

Relating Dietary Health to Accurate Quantification of the Gut Microbiome Using xMAP® Technology from the Molecular Diagnostic Company Genetic Analysis AS

By Dominic Andrada

Problem: The need to accurately measure DNA abundancies in microbiome research

The human gut microbiome has been a major topic of medical research. Bacteria residing in the gastrointestinal tract modulate many bodily functions from nutrient metabolism to immune system regulation.¹ Microbiome researchers generate these insights by comparing the abundance of DNA sequences mapped to different bacterial taxonomic groups between experimental samples. However, these abundance are typically based on changes in the proportions of mapped reads between samples. Conclusions drawn from these measurements can generate systemic biases that make comparing datasets and obtaining reproducibility challenging.² Hence, a means to quantify the actual amount of DNA belonging to a taxonomic group would help researchers examine the gut microbiome in human health.

Method: Identifying microbiome-based markers of human health with xMAP® Technology

To address the problem of accurate DNA quantification in microbiome research, scientists from Oslo Metropolitan University, Norway, utilized the GA-map™ Dysbiosis Test from the molecular diagnostic company Genetic Analysis AS (Oslo, Norway). This test which utilizes a fecal sample from its home collection kit is based on Luminex's xMAP Technology for multiplex molecular testing. The genomic test is composed of 48 single-stranded DNA probes coupled to xMAP MagPlex® Microspheres that can target ≥ 300 bacteria on different taxonomic levels. These sequences are complementary to different 16S rRNA sequences from the V3-V7 regions, providing higher taxonomic mapping resolution of selected bacteria in the intestinal microbiota. Specific 16S rRNA target sequences hybridize to their complementary DNA probe on the microspheres, after sample extraction, fluorescent labelling, and amplification by PCR.

For quantifying DNA belonging to a taxonomic group, prepared samples were read on the Luminex® 200™ instrument. The readout consisted of median fluorescent intensity of the labelled target sequences and the unique signature of the bead to which they were hybridized. Measured fluorescent intensity indicates bacterial abundance, while bead signature delineates the taxonomic group being measured. Measuring fluorescent signal

intensities with xMAP Technology provides researchers with a standardized approach to quantify the abundance of many taxonomic groups within a sample beyond its proportions.

With diverse microbial taxa from Bacteroidetes to Verrucomicrobiota covered by probes in the GA-map® Dysbiosis Test, the researchers could now investigate the relationship between the gut microbiome and dietary markers of health with a cross-sectional study.³

Results: Generating robust correlations with human microbiome data

Using the GA-map® Dysbiosis Test allowed the researchers to identify a series of trends between the gut microbiome and dietary indicators of health. Here are just some of the correlations they observed through Pearson's correlation analyses (**Figure 1**):

- **Streptococcus spp.:** Elevated *Streptococcus* abundance were positively associated with whole-flour bread and trans-fat consumption in healthy participants (Pearson correlation ≥ 0.3) (**Figure 1**).
- **Bacteroides stercoris:** The researchers observed increased *B. stercoris* abundance that were correlated with increased fiber consumption among healthy participants (Pearson correlation ≥ 0.3) (**Figure 1**). This feature aligns with observations that *Bacteroides* can degrade fibers and complex sugars with its array of carbohydrate-active enzymes (CAZymes).⁴
- **Bacilli:** The abundance of bacilli within Firmicutes was also significantly negatively correlated with systolic and diastolic BP values (Pearson correlation ≤ -0.3) (**Figure 2**).
- **Lactobacillus spp.:** Lower abundance of *Lactobacillus* spp. were significantly correlated with higher systolic and diastolic blood pressure ($P < 0.05$) after adjusting for age, sex, and body mass index (BMI) (**Table 1**). This result corroborates previous research suggesting that *Lactobacillus*-based probiotics can improve blood pressure control.⁵
- **Other bacterial taxa:** The researchers also observed the abundance of *Alistipes onderdonkii* and *Akkermansia muciniphila* being correlated with fat levels (%) (**Figure 2**). The latter is surprising given their correlation with reduced body fat after fecal microbiota transplantation.⁶

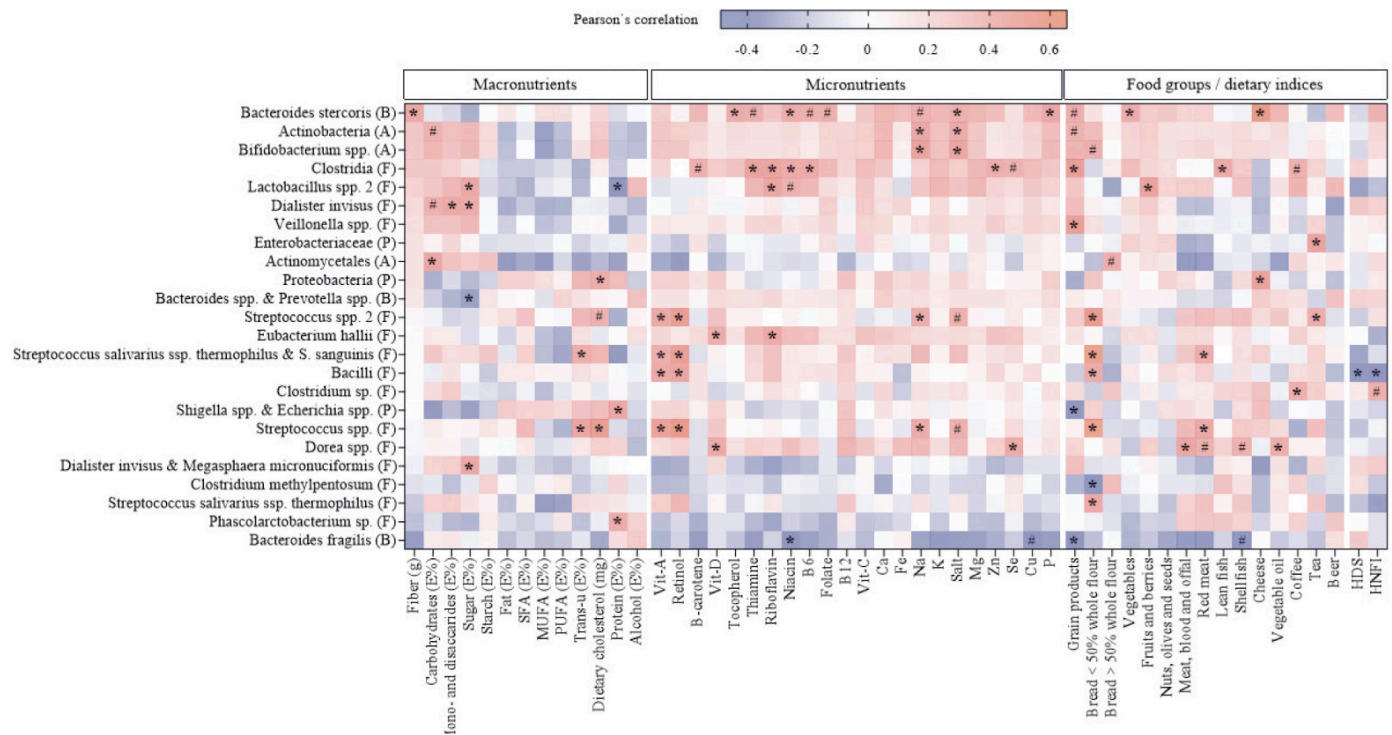


Figure 1. Heat map correlating the abundance of microbial taxa with nutrient and food group intake.³ Significant correlations (Pearson's correlation coefficient ≥ 0.3) are marked with an asterisk. The taxonomic level of each taxon is indicated beside their names in brackets. A, Actinobacteria; B, Bacteroidetes; F, Firmicutes; P, Proteobacteria.

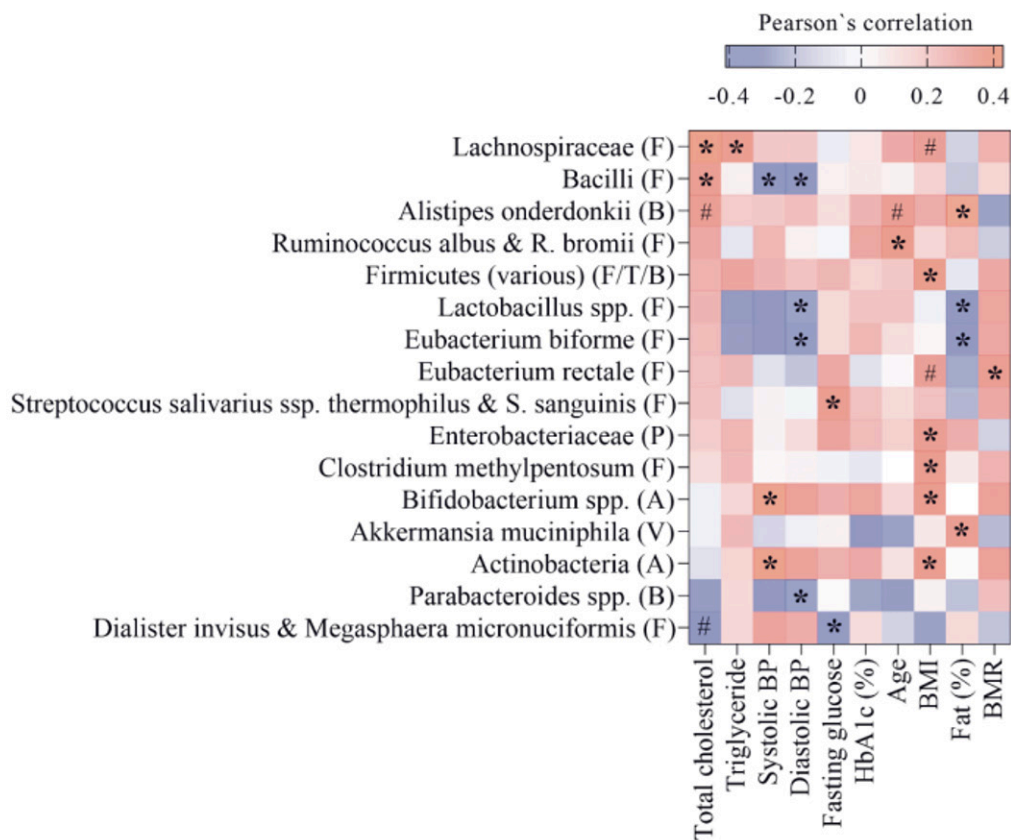


Figure 2. Heat map correlating the abundance of specific gut microbial taxa with metabolic and anthropometric markers.³ Significant correlations (Pearson's correlation coefficient ≥ 0.3) are marked with an asterisk. The taxonomic level of each taxon is indicated beside their names in brackets. A, Actinobacteria; B, Bacteroidetes; F, Firmicutes; P, Proteobacteria; T, Tenericutes; V, Verrucomicrobiota.

Table 1. Linear regression model for correlating gut bacteria abundance with blood pressure and total cholesterol levels.³

Gut bacteria	Unadjusted values		Adjusted values		P [†]	P [‡]
	B	95% CI	B	95% CI		
Systolic BP (≥ 120 mmHg) (n = 24)						
(A) <i>Actinobacteria</i>	0.285	0.014, 2.028	0.238	-0.196, 1.903	0.047	0.108
(A) <i>Bifidobacterium</i>	0.305	0.097, 2.201	0.251	-0.152, 2.045	0.033	0.090
(F) <i>Lactobacillus</i> spp.	-0.229	-2.269, 0.251	-0.309	-2.583, -0.140	0.114	0.030
Diastolic BP (≥ 80 mmHg) (n = 10)						
(B) <i>Bacteroides stercoris</i>	-0.300	-2.570, -0.087	-0.320	-2.718, -0.123	0.036	0.033
(B) <i>Bacteroides</i> spp. and <i>Prevotella</i> spp.	-0.288	-1.259, -0.015	-0.225	-1.163, 0.166	0.045	0.138
(B) <i>Bacteroides</i> spp.	-0.279	-2.348, 0.010	-0.335	-2.635, -0.166	0.052	0.027
(F) <i>Bacilli</i>	-0.350	-1.286, -0.155	-0.387	-1.404, -0.189	0.014	0.011
(F) <i>Dialister invisus</i> and <i>Megasphaera micronuciformis</i>	0.330	0.363, 4.138	0.384	0.644, 4.584	0.020	0.010
(F) <i>Eubacterium bifforme</i>	-0.345	-3.487, -0.389	-0.313	-3.361, -0.157	0.015	0.032
(F) <i>Eubacterium rectale</i>	-0.325	-2.661, -0.209	-0.357	-2.814, -0.343	0.023	0.013
(F) <i>Lactobacillus</i> spp.	-0.378	-3.553, -0.580	-0.348	-3.400, -0.407	0.007	0.014
(F) <i>Streptococcus</i> spp. 2	-0.294	-1.217, -0.029	-0.305	-1.284, -0.007	0.040	0.048
Total cholesterol (≥ 5.0 mmol/L) (n = 20)						
(F) <i>Ruminococcus albus</i> and <i>R. bromii</i>	0.374	0.433, 2.739	0.245	-0.579, 2.659	0.008	0.202

[†]Gut bacteria values were log-transformed before analysis.

[‡]P for unadjusted values assessed by a linear regression model.

#P for values adjusted for age, sex, and BMI, assessed by a linear regression model.

Phyla are indicated within parentheses; A, Actinobacteria; B, Bacteroidetes; F, Firmicutes.

The level of significance was set at $P < 0.05$ and are indicated in bold italic.

Conclusion: Elucidating microbiome-based markers of human health

Together, the GA-map[®] Dysbiosis Test with xMAP Technology provided researchers with the platform to generate quantitative insights into the human gut microbiome. On one hand, the data agreed with the previous literature, strengthening possible connections between gut microbes and health status. However other correlations differed from the literature, providing room for further research to resolve the contrasting results. This standardized, multiplex test provides scientists with strong opportunities to complement existing microbiome research efforts with accurate, reproducible quantification of microbial taxa. Continued efforts with the GA-map[®] Dysbiosis Test will allow scientists to develop confident insights relating aberrant changes to the gut microbiome and its' association with disease.

xMAP Technology for easy quantitative, multiplex genetic analysis

In addition to the GA-map[®] Dysbiosis Test, xMAP Technology is utilized in other customized genetic tests. Various assay formats can be developed by your lab or chosen from ready-made kits from Partner vendors. Our LuminexPLORE Lab* provides technical expertise and assay services, as Luminex is an established leader in reliable and proven proteomic and genomic assays in medical research and clinical diagnostics.

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Change this statement to:

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