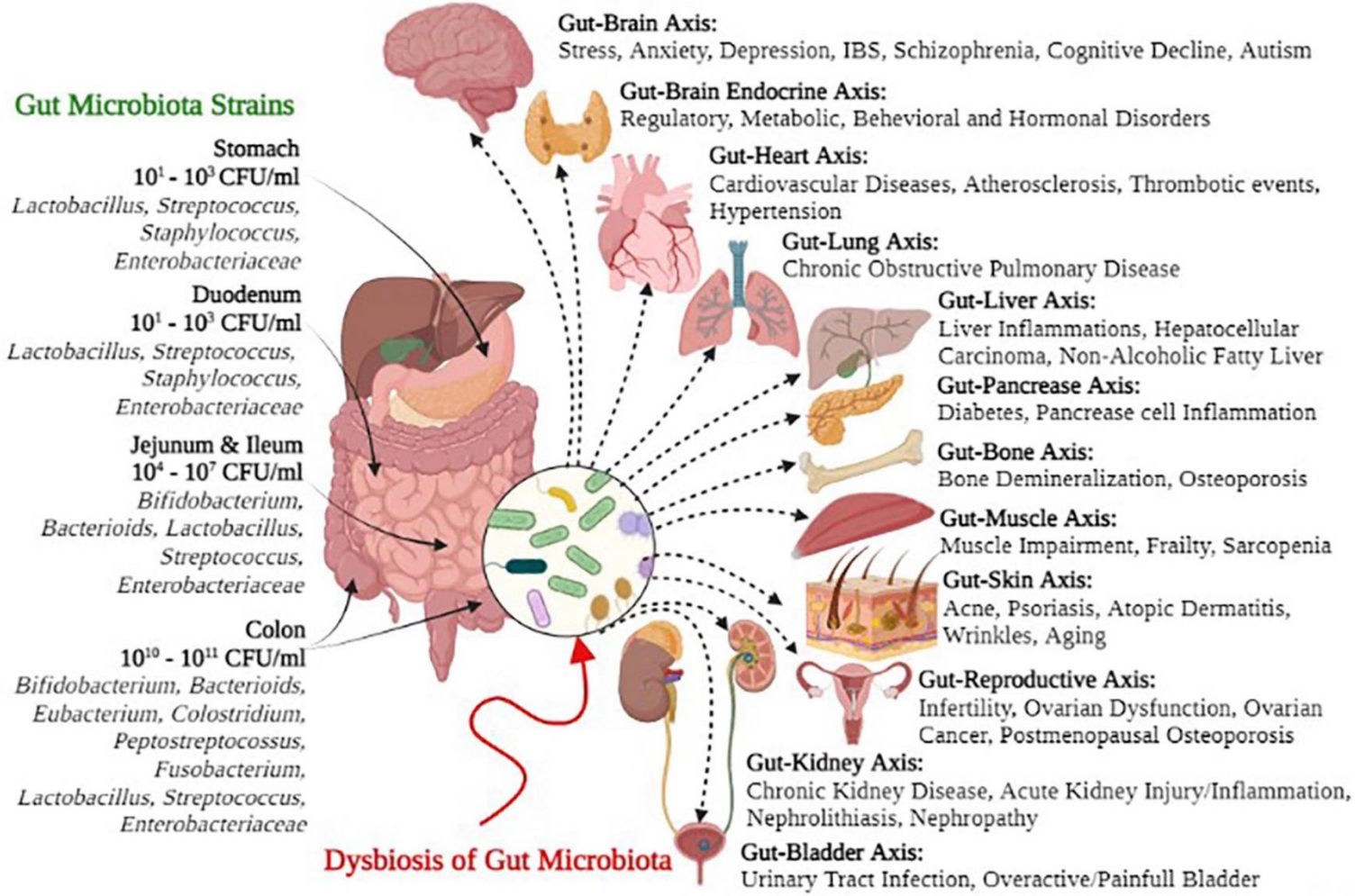
Impact on Health and Wellbeing

Body site	Main taxa in healthy individuals	Main alterations in disease	Associated diseases	References
Vagina	*Lactobacillus crispatus*, *L. iners*, *L. gasseri*, *L. jensenii Streptococcus*, *Bifidobacterium*	↑ *Sneathia*, *Atopobium*, *Gardnerella*	Bacterial vaginosis, Vulvovaginal infections (RVVI), HPV infections and cervical cancer	Felten et al. (1999), Zhou et al. (2007), Di Paola et al. (2017)
	Very low abundance of anaerobes, *Prevotella*, *Atopobium*, Sneathia Gardnerella	↓ Lactobacilli	Symptoms associated with these include discomfort, odor, discharge, infertility, and, if pregnant, could even lead to miscarriages	
Skin	*Staphylococcus*, *Propionibacterium*, *Corynebacterium*, and *Streptococcus*	↑ *S. aureus*, *S. epidermidis*, *P. acnes*, Proteobacteria ↓ *Acinetobacter Cutibacterium*, *Propionibacterium*, *Corynebacterium*, and *Staphylococcus*	Psoriasis, atopic dermatitis, systemic lupus erythematosus and alopecia	Chang et al. (2018), Ho et al. (2019), Paller et al. (2019), Bay et al. (2020), Huang et al. (2020)
Eye	*Staphylococcus*, *Propionibacterium*, and *Pseudomonas*	↑*Delftia* and *Bacteroides* ↓Proteobacteria and Acinetobacter	Keratoconjunctivitis, mucosa-associated lymphoid tissue (MALT) lymphoma, and high glucose levels on the ocular surface due to diabetes	Asao et al. (2019), Li et al. (2019), Suzuki et al. (2020)
Ear	*Corynebacterium*, *Staphylococcus*, and *Propionibacterium*	↑ *Haemophilus*, *Alloiococcus Staphylococcus*, *Turicella*, *Moraxella*, *Streptococcus* and *Stenotrophomonas*	Otitis media infections: Acute Otitis Media (AOM) or Chronic Otitis Media with Effusion (COME)	Lappan et al. (2018), Jervis-Bardy et al. (2019), Kolbe et al. (2019)

Reynoso-García J, Miranda-Santiago AE, Meléndez-Vázquez NM, Acosta-Pagán K, Sánchez-Rosado M, Díaz-Rivera J, Rosado-Quiñones AM, Acevedo-Márquez L, Cruz-Roldán L, Tosado-Rodríguez EL, Figueroa-Gispert MDM, Godoy-Vitorino F. A complete guide to human microbiomes: Body niches, transmission, development, dysbiosis, and restoration. Front Syst Biol. 2022;2:951403. doi: 10.3389/fsysb.2022.951403. Epub 2022 Jul 22. PMID: 38993286; PMCID: PMC11238057.

Microbiota Matters

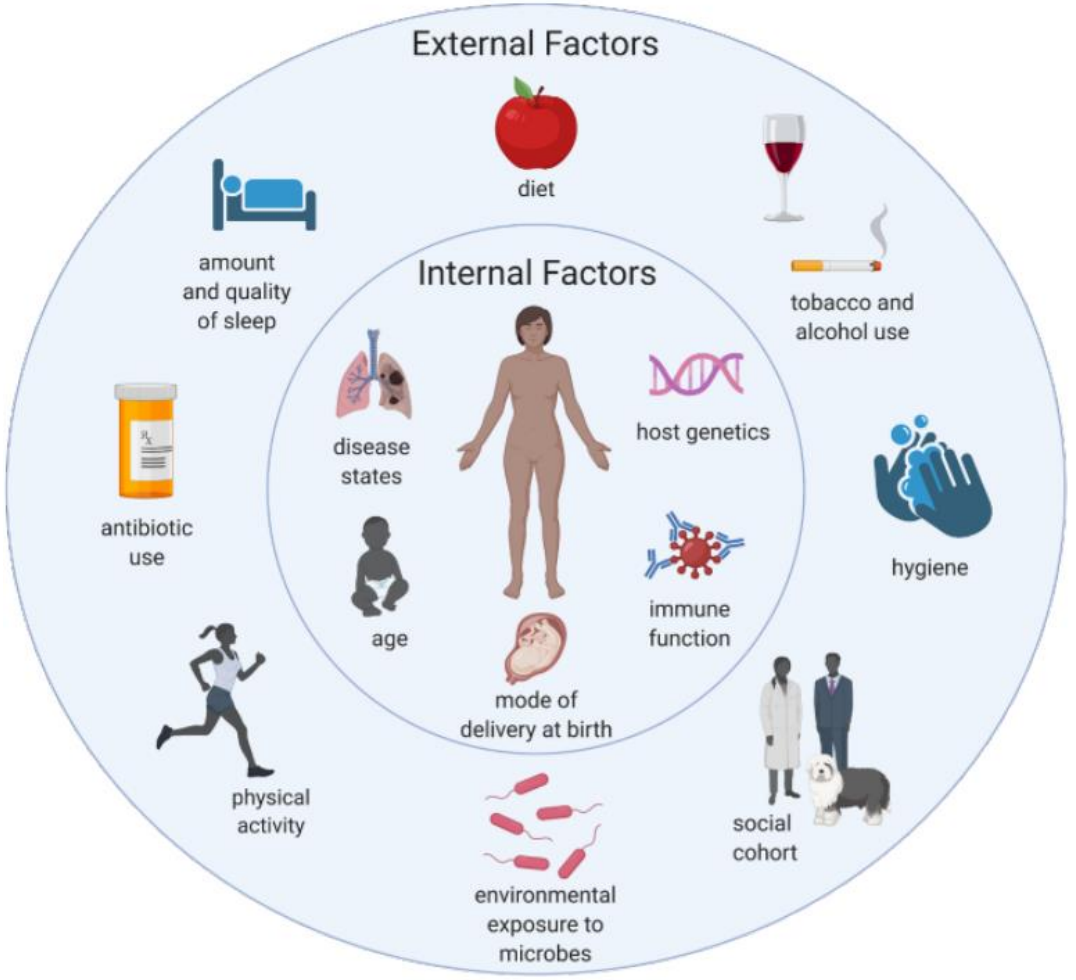
Impact on Health and Wellbeing



Afzaal M, Saeed F, Shah YA, Hussain M, Rabail R, Socol CT, Hassoun A, Pateiro M, Lorenzo JM, Rusu AV, Aadil RM. Human gut microbiota in health and disease: Unveiling the relationship. Front Microbiol. 2022 Sep 26;13:999001. doi: 10.3389/fmicb.2022.999001. PMID: 36225386; PMCID: PMC9549250.

Microbiota Matters

Modulation



Waynick, Geneva & Carmichael, Mary. (2022). The Influence of Infant Formulae on the Growth of Commensal and Pathogenic Streptococcus Species in the Infant Oral Cavity. Fine Focus. 8. 48-72. 10.33043/FF.8.1.48-72.

Study Design Considerations

Study Design Considerations

Understanding the “Why?”

Measure the impact on microbiota by dietary supplement / natural health product

- Understand mechanism of action
- Correlate microbiota changes with health outcomes
- Substantiate Structure/Function Claims (USA and Europe) or Health Claims (Canada)

Study Design Considerations

Prebiotic Claim Examples

Canada

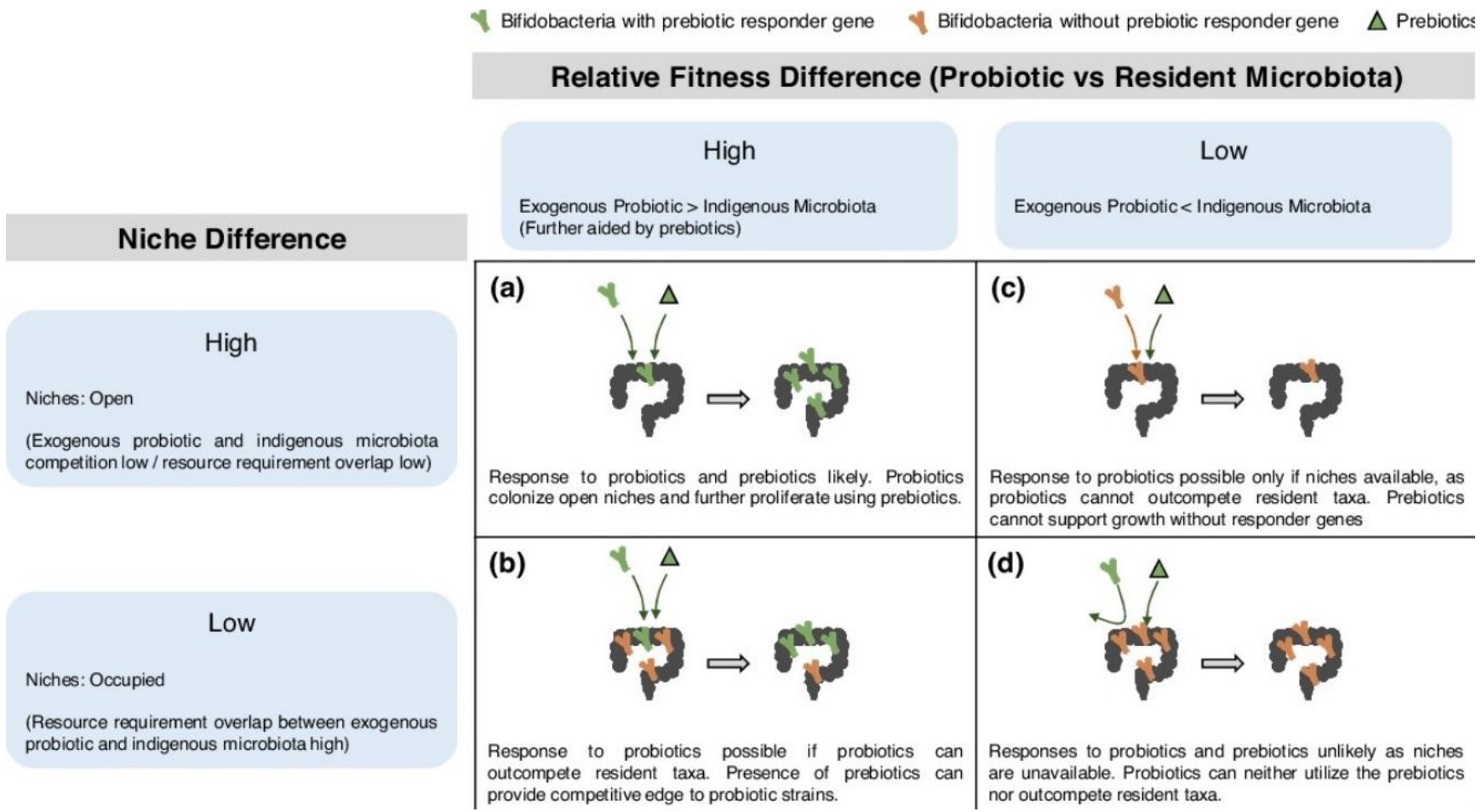
- *“Prebiotic”*
- *“Helps stimulate the growth of healthy bacteria (such as Bifidobacteria) in the intestine/gut”*
- *“(Clinically shown to help) / helps increase levels of healthy gastrointestinal bacteria such as Bifidobacteria”*
- *“Prebiotic / A source of Prebiotic Fibre"*

USA

- *“Prebiotic”*
- *“GI balance/environment support”*
- *“Increase levels of good bacteria in your gut”*

Study Design Considerations

Responders vs. Non-Responders



Ojima MN, Yoshida K, Sakanaka M, Jiang L, Odamaki T, Katayama T. Ecological and molecular perspectives on responders and non-responders to probiotics and prebiotics. Curr Opin Biotechnol. 2022 Feb;73:108-120. doi: 10.1016/j.copbio.2021.06.023. Epub 2021 Aug 7. PMID: 34375845.

Study Design Considerations

Microbiota as a Screening Tool

Variations in gut microbiota of participants can't be avoided

- Baseline microbiota as an inclusion/exclusion criteria (IC/EC) or during stratification
  - Richness
  - Diversity
  - Enterotypes
  - Specific bacterial groups

Baseline characteristics known to impact the gut microbiota as IC/EC

- Age >65 years
- Gender
- BMI
- Health Status
- Drug / Medication
- Diet
- Lifestyle (exercise level, long-distance travel (jet lag)…)
- Environmental Factors (crowding, family size, pets, hygiene practices…)

Swann JR, Rajilic-Stojanovic M, Salonen A, Sakwinska O, Gill C, Meynier A, Fança-Berthon P, Schelkle B, Segata N, Shortt C, Tuohy K, Hasselwander O. Considerations for the design and conduct of human gut microbiota intervention studies relating to foods. Eur J Nutr. 2020 Dec;59(8):3347-3368. doi: 10.1007/s00394-020-02232-1. Epub 2020 Apr 3. PMID: 32246263; PMCID: PMC7669793.

Study Design Considerations

Primary vs. Secondary Outcome

Primary outcome

- When the trial's key focus is altering gut microbiota itself, such as in probiotic, prebiotic, or gut health studies

Secondary/exploratory outcome

- When the trial's primary focus is on broader clinical outcomes, and microbiota is measured to help explain how the intervention works (MoA)

Key challenges in microbiota sample size determination

- Uncertainty in target bacteria
- No clear normal vs. abnormal microbiota
- Biologically relevant changes
- No guidelines from regulatory bodies
- Sample size based on other outcomes

Study Design Considerations

Placebos and Microbiota

Common placebos

- Silica
- Maltodextrin
- Potato Starch
- Hypromellose (HPMC)

Considerations

- Similar physicochemical properties in the dosage form consumed
- Safety at the dose consumed
- Impact on gut microbiota

Study Design Considerations

Placebos and Microbiota

	Silica (silicon dioxide)	Maltodextrin	Potato starch	Hypromellose
Uses	• Commonly used food additive • Current safety data in male adult humans is with an oral dosage up to 9 g/day	• Commonly used food additive	• Commonly consumed	• Commonly used food additive
Dissolution	• Difficult to dissolve in cold water	• Easily dissolved in water	• When dispersed/dissolved water, it appears as a pale white color	• Easily dissolved/dispersed in water

Study Design Considerations

Placebos and Microbiota

	Silica (silicon dioxide)	Maltodextrin	Potato starch	Hypromellose
Microbiota (Animal Studies)	• In a rat study, high doses of amorphous silica nanoparticles (1 g/kg bw/day for 28 days in rats = in human 0.161 g/kg bw/day) led to substantial modifications of the gut microbiota and plasma metabolomics. This dose is relevant to someone of 60 kg bw taking 10 g silica per day.	• Possible effects on epithelial cells and subsequently the intestinal epithelial barrier, which may be related to the pathogenesis of inflammatory bowel disease – shown in an animal study		• Possible effects on gut microbiota, as shown by an in vitro study using a model and an animal study
Microbiota (Human Studies)	• Possible effects on gut microbiota, shown in some studies (1, 2, 3)	• Possible effects on gut microbiota, shown by a systematic review. Specifically, a randomized controlled study using maltodextrin at 10 g per day as the placebo demonstrated decreased alpha diversity in healthy adults and stressed adults	• Resistant potato starch has demonstrated potential prebiotic effects (1, 2). Resistant potato starch dosage levels < 10 g per day showed effects	• Least amount of evidence gut microbiota modulation (no human data demonstrating modulation of gut microbiota)

Sampling, Storage & Analysis

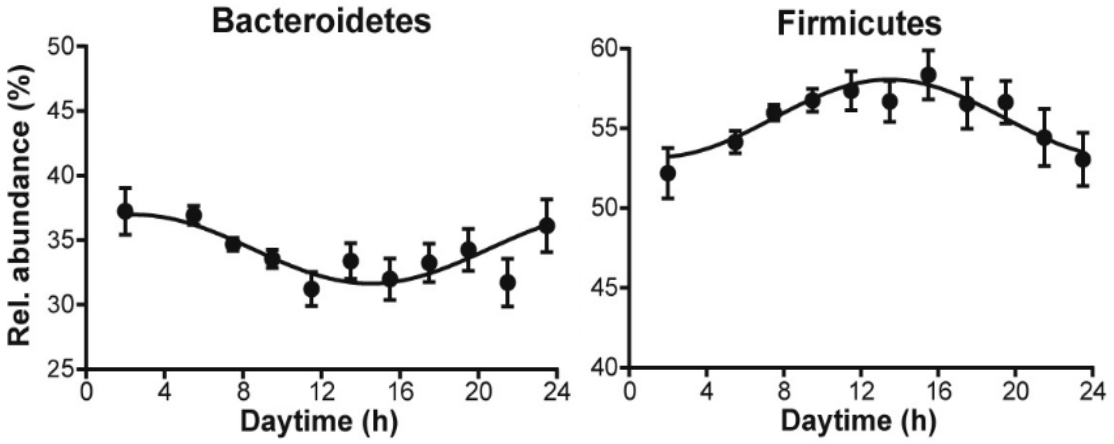
Sampling

Timepoints and Timing

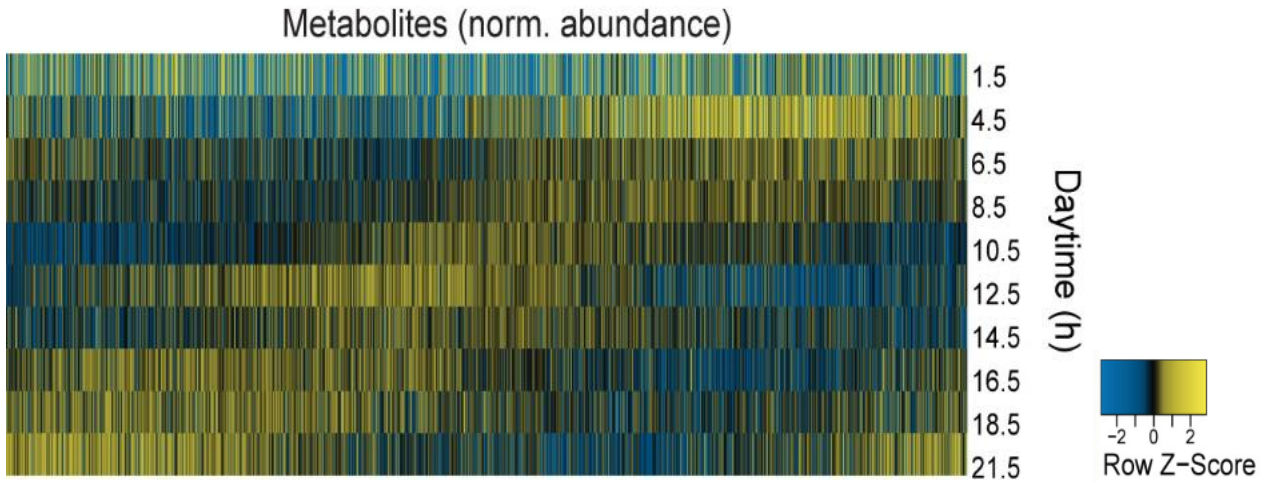
- Collection timepoints

  Multiple samples collected at baseline over multiple days to be more representative of “true baseline”

- Timing in terms of sample collection during the day (morning vs. night)



Reitmeier, S., Kiessling, S., Clavel, T., List, M., Almeida, E. L., Ghosh, T. S., ... & Haller, D. (2020). Arrhythmic gut microbiome signatures predict risk of type 2 diabetes. *Cell Host & Microbe, 28*(2), 258-272.e6. Doi: 10.1016/j.chom.2020.06.004



Heppner, N., Reitmeier, S., Heddes, M., Merino, M. V., Schwartz, L., Dietrich, A., List, M., Gigl, M., Meng, C., van der Veen, D. R., Schirmer, M., Kleigrewe, K., Omer, H., Kiessling, S., & Haller, D. (2024). Diurnal rhythmicity of infant fecal microbiota and metabolites: A randomized controlled interventional trial with infant formula. Cell Host & Microbe, 32(2), 573-587.e5. https://doi.org/10.1016/j.chom.2024.02.015

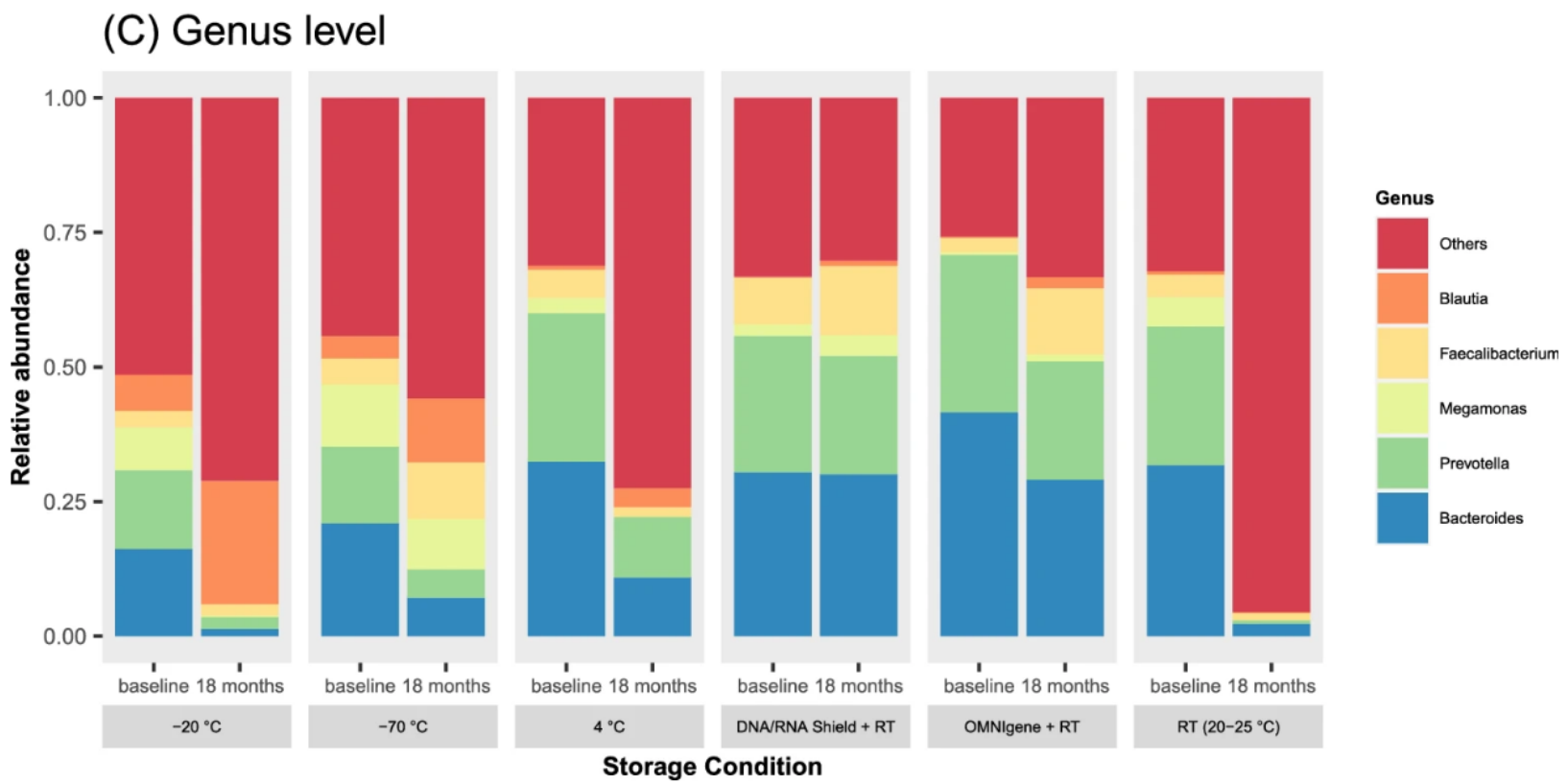
Sampling
Methods of collection

	Fresh Stool Samples	Preserved Stool Sample	Ingestible devices for sampling
			
Measurement	Stool (Bacterial DNA/Proteins/SCFA)	Stools (Bacterial DNA)	Intestinal Fluid
Pros	• Homogenized is more reproducible and representative of whole stool sample • Gut microbiota profiling gold standard	• Convenient • Preserves the DNA sample (stored and shipped at room T°)	• Convenient • Precise and regional sampling of the intestine (pH)
Cons	• Burden for participants • Must be processed, aliquoted & frozen rapidly (-80°C) • Representative of colon lumen content (not mucosal microbiota)	• Only preserves DNA (no proteins) • Uneven distribution of bacteria within feces • Representative of colon lumen content (not mucosal microbiota)	• Relatively expensive • Great technical difficulty • Previous reports of contamination by downstream liquid

Tang Q, Jin G, Wang G, Liu T, Liu X, Wang B, Cao H. Current Sampling Methods for Gut Microbiota: A Call for More Precise Devices. Front Cell Infect Microbiol. 2020 Apr 9;10:151. doi: 10.3389/fcimb.2020.00151. PMID: 32328469; PMCID: PMC7161087.

Storage

Preservation and Storage Conditions



Kim JH, Jeon JY, Im YJ, Ha N, Kim JK, Moon SJ, Kim MG. Long-term taxonomic and functional stability of the gut microbiome from human fecal samples. Sci Rep. 2023 Jan 3;13(1):114. doi: 10.1038/s41598-022-27033-w. PMID: 36596832; PMCID: PMC9810722.

Analysis

Bacteria and Metabolites to Measure

When known

- Measure beneficial bacteria that utilise your substrate to grow/thrive (e.g. linkages of prebiotics)

Table 1 Examples of bacterial, host, outcome markers that have been used in studies

Endpoint/marker	Type	Sample type	Associated health benefit
Change in abundance of lactobacilli	Bacteria	Feces	Compliance for probiotic supplementation, probiotic activity if linked to health outcome
Change in abundance of bifidobacteria (bifidogenic effect)	Bacteria	Feces	Compliance for probiotic supplementation, probiotic activity if linked to health outcome
Effect on gut commensals e.g., *Akkermansia muciniphila*, *Prevotella copri*, *Faecalibacterium prausnitzii*, *Prevotella*-to-*Bacteroides* ratio, Christensenella, *Faecalibacterium* and *Coprococcus*	Bacteria	Feces	Various health outcomes such as metabolic health, gut and immune health, brain health
Alpha-diversity: Microbiota richness and evenness in a specific sample	Bacteria	Feces	
Beta-diversity: Heterogeneity of the microbiota among the analyzed samples	Bacteria	Feces	
Gene richness	Bacteria	Feces	Metabolic health

European Journal of Nutrition (2020) 59:3347–3368

When unknown

- *In vitro* screening tools (human gut modeling systems e.g. SHIME)
- The use of available generic “panels” in clinical trial setting

Analysis

Bacteria and Metabolites to Measure

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Table 1 Examples of bacterial, host, outcome markers that have been used in studies

Endpoint/marker	Type	Sample type	Associated health benefit
Acetate	Metabolite	Urine, blood, feces	Reduced intestinal pH, pathogen exclusion, mineral absorption, appetite regulation, energy source, alters intestinal motility
Butyrate	Metabolite	Urine, blood, feces	Reduced intestinal pH, pathogen exclusion, mineral absorption, enterocyte energy source, stimulates apoptosis, glutathione regulation, modifies tight junction permeability, alters intestinal motility, anti-inflammatory properties in colonocytes, stimulates gut mucin production
Propionate	Metabolite	Urine, blood, feces	Reduced intestinal pH, pathogen exclusion, mineral absorption, appetite regulation, improves insulin sensitivity and glucose tolerance and modifies lipid metabolism, hepatic gluconeogenesis, alters intestinal motility
Succinate	Metabolite	Urine, blood	Inhibits hepatic glucose output to improve glucose and energy metabolism
Imidazole propionate	Metabolite	Blood	Impairs glucose tolerance and insulin signaling by activation of mTORC1
Hydrogen sulfide (H_2S)	Metabolite	Feces	DNA damage, intestinal inflammation
Polyamines (agmatine, tyramine, histamine, cadaverine, putrescine, spermidine, spermine)	Metabolite	Urine, blood, feces	Immunoregulatory effects, oxidative stress, inflammation, genotoxicity
Chenodeoxycholic acid	Metabolite	Urine, blood, feces	Thermogenesis (energy expenditure) in brown adipose tissue
Secondary bile acids	Metabolite	Urine, blood, feces	Cholesterol gallstone formation, colorectal cancer, modifies *C. difficile* infection susceptibility, lipid digestion/absorption, metabolic signaling/regulation
Indole and indoxyl-sulfate	Metabolite	Urine, plasma	Gut microbial proteolysis markers, aryl-hydrocarbon receptor (AHR) ligands,
4-Cresyl sulfate (*p*-cresyl sulfate)	Metabolite	Urine	Gut microbial proteolysis, genotoxicity, impact on microbiota diversity, modulates host phase II drug metabolism
Hippurate	Metabolite	Urine	Inversely correlated with blood pressure and BMI, marker of microbial polyphenol metabolism
Trimethylamine-*N*-oxide (TMAO)	Metabolite	Urine and blood	Associated with CVD, possible marker of renal function

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Reporting

The S.T.O.R.M.S. checklist

S trengthening
T he
O rganization and
R eporting of
M icrobiome
S tudies

Reporting Microbiota Results

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The S.T.O.R.M.S. Checklist

What is it?

- Checklist for reporting on human microbiome studies
- Six sections covering all sections of a scientific publication
- Presented as a table with space for author-provided comments
- Intended for inclusion in supplementary materials

Reporting

S.T.O.R.M.S. Checklist

Who developed it?

Bioinformaticians, epidemiologists, biostatisticians and microbiologists

Why is it needed?

Ensure standardized reporting in microbiome studies
Facilitate peer review
Comparative analysis of research findings
Aid in effective communication

Who should use it?

Researchers in the microbiome field conducting studies

When to use?

During the reporting of microbiome studies

https://www.stormsmicrobiome.org/

Reporting

S.T.O.R.M.S. Checklist

Version:	1.03		
Number	**Item**	**Recommendation**	**Item Source**
Abstract			
1.0	**Structured or Unstructured Abstrac**	Abstract should include information on background, methods, results, ar	STORMS
1.1	**Study Design**	State study design in abstract.	STORMS
1.2	**Sequencing methods**	State the strategy used for metagenomic classification.	STORMS
1.3	**Specimens**	Describe body site(s) studied.	STORMS
Introduction			
2.0	**Background and Rationale**	Summarize the underlying background, scientific evidence, or theory driv	STORMS
2.1	**Hypotheses**	State the pre-specified hypothesis. If the study is exploratory, state any	STORMS
Methods			
3.0	**Study Design**	Describe the study design.	STORMS
3.1	**Participants**	State what the population of interest is, and the method by which particip	STORMS
3.2	Geographic location	State the geographic region(s) where participants were sampled from.	MIxS: geographic
3.3	Relevant Dates	State the start and end dates for recruitment, follow-up, and data collecti	STORMS
3.4	Eligibility criteria	List any criteria for inclusion and exclusion of recruited participants.	Modified STROBE
3.5	Antibiotics Usage	List what is known about antibiotics usage before or during sample colle	STORMS
3.6	Analytic sample size	Explain how the final analytic sample size was calculated, including the	STORMS
3.7	Longitudinal Studies	For longitudinal studies, state how many follow-ups were conducted, des	STORMS
3.8	Matching	For matched studies, give matching criteria.	Modified STROBE
3.9	Ethics	State the name of the institutional review board that approved the study a	STORMS
4.0	**Laboratory methods**	State the laboratory/center where laboratory work was done.	STORMS
4.1	Specimen collection	State the body site(s) sampled from and how specimens were collected.	MIxS: sample coll
4.2	Shipping	Describe how samples were stored and shipped to the laboratory.	STORMS
4.3	Storage	Describe how the laboratory stored samples, including time between coll	STORMS
4.4	DNA extraction	Provide DNA extraction method, including kit and version if relevant.	MIxS: nucleic acid
4.5	Human DNA sequence depletion o	Describe whether human DNA sequence depletion or enrichment of micr	STORMS
4.6	Primer selection	Provide primer selection and DNA amplification methods as well as varia	MIxS: pcr primers
4.7	Positive Controls	Describe any positive controls (mock communities) if used.	STORMS
4.8	Negative Controls	Describe any negative controls if used.	STORMS
4.9	Contaminant mitigation and identifi	Provide any laboratory or computational methods used to control for or id	STORMS
4.10	Replication	Describe any biological or technical replicates included in the sequencin	STORMS
4.11	Sequencing strategy	Major divisions of strategy, such as shotgun or amplicon sequencing.	MIxS: sequencing
4.12	Sequencing methods	State whether experimental quantification was used (QMP/cell count bas	STORMS

Reporting

S.T.O.R.M.S. Checklist

Version:	1.03		
Number	**Item**	**Recommendation**	**Item Source**
4.13	Batch effects	Detail any blocking or randomization used in study design to avoid confo	STORMS
4.14	Metatranscriptomics	Detail whether any mRNA enrichment was performed and whether/how re	STORMS
4.15	Metaproteomics	Detail which protease was used for digestion. Provide details on proteom	STORMS
4.16	Metabolomics	Specify the analytic method used (such as nuclear magnetic resonance	STORMS
5.0	**Data sources/measurement**	For each non-microbiome variable, including the health condition, interver	MIxS: host diseas
6.0	**Research design for causal infere**	Discuss any potential for confounding by variables that may influence bo	STORMS
6.1	Selection bias	Discuss potential for selection or survival bias.	STORMS
7.0	**Bioinformatic and Statistical Meth**	Describe any transformations to quantitative variables used in analyses (	STORMS
7.1	Quality Control	Describe any methods to identify or filter low quality reads or samples.	MIxS: sequence q
7.2	Sequence analysis	Describe any taxonomic, functional profiling, or other sequence analysis	MIxS: feature pred
7.3	Statistical methods	Describe all statistical methods.	Modified STROBE
7.4	Longitudinal analysis	If the study is longitudinal, include a section that explicitly states what a	STORMS
7.5	Subgroup analysis	Describe any methods used to examine subgroups and interactions.	STROBE
7.6	Missing data	Explain how missing data were addressed.	STROBE
7.7	Sensitivity analyses	Describe any sensitivity analyses.	STROBE
7.8	Findings	State criteria used to select findings for reporting.	STORMS
7.9	Software	Cite all software (including read mapping software) and databases (includ	Modified STREGA
8.0	**Reproducible research**	Make a statement about whether and how others can reproduce the repo	STORMS
8.1	Raw data access	State where raw data may be accessed including demultiplexing informa	STORMS
8.2	Processed data access	State where processed data may be accessed.	STORMS
8.3	Participant data access	State where individual participant data such as demographics and other	STORMS
8.4	Source code access	State where code may be accessed.	STORMS
8.5	Full results	Provide full results of all analyses, in computer-readable format, in supple	STORMS
Results			
9.0	**Descriptive data**	Give characteristics of study participants (e.g. dietary, demographic, clin	STROBE
10.0	**Microbiome data**	Report descriptive findings for microbiome analyses with all applicable ou	STORMS
10.1	Taxonomy	Identify taxonomy using standardized taxon classifications that are suffic	STORMS
10.2	Differential abundance	Report results of differential abundance analysis by the variable of interes	STORMS
10.3	Other data types	Report other data analyzed--e.g. metabolic function, functional potential,	STORMS
10.4	Other statistical analysis	Report any statistical data analysis not covered above.	STORMS
Discussion			
11.0	**Key results**	Summarise key results with reference to study objectives	STROBE
12.0	**Interpretation**	Give a cautious overall interpretation of results considering objectives, lin	STROBE
13.0	**Limitations**	Discuss limitations of the study, taking into account sources of potential	STROBE
13.1	Bias	Discuss any potential for bias to influence study findings.	STORMS
13.2	Generalizability	Discuss the generalisability (external validity) of the study results	STROBE
14.0	**Ongoing/future work**	Describe potential future research or ongoing research based on the stud	STORMS

Thank You

For more information, please contact

Stephanie-Anne Girard, PhD
Director, Scientific Affairs
sgirard@nutrasource.ca