GI EcologiX™
Gastrointestinal Health & Microbiome Profile

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INTRODUCTION

Due to recent advancements in culture-independent molecular techniques, it is now possible to measure the composition of the human microbiota. Billions of microorganisms colonise the gastrointestinal tract, which extends from the stomach to the rectum. The presence and activity of these microorganisms is fundamental for the homeostasis of the organism. They play a key role in the development of the immune system, digestion of fibres, production of energy metabolites, vitamins and neurotransmitters and in the defence against pathogen colonisation. The disruption of these microbial communities, defined as dysbiotic profiles, has been associated with several diseases including metabolic syndrome, systemic inflammation, autoimmune and mental health conditions.

Monitoring the gut microbiota is fundamental to obtain a holistic view of host current health and predict future health trajectories. The obtained information can be used to tailor specific interventions and to informatively adjust personal lifestyle choices in order to promote health.

To this end, Phylobioscience have developed the GI EcologiX™ Gastrointestinal Health and Microbiome Profile, a ground-breaking tool for analysis of gastrointestinal microbiota composition and host immune responses. Using innovative microbial culture-independent technologies, including quantitative real-time PCR (qRT-PCR) and enzyme-linked immunosorbent assay (ELISA), the profile provides an accurate, reliable and quantifiable measurement of microbiota abundance and host inflammatory markers.

For microbiota composition analysis, the technology detects:

- Abundance of commensal bacteria (SCFA producers and homeostasis-inducing organisms)
- Abundance of pathobionts (opportunistic organisms that can provoke dysbiosis in immunocompromised subjects)
- Presence of pathogens (bacteria and viruses that can cause disease)
- Presence of commensal and pathogenic parasitic organisms

For host biomarker analysis, the technology detects:

- Beta-defensin 2
- Calprotectin (protein marker of IBD and colorectal cancer)
- Elastase (enzymatic marker of exocrine pancreatic insufficiency)
- SigA (antibody marker of immunity in mucosal surfaces)
- Zonulin (protein marker of intestinal permeability)

Dependent on microbiota composition and host biomarkers, the GI the profile will report three different states:

- Healthy gastrointestinal microbiome (homeostasis of host and microbiome)
- Gastrointestinal dysbiosis detected (imbalance of the GI microbiota and/or host immune response)
- Gastrointestinal pathogens detected (bacteria and amoebae)

GLOSSARY

<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commensal</td>
<td>Microorganism (i.e. bacteria, fungi) that lives in symbiosis with host when residing within its specific environment</td>
<td>1</td>
</tr>
<tr>
<td>Pathogen</td>
<td>Microorganism (e.g. bacteria, fungi, virus) that may cause disease</td>
<td>2</td>
</tr>
<tr>
<td>Pathobiont</td>
<td>Potential pathogen that lives in symbiosis under normal conditions</td>
<td>2</td>
</tr>
<tr>
<td>Homeostasis</td>
<td>Ability to maintain internal stability in an organism despite environmental changes</td>
<td>3</td>
</tr>
<tr>
<td>Dysbiosis</td>
<td>Imbalance or disturbance in the human microbiota</td>
<td>4</td>
</tr>
<tr>
<td>Microbiota</td>
<td>Collective ecosystem of microorganisms that inhabit the human body</td>
<td>5</td>
</tr>
<tr>
<td>Nosocomial</td>
<td>Referred to diseases that are originating in hospitalised patients</td>
<td>6</td>
</tr>
</tbody>
</table>
**IBD**  
Inflammatory Bowel Disease – term used to describe two different pathological conditions: ulcerative colitis (UC) and Crohn’s disease (CD). Both diseases are characterised by high inflammation in different areas of the gastrointestinal tract

**IBS**  
Irritable Bowel Syndrome – conditions characterised by stomach cramps, bloating, diarrhoea and constipation. Exact cause is unknown and there is not a defined cure

**Immunocompromised**  
Individuals with an impaired immune system, usually due to systemic disease, transplants or surgeries. These subjects are more prone to develop infections

**BACKGROUND**

**Gastrointestinal Microbiota**

The human body is densely colonised by microorganisms, with an estimated 1:1 ratio of human to bacterial cells. From the skin to the gastrointestinal (GI) tract, almost all external and internal surfaces harbour specific communities of bacteria, viruses, archaea and unicellular organisms, collectively referred to as ‘microbiota’. The gastrointestinal microbiota is the most rich and diverse, consisting of more than 2,000 unique species of bacteria classified into 12 different phyla. Nevertheless, the majority of these bacteria (93.5%) belong to only four phyla: Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria. Species composition and abundance change and increase throughout the GI tract, reaching $10^{12}$ CFU/g of lumen content in the colon. The composition of this last portion of GI tract is the one usually investigated through the analysis of the faecal content. Bacteria in the colon, and in the remaining tracts of the intestine, play a key role in executing fundamental processes for the human organism. An equilibrated interaction between these bacteria and the host promotes systemic health, a balance defined as ‘homeostasis’.

**Homeostasis**

The mutualistic relationship established throughout evolution between the human host and the microorganisms led to the modern concept of humans as ‘super-organisms’. The collection of all bacterial genomes – microbiome – encodes fundamental functionalities that are not present in the human genome. Bacteria are able to digest complex polysaccharides and to produce beneficial metabolites for the host, like the short chain fatty acids (SCFAs), vitamins and neurotransmitters. The most produced SCFAs are acetate, propionate and butyrate in a 3:1:1 ratio. These molecules are absorbed by the epithelial cells of the intestine and they have a fundamental role in human metabolic pathways, gene expression, cell proliferation, apoptosis and immune system regulation. For example, butyrate is the main energy source for the colonic enterocytes and can induce apoptosis in colorectal cancer cells via its histone deacetylases (HDAC) inhibitory activity. Additionally, it can reduce the production of proinflammatory cytokines through the inhibition of the NF-$\kappa$B pathway. Impaired butyrate metabolism has been associated with several diseases, including inflammatory bowel diseases (IBD) and other systemic disorders. Perturbed balance in the composition and function of the gut microbiota – defined as dysbiosis – has been associated to numerous diseases, including systemic inflammation, cancer, autoimmunity, atopic diseases, autism and depression.

**Dysbiosis**

The complex microbial communities of our organism are in a dynamic balance, constantly influenced by external factors such as the environment, diet, physical activity and use of pharmaceutical drugs such as antibiotics. Losing key bacterial species or having an overgrowth of others can disrupt this balance, influencing host physiology and leading to the development of disease.

**Atopic Diseases**

The early-life composition of the gut microbiota has a strong influence on the development of the immune system. C-section infants and infants who received formula milk instated of breastmilk have lower abundance of Bifidobacteria and Lactobacilli. This deficit has been associated with the development of atopic diseases, such as asthma and eczema, later in life. Additionally, the administration of probiotic strains belonging to both Bifidobacteria and Lactobacilli appear to reduce the incidence of the onset of such diseases and ameliorate symptoms of those affected.
Obesity and Related Disorders

Diet is a critical factor in shaping the composition of the gut microbiota. Diets high in fat reduce the biodiversity of the gut microbiota and increase the ratio of Firmicutes to Bacteroidetes\(^{20}\). Obesity and a sedentary lifestyle increase the risk of metabolic syndrome, characterised by high blood pressure, insulin resistance and fat accumulation. These subjects have less Bifidobacteria, Desulfovibrio, Akkermansia muciniphila and increased Staphylococcus, Enterobacteriaceae and Escherichia coli\(^{21}\). Aggravated conditions lead to the development of severe conditions like type II diabetes (T2D), cardiovascular diseases (CVD) and non-alcoholic fatty liver disease (NAFLD). In all of these disease states there is a reduction in SCFA-producing bacteria, such as Akkermansia muciniphila and Faecalibacterium prausnitzii. When the diet is enriched with fibres (prebiotics) and SCFA-producing bacteria (probiotics), research shows the conditions improve\(^{21}\).

**IBD, IBS and Colorectal Cancer**

Inflammatory bowel diseases (IBD) affect around 620,000 people in the UK\(^{22}\) and 3.1 million people in the USA. Its incidence it is rising worldwide and is increasingly affecting younger age groups\(^{22}\). IBD is characterised by chronic inflammation of the gastrointestinal mucosa. It is caused by genetic predisposition and environmental factors, which have an impact on gut microbiota composition\(^{23}\). Dysbiotic profiles associated with IBD usually have decreased abundance of *F. prausnitzii* and increased presence of Enterobacteriaceae, including *E. coli*. Other bacteria possibly involved with the onset of IBD are *Mycobacterium avium*, *Fusobacterium nucleatum* and *Ruminococcus gnavus* but viruses and fungi (*S. cerevisiae* and *C. albicans*) are also believed to play a role in the development of the disease\(^{23}\).

Irritable bowel syndrome (IBS) is characterised by an altered host immune function (abnormal cytokine secretion) and intestinal physiology (abnormal gut motility, increased permeability and mucin secretion), often followed with psychiatric comorbidity and stress\(^{22}\). Changes in the gut microbiota often involves increased Firmicutes and decreased Bacteroides. Additionally, in constipated patients, a higher abundance of *Methanobrevibacter smithii* has been found\(^{21}\).

Colorectal cancer (CRC) is the third most common cause of cancer mortality in the world. Diets rich in fibre reduces the risk of CRC development, while red meat, fat and alcohol consumption are associated with increased incidence of CRC. Fibrous diets increase the abundance of SCFA producers and beneficial bacteria like Roseburia, Eubacterium, Bifidobacterium and Prevotella. Pathogens known to promote CRC are enterotoxigenic *B. fragilis*, adherent-invasive *E. coli*, Fusobacterium spp. and Campylobacter spp.\(^{21}\).

**Neurological Disorders and Autism**

Animal studies have elucidated a central role of the gut microbiota in influencing and promoting the development of the central nervous system (CNS). Bacteria can synthesize several neurotransmitters such as dopamine and GABA, which interact with the enteric nervous system and signal to the CNS. The administration of Bifidobacteria and Lactobacilli has been shown to reduce anxiety and depression, while pathogens such as Citrobacter and Campylobacter, activate stress circuits\(^{27}\).

Dysbiotic gut microbiota profiles have been associated to neurodegenerative diseases like Alzheimer diseases (AD), Parkinson’s diseases (PD) and amyotrophic lateral sclerosis (ALS). In most cases, subjects have higher abundance of pro-inflammatory bacterial species\(^{24}\).

Autism spectrum disorder (ASD) is a neurodevelopmental disorder which is manifested through impaired social communication and repetitive behaviour, often with associated immune dysregulation and gastrointestinal disorders. Dysbiotic gut microbial profiles have been associated with ASD. Researchers have found higher abundance of Clostridia, Lactobacilli and Desulfovibrio species and decreased abundance of Bifidobacteria, Prevotella, Coprococcus and Veillonella species in subjects affected with ASD\(^{29}\).

**Nosocomial Infections**

The gut microbiota is composed by several pathobiont species. These microorganisms are asymptomatic in normal conditions and they participate in maintaining the homeostasis of the organism. Nevertheless, when disequilibrium in the microbiota composition, or in the regulation of the immune system is introduced through external factors, they can cause the onset of several diseases. Usually this happens in immunocompromised subjects, who have had surgery.
or that are infected with HIV. Infections can involve the respiratory system, gastrointestinal tract or they can cause systemic inflammation and sepsis when entering the blood circulation.

**Pathogenic Infections**

Several pathogens can colonise the gastrointestinal tract, causing gastroenteritis. Salmonella and Shigella are common examples of food poisoning while Adenovirus and Rotavirus cause the typical gastrointestinal flu. Unicellular parasites like Giardia and Cryptosporidium can also colonise the gut, causing diarrhoea. As well as the acute discomfort, these pathogens may be triggers for chronic conditions such as post-infective Irritable Bowel Syndrome (IBS) and extra-intestinal manifestations.

**METHODOLOGY**

The Phylo Bioscience methodology is comprised of two key culture-independent techniques: quantitative real-time PCR (qRT-PCR), using Taqman technology, and enzyme-linked immunosorbent assays (ELISA). These techniques are used to quantify abundance of gastrointestinal microbiota species and host immune biomarkers. The selected microbiota species and host biomarkers are based on clinically relevant research.

**METHODOLOGY FLOWCHART**

```
Faecal sample
  DNA extraction
       ↓
    qRT-PCR
  Supernatant preparation
       ↓
ELISA
       ↓
Microbiota composition and abundance
       ↓
Host immune response profile
```

**BIOMARKERS**

**HEALTH MARKERS**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Type</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Beta-defensin 2</td>
<td>Peptide</td>
<td>N/A</td>
</tr>
<tr>
<td>Calprotectin</td>
<td>Protein</td>
<td>N/A</td>
</tr>
<tr>
<td>Pancreatic Elastase</td>
<td>Enzyme</td>
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</tr>
<tr>
<td>SlgA</td>
<td>Antibody</td>
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</tr>
<tr>
<td>Zonulin</td>
<td>Protein</td>
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</tr>
</tbody>
</table>

**COMMENSAL BACTERIA**

Invivo Healthcare, The New Warehouse, Libby’s Drive, Stroud, GL5 1RN
Please call us on 0333 241 2997, or visit us at invivohealthcare.com
<table>
<thead>
<tr>
<th>Bacteria Species</th>
<th>Type</th>
<th>Gram Status</th>
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<tbody>
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<td>Akkermansia muciniphila</td>
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<tr>
<td>Anaerostipes c caccae</td>
<td>Bacteria</td>
<td>Gram-variable</td>
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<tr>
<td>Bacteroides spp.</td>
<td>Bacteria</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>Bifidobacterium spp.</td>
<td>Bacteria</td>
<td>Gram-positive</td>
</tr>
<tr>
<td>Clostridium spp.</td>
<td>Bacteria</td>
<td>Gram-positive</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Bacteria</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>Eubacterium rectale</td>
<td>Bacteria</td>
<td>Gram-positive</td>
</tr>
<tr>
<td>Faecalibacterium prausnitzii</td>
<td>Bacteria</td>
<td>Gram-positive</td>
</tr>
<tr>
<td>Lactobacillus spp.</td>
<td>Bacteria</td>
<td>Gram-positive</td>
</tr>
<tr>
<td>Roseburia homini</td>
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<td>Gram-variable</td>
</tr>
<tr>
<td>Ruminococcus bromi</td>
<td>Bacteria</td>
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</tr>
<tr>
<td>Subdoligranulum variabile</td>
<td>Bacteria</td>
<td>Gram-positive</td>
</tr>
<tr>
<td>Bacteroides dorei</td>
<td>Bacteria</td>
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</tr>
<tr>
<td>Bacteroides fragilis</td>
<td>Bacteria</td>
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</tr>
<tr>
<td>Bacteroides fragilis (Enterotoxigenic)</td>
<td>Bacteria</td>
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</tr>
<tr>
<td>Bacteroides ovatus</td>
<td>Bacteria</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>Bacteroides thetaiotaomicron</td>
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</tr>
<tr>
<td>Bacteroides uniformis</td>
<td>Bacteria</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>Bacteroides vulgatus</td>
<td>Bacteria</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>Clostridium difficile</td>
<td>Bacteria</td>
<td>Gram-positive</td>
</tr>
<tr>
<td>Clostridium difficile TcdA-producing strains</td>
<td>Bacteria</td>
<td>Gram-positive</td>
</tr>
<tr>
<td>Clostridium difficile TcdB-producing strains</td>
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</tr>
<tr>
<td>Clostridium perfringens</td>
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<td>Gram-positive</td>
</tr>
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<td>Clostridium sporogenes</td>
<td>Bacteria</td>
<td>Gram-positive</td>
</tr>
<tr>
<td>Bilophila Wadsworthia</td>
<td>Bacteria</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>Bacteria</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>Citrobacter koseri</td>
<td>Bacteria</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>Citrobacter spp.</td>
<td>Bacteria</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>Desulfovibrio spp.</td>
<td>Bacteria</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>Bacteria</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>Bacteria</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>Fusobacterium nucleatum</td>
<td>Bacteria</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>Hafnia alvei</td>
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</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>Bacteria</td>
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</tr>
<tr>
<td>Klebsiella pneumoniae</td>
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</tr>
<tr>
<td>Morganella Morgani</td>
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<td>Gram-negative</td>
</tr>
<tr>
<td>Kluyvera ascorbata</td>
<td>Bacteria</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>Oxalobacter formigenes</td>
<td>Bacteria</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>Prevotella copri</td>
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</tr>
<tr>
<td>Proteus Mirabilis</td>
<td>Bacteria</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Bacteria</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>Veillonella spp.</td>
<td>Bacteria</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>Bacteria</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>Bacteria</td>
<td>Gram-negative</td>
</tr>
<tr>
<td><strong>BACTEROIDES SUB-GROUP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CLOSTRIDIUM SUB-GROUP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>GRAM NEGATIVE BACTERIA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>GRAM POSITIVE BACTERIA</strong></td>
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### MYCOLOGY

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Trichocomaceae</th>
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<tbody>
<tr>
<td>Aspergillus fumigatus</td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>Saccharomycetes</td>
</tr>
<tr>
<td>Candida krusei</td>
<td>Saccharomycetes</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>Saccharomycetes</td>
</tr>
<tr>
<td>Malassezia restricta</td>
<td>Basidiomycota</td>
</tr>
</tbody>
</table>

### HELICOBACTER PYLORI

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Gram-negative</th>
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</thead>
<tbody>
<tr>
<td>H. pylori qPCR</td>
<td></td>
</tr>
<tr>
<td>H. pylori Antigen (as confirmatory reflex)</td>
<td></td>
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</table>

### PARASITOLOGY

<table>
<thead>
<tr>
<th>Protista</th>
<th>Heterokonta</th>
</tr>
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<tbody>
<tr>
<td>Blastocystis hominis</td>
<td></td>
</tr>
<tr>
<td>Dientamoeba fragilis</td>
<td>Metamonada</td>
</tr>
<tr>
<td>Entamoeba histolytica</td>
<td>Amoebozoa</td>
</tr>
<tr>
<td>Giardia</td>
<td>Metamonada</td>
</tr>
</tbody>
</table>

### DATA INTERPRETATION Quality, Reproducibility & Standardisation

GI Ecologix have a two-step quality and control process that underlies the methodology: standardisation of the samples to a particular concentration of extracted DNA, and the use of an endogenous control to normalise the data.

**Standardisation**

Stool samples vary significantly in terms of their composition – the human DNA content, microbial DNA content, fibre, water, etc. Therefore, for accuracy and reproducibility, Phylo extract a set amount of total DNA from the sample to analyse, as opposed to using a particular weight of stool. This removes many variables, limiting the risk of two samples from the same individual giving rise to different results – because of the varied components of the stool – and, therefore, allowing better comparisons between samples after time and/or treatment.

**Relative Abundance**

The data is represented as a relative abundance of the target microbes, compared to the ‘general’ microbial population available in the extracted DNA. We do this by using an endogenous control – the conserved portion of DNA for all microorganisms – to measure your ‘general microbial load within the stool sample. This is your baseline – a representation of the general population of your gut microbiome in the given sample. This is the most precise way of using DNA to establish microbial gene abundance, as is used throughout research. We then target specific markers using TaqMan probes to understand the levels of gene copy number per target microbe, which we can present relative to the total microbial abundance.
Because we use relative quantification, you will not see ‘healthy’ reference ranges with which to compare your sample to. There is no need for external reference ranges, as the results are high or low as compared to your individual total microbial load.

In line with microbiome research, the results of GI EcologiX are to be viewed in light of the context of your patient and the patterns of microbial expression. This is what we refer to as ‘Pattern and Context Recognition’.

**Pattern & Context**

Microorganisms are in constant flux. They have a dynamic relationship with each other and with the host, largely mediated by the host’s immune function, diet, lifestyle and hormones.

The host markers help us to understand the functioning of the host – the immune function – including inflammation – at the gut wall, digestive capacity, and potential loss of integrity of the epithelial lining of the gut. If these markers are presenting as out of balance, then we must view the microbial markers in light of that, as well as the context – the host markers, symptoms, and medical history – of the patient.

**HOST BIOMARKERS**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Range</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-defensin 2</td>
<td>&gt;62ng/g</td>
<td>&gt;62ng/g</td>
</tr>
</tbody>
</table>

Beta-defensins are anti-microbial peptides that play a large role in the innate immune response to potential pathogenic microorganisms. Beta-defensins display antibiotic activity toward gram-positive and gram-negative bacteria and enveloped viruses and fungi.

Human Beta-defensin 2 is an inducible peptide produced by intestinal cells. They are antibiotic and have abilities to recruit dendritic and T-Cells to the site of microbial invasion.

It is highly elevated in cases of irritable bowel syndrome (IBS), and in cases of those with active inflammatory bowel disease (IBD), but normal in healthy controls. It is helpful at showing a raised inflammatory response even in light of a lack of macroscopic inflammation.

Beta-defensin 2 has been shown to be consistently higher in cases of ulcerative colitis in compared to Crohn’s disease (even though still both over the normal level), hinting to different pathophysiology in the differing IBD presentations.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Cut off:</th>
<th>IBD or CRC:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calprotectin</td>
<td>50 µg/g</td>
<td>&gt;175 µg/g</td>
</tr>
</tbody>
</table>

*Calprotectin* is a protein expressed mainly by myeloid cells. It recruits monocytes to the site of inflammation and is thus believed to be a mediator of chronic inflammation. It is used as a clinical marker for IBD.

Concentrations <50 µg/g suggest presence of IBS while concentration >150 µg/g are indicative of IBD or possible presence of colorectal cancer. Values 50-150 µg/g may derive from infections or the use of nonsteroidal anti-inflammatory drugs.

The NICE guidelines recommend retesting two weeks after an initial raised result of <100 µg/g, whilst avoiding NSAID and aspirin use. If there is a sustained high on retest of 100-250 µg/g, it is recommended to refer to a gastroenterologist appointment, or if there is a sustained high of greater than 250 µg/g it may trigger immediate colonoscopy investigation.

Calprotectin levels vary for age, with children and elderly people having a higher ‘normal’ range. Reference ranges for under 50 year olds are valid for ages between 4-49.
**Age**  |  **Normal values, µg/g**
---|---
1–6 months  | <538
7 months to 3 years  | <214
3–4 years  | <75
4–49 years  | <50
50–70 years  | 24.7% To supress – higher than 65
Above 70 years  | No data; probably higher than 100

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**Elastase** is a proteolytic enzyme excreted by the pancreas. Faecal concentrations reflect the secretory capacity of the pancreas since it does not breakdown in the intestinal tract. Faecal elastase is used to detect exocrine pancreatic insufficiency which co-occur with several gastro- and non-gastro- intestinal diseases such as: coeliac disease, cystic fibrosis, HIV, IBS, IBD, pancreatitis, alcohol-related liver disease and diabetes mellitus. Faecal elastase concentration is also used to predict survival rates in patients with pancreatic cancer.

There are good correlations between faecal elastase levels and pancreatic enzyme output including amylase, trypsin and lipase. When elastase PE-1 is low, the best symptom to check for are a floating, difficult to flush stool, and weight loss. A single low reading should be repeated before further investigations are made.

**Secretory IgA**

| Range: 0 – 2000 µg/g |
---|---|
High >750 µg/g

Faecal secretory immunoglobulin A (sIgA) is the most abundant secreted antibody and it has a fundamental role in preventing pathogen colonisation in the gastrointestinal tract. Low levels of S IgA (<100 µg/g) correlate with increased susceptibility to GIT infection. Subjects affected by asthma, autoimmune disease, coeliac disease, food allergies and IBD have low levels of sIgA. On the other hand extreme levels of sIgA may indicate presence of acute gut infections, or chronic infections due to, for example, the presence of CMV, EBV or HIV.

**Zonulin**

| Range: 0 – 500 µg/g | High >100 µg/g |
---|---|

Zonulin, also known as prehaptoglobin-2, is a human protein that causes increased intestinal permeability through tight junction disassembly. An increased intestinal permeability status is known to increase inflammation and has been associated to several diseases. Increased levels of zonulin have been specifically associated to coeliac disease, type I and II diabetes, non-alcoholic fatty liver disease, hyperlipidaemia, obesity, autism, autoimmune disease, nervous system diseases and certain cancers.

**MICROBIOTA**

| Akkermansia muciniphila | COMMENSAL BACTERIA  | Negatively associated with metabolic disorders |
---|---|---|

Akkermansia muciniphila is gram-negative, strictly anaerobic bacterium. It is commonly found in the human gut and it is specialised in mucin degradation. It is hard to culture, so can only really be measured with qPCR techniques. Different human clinical studies reported a negative association between A. muciniphila and metabolic disorders. Obese subjects tend to have lower abundance of this bacterium, which increases after weight loss and bariatric surgery.
A. muciniphila is also decreased in pre-diabetic and type II diabetic patients and may be involved in the modulation of glucose homestasis.\textsuperscript{71,72} 16s RNA sequencing of patients with multiple sclerosis showed an increase of \textit{Methanobrevibacter} and \textit{Akermansia} species in untreated individuals\textsuperscript{73}. Low levels of \textit{Akermansia} are associated with decreased production of short chain acids (SCFAs), which in turn can be associated with increased intestinal permeability\textsuperscript{74}.

<table>
<thead>
<tr>
<th>Anaerostipes caccae</th>
<th>COMMENSAL BACTERIA</th>
<th>Butyrate producer</th>
</tr>
</thead>
</table>

\textit{Anaerostipes caccae} is a gram-variable, saccharolytic bacterium. It can utilise lactate and the end product of its metabolism is butyrate\textsuperscript{76,77}. A recent study described a trophic interaction between \textit{A. muciniphila} and \textit{A. caccae}, highlighting increased mucin degradation activity by \textit{A. muciniphila} resulting in a higher butyrate production by \textit{A. caccae}\textsuperscript{80}. Butyrate has been associated with several beneficial effects for the host's physiology, as previously described.

<table>
<thead>
<tr>
<th>Bacteroides spp.</th>
<th>COMMENSAL BACTERIA</th>
<th>Acetate producer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroides dorei</td>
<td>COMMENSAL BACTERIA</td>
<td>Inversely linked with CVD</td>
</tr>
<tr>
<td>Bacteroides fragilis</td>
<td>PATHOBIONT</td>
<td>Extraintestinal pathogen</td>
</tr>
<tr>
<td>Bacteroides fragilis (Enterotoxigenic)</td>
<td>OPPORTUNISTIC PATHOGEN</td>
<td>Associated with increased intestinal permeability</td>
</tr>
<tr>
<td>Bacteroides ovatus</td>
<td>COMMENSAL BACTERIA</td>
<td>Symbiotrophic effect with \textit{B. vulgatus}</td>
</tr>
<tr>
<td>Bacteroides thetaiotaomicron</td>
<td>COMMENSAL BACTERIA</td>
<td>Mucin degrading bacteria</td>
</tr>
<tr>
<td>Bacteroides uniformis</td>
<td>COMMENSAL</td>
<td></td>
</tr>
<tr>
<td>Bacteroides vulgatus</td>
<td>COMMENSAL</td>
<td>Inversely linked with CVD</td>
</tr>
</tbody>
</table>

\textit{Bacteroides} is a genus of gram-negative, obligate anaerobic and saccharolytic bacteria, which mainly produce succinate and acetate SCFAs. It is one of the most abundant genera in the gut of western people making up around 25% of the gut microbiome\textsuperscript{85}. \textit{Bacteroides} strains directly modulate gut function. \textit{Bacteroides} play an important role in the maturation of the peyers patches of the immune system.

Family members of \textit{Bacteroides} possess the capacity to process and utilize complex dietary glycans, such as the mixed-linkage β-glucans that are abundant in cereal grains such as oats and barley\textsuperscript{82,83}.

\textit{Bacteroides fragilis} is well known as an extra-intestinal pathogen, which can be antibiotic resistant and cause inflammation or infection through the production of metalloclopeptase or enterotoxins\textsuperscript{86}. Nevertheless, several strains belonging to \textit{B. fragilis} are currently under investigation as potential next generation probiotics. Preliminary data shows reduced Helicobacter infections, relieved antibiotic-associated diarrhoea, decreased diseases and autism spectrum disorders\textsuperscript{87}. \textit{Bacteroides dorei} abundance in paediatric microbiomes is correlated with a higher occurrence of type 1 juvenile diabetes\textsuperscript{88}. \textit{Bacteroides} species are thought to come from the mother during birthing, but breastfed babies have lower levels, with more of an abundance of \textit{Bifidobacterium}, until they reach the age of weaning. \textit{Bacteroides vulgatus} and \textit{B. dorei} have been shown to be in lower abundance in patients with cardiovascular arterial disease\textsuperscript{89}. Diets higher in fibre have been shown to increase the prevalence of \textit{Bacteroides acidifaciens}, which in turn has been linked with lower heart disease risk factors\textsuperscript{90}.

\textit{Enterotoxigenic Bacteroides fragilis (ETBF)} is an opportunistic pathogen normally found in the GIT and it is associated with inflammatory diarrhoeal diseases\textsuperscript{91,92}. The toxin destroys the zonula adherens tight junctions in the intestinal epithelium, resulting in barrier leakage and diarrhoea. ETBF is also associated with IBD, colorectal cancer, and colitis\textsuperscript{91,92}. \textit{Bacteroides thetaiotaomicron} lives near the mucus lining of the intestine and can bind and metabolize mucin glycans as an energy source. It appears to possess the ability to increase fucosylation in the mucus producing cells of the epithelium, thereby having some control in mucous production to help maintain its niche in the GIT.

\textit{B. thetaiotaomicron} and \textit{Bacteroides caccae} both have the ability to switch between carbohydrate metabolism and mucin metabolism as a fuel source, and depriving the bacteria of polysaccharide sources results in a thinner mucin layer, which in turn has been shown to result in increased infection rate\textsuperscript{84}. Increased serum titres of agglutinins to anaerobic intestinal bacteria, especially \textit{Bacteroides vulgatus}, and \textit{Bacteroides ovatus} has been found in IBD patients.
**Bacteroides ovatus** had been shown to elicit the largest IgG and IgA responses in Crohn's patients of the commensal bacteria. Why *Bacteroides ovatus* induces such as response is not totally known, but it does produce esterase and lipase which can be potentially hazardous to the intestinal tissue, especially if initial damage has been created by a bacterial toxin such as *Enterotoxigenic Bacteroides fragilis* (ETBF). *Bacteroides ovatus* plays an important role of degrading inulin, which the metabolites of in turn feeds *Bacteroides vulgatus* and supports the colony, so they have a syntrophic relationship.

<table>
<thead>
<tr>
<th>Bifidobacterium spp.</th>
<th>Lactate and acetate producer</th>
</tr>
</thead>
</table>

*Bifidobacterium* is a genus of gram-positive, anaerobic bacteria. Species of this genus are highly abundant in infants, especially in breastfed ones. Reduced levels of Bifidobacteria, with a consequent depletion of acetate production, in early life have been correlated to the insurgence of atopic diseases later in life, such as asthma and eczema.

Several strains are now used as probiotics in order to prevent the development of these diseases and ameliorate symptoms. For example, they have been used to treat or prevent colorectal cancer, treat antibiotic-associated diarrhoea, decrease incidence of necrotising enterocolitis, reduce symptoms of IBD, improve colon regularity and decrease pathogen colonisation in the gut.

<table>
<thead>
<tr>
<th>Clostridium spp.</th>
<th>COMMENSAL BACTERIA</th>
<th>Butyrate producer</th>
</tr>
</thead>
</table>

*Clostridium* is a genus of gram-positive bacteria. Species belonging to this genus make up to 10-40% of the total bacteria in the gut microbiota and are involved in the homeostasis of the organism. Colonising the host since early-life, they have a fundamental role in immune system priming. They are also one of the main producers of butyrate, which is an essential fuel for colonocytes. They also produce norepinephrine and dopamine, making them relevant bacteria in the gut-brain axis interaction. The *Clostridium* cluster XIVa and IV make up a substantial part (10-40%) of the total bacteria in the gut microbiota. There are pathogenic *Clostridium* species which can cause botulism and tetanus.

<table>
<thead>
<tr>
<th><strong>Clostridium sub-group</strong></th>
<th><strong>PATHOGEN</strong></th>
<th><strong>Diarrhoea and colitis</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Clostridium difficile</td>
<td>PATHOGEN</td>
<td>Diarrhoea and colitis</td>
</tr>
<tr>
<td>Clostridium diff TcdA-producing strains</td>
<td>PATHOGEN</td>
<td>Diarrhoea and colitis</td>
</tr>
<tr>
<td>Clostridium diff TcdB-producing strains</td>
<td>PATHOGEN</td>
<td>Diarrhoea and colitis</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>PATHOGEN</td>
<td>Food poisoning, autoimmune</td>
</tr>
<tr>
<td>Clostridium sporenges</td>
<td>Commensal</td>
<td>Produces IPA from tryptophan</td>
</tr>
</tbody>
</table>

*Clostridium difficile* is a gram-positive, toxin-producing, pathogenic bacterium usually associated with nosocomial infections in developed countries. In the past decade, *Clostridium difficile* became one of the most well-known pathogens due to its increased incidence in developed countries and its antibiotic resistance.

Colonisation can be asymptomatic, but the secreted toxins (toxin A and toxin B) cause colitis and acute diarrhoea. Antibiotic treatments are often ineffective in curing chronic infection; however, recently faecal microbiota transplant (FMT) has shown promise. *Clostridium cluster* includes some pathogenic species, such as *Clostridium Perfringens* and *Clostridium Tetani*. *C. perfringens* is found regularly in soil, decaying matter and in the human intestine. It is commonly found in high amounts on raw meat and poultry. Some strains of *C. perfringens* produce a toxin known as clostridium perfringens enterotoxin (CPE) which can cause food poisoning when found in high amounts, with symptoms that include abdominal cramping, diarrhoea, vomiting, and fever. A strain of *C. perfringens* can produce Epsilon toxin (Etx), which can cross the blood brain barrier and bind to myelin sheath, and it is thought to play a role in the aetiology of multiple sclerosis (MS) and other autoimmune diseases.

*C. sporogenes* uses dietary tryptophan to produce indolepropionic acid (IPA). IPA is a metabolite produced exclusively by the microbiota and has been shown that it can fortify the intestinal barrier by directly engaging the pregnant X
Escherichia coli is a gram-negative bacterium. Ubiquitous in nature, it colonises the human intestine during the first days of life. In its commensal role, it does not cause disease and it can mainly be found in the mucus layer of the colon.

Little is known about the role of commensal Escherichia coli, its ability to metabolize glucose probably gives selective advantage to other species. It is a reservoir of antibiotic resistance and it can acquire virulent factors which lead to the production of enterotoxins, causing different diseases (see enterotoxigenic Escherichia coli).

Faecalibacterium prausnitzii is a gram-positive, strictly anaerobic bacterium, which is one of the major producers of butyrate in the GIT. The main end products of its metabolism are formate and butyrate.

Decreased abundance of Faecalibacterium prausnitzii is usually observed in Crohn’s disease, but not ulcerative colitis patients. This bacterium is also negatively correlated to the presence of IBS and the development of colorectal cancer. Other diseases that show a concomitant lowered concentration of Faecalibacterium prausnitzii are Parkinson’s disease, T2D and hypovitaminosis D.

Lactobacillus spp. is a genus of gram-positive, facultative anaerobes. Species belonging to this genus normally colonise the mouth, gastrointestinal- and vaginal- tract of humans. They are also known as lactic acid bacteria since the main product of their metabolism is lactate and acetate.

Lactobacillus reduce pathogen colonisation in the GI tract by lowering the pH and by producing antimicrobial compounds like reuterin. Species belonging to this genus are mostly considered beneficial for the organism, helping maintain homeostasis, and are considered probiotics. They help to reduce inflammation through immunomodulation; act on the nervous system and on gut permeability. They are also protective from early-life disorders such as autoimmune diseases, allergies and obesity.

Roseburia hominis is a gram-negative or gram-variable, anaerobic bacterium. Being a butyrate-producing bacterium, the derived anti-inflammatory properties have been inversely associated to the active status of Crohn’s disease. Decreased abundance of Roseburia hominis has been also associated to IBS and colorectal carcinogenesis. Roseburia negatively correlates with plasma glucose in T2D patients, suggesting a possible role in glucose homeostasis.

Lower levels of Roseburia have been detected in subjects affected by Parkinson’s diseases and gallstones.

Ruminococcus bromii is a keystone species, playing a large role in the digestion of resistant starches. It has been proposed that the primary role played by Ruminococcus bromii is to release energy from resistant starch to other members of the

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microbial community, giving it an important role for maintaining microbial community balance\textsuperscript{97}. \(R.\) gnavus can efficiently cross-feed on starch degradation products released by \(R.\) bromii, even though it is normally a mucin degrading bacterium.

### Subdoligranulum variabile

\textit{Subdoligranulum variabile} is a gram-negative, strictly anaerobic bacterium\textsuperscript{100}. It produces butyrate through the butyrate kinase route instead of the butyryl-CoA:acetate CoA-transferase route commonly used by other species commonly present in the human gut (e.g. \(Eubacterium, Roseburia, Faecalibacterium\))\textsuperscript{101}.

#### Gram-negative bacteria

<table>
<thead>
<tr>
<th><strong>Bilophila Wadsworthia</strong></th>
<th>PATHOBIONT</th>
<th>Positively correlates with metabolic impairment</th>
</tr>
</thead>
</table>

\textit{Bilophila Wadsworthia} is a gram negative, anaerobic, sulfidogenic bacterium resistant to \(\beta\)-lactam antibiotics\textsuperscript{102}. This pathobiont is commonly found in patients with appendicitis and it has been associated to the Western diet (high in fats and animal proteins), as well as severe malnutrition\textsuperscript{103}-\textsuperscript{104}.

A recent study in animals showed that a high fat diet stimulates the growth of \(B.\) Wadsworthia, which causes inflammation, dysfunction in the intestinal barrier and bile acid metabolism, hepatic steatosis and dysfunctional glucose metabolism\textsuperscript{105}. Interestingly, the co-administration of a probiotic strain (\textit{Lactobacillus rhamnosus}) reduces the generated inflammation and limits the metabolic impairment.

<table>
<thead>
<tr>
<th><strong>Citrobacter freundii</strong></th>
<th>PATHOBIONT</th>
<th>Infections in immunocompromised</th>
</tr>
</thead>
</table>

\textit{Citrobacter freundii} is a facultative anaerobe, gram-negative bacterium\textsuperscript{106}. It is involved on the onset of several infections of the intestine, liver, biliary and urinary tract, respiratory tract and brain\textsuperscript{107}-\textsuperscript{109}.

Immunocompromised subjects are vulnerable to this bacterium, with a reported 6.8% mortality rate among infected hospitalised patients. The rate increases to 17.8-56% in case of \textit{Citrobacter bacteraemia}\textsuperscript{110}. Recently, multidrug-resistant strains of Citrobacter have emerged\textsuperscript{111}.

<table>
<thead>
<tr>
<th><strong>Citrobacter koseri</strong></th>
<th>PATHOBIONT</th>
<th>Infections in immunocompromised</th>
</tr>
</thead>
</table>

\textit{Citrobacter koseri} is a gram-negative, non-lactose fermenting bacterium. It is mainly associated with infant meningitis and fatal cerebral abscess in premature neonates\textsuperscript{112}.

In adults, it commonly causes infection of the urinary tract\textsuperscript{113}. As with \textit{C. freundii}, it is reported to cause liver failure and infectious aneurysms in immunocompromised subjects\textsuperscript{114,115}.

<table>
<thead>
<tr>
<th><strong>Desulfovibrio spp.</strong></th>
<th>COMMENSAL BACTERIA</th>
<th>Sulphate production</th>
</tr>
</thead>
</table>

\textit{Desulfovibrio} is a genus of gram-negative sulphate-reducing bacteria. This genus has been positively correlated to IBD, colorectal cancer, ulcerative colitis, liver disease and autism\textsuperscript{116-118}.

<table>
<thead>
<tr>
<th><strong>Enterobacter aerogenes</strong></th>
<th>PATHOBIONT</th>
<th>Nosocomial infections</th>
</tr>
</thead>
</table>

\textit{Enterobacter aerogenes} is a facultative anaerobe, gram-negative bacterium. Little is known about the pathogenicity of \textit{Enterobacter} spp. but they are commonly involved in nosocomial infections in intensive care patients\textsuperscript{119}. \(E.\) aerogenes is highly resistant to antibiotic treatments\textsuperscript{120}.

<table>
<thead>
<tr>
<th><strong>Enterobacter cloacae</strong></th>
<th>PATHOBIONT</th>
<th>Nosocomial infections</th>
</tr>
</thead>
</table>

\textit{Enterobacter cloacae} is a gram-negative, multidrug-resistant bacterium\textsuperscript{121}. \(E.\) cloacae is often isolated from patients with a clinical infection, especially when immunocompromised.

It has been associated to peritonitis, sepsis, pneumonia and urinary tract infection\textsuperscript{122}.
**Fusobacterium nucleatum** is a gram-negative bacterium that is found in the human oral cavity and can play a role in the progression of periodontal disease. High levels of *F. nucleatum* have been found gastrointestinal diseases including colorectal cancer (human adenomas), inflammatory bowel disease (IBD) and appendicitis153,154.

Colorectal tumours which contain *F. nucleatum* at time of surgery have been linked with increased mortality and poorer outcomes155. *F. nucleatum* has been implicated in cardiovascular diseases (CVD), rheumatoid arthritis and Alzheimer’s disease, but with the main source of LPS intrusion being the oral cavity156.

<table>
<thead>
<tr>
<th>Hafnia alvei</th>
<th>PATHOBIONT</th>
<th>Associated with CFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella oxytoca</td>
<td>PATHOBIONT</td>
<td>Nosocomial infections</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>PATHOBIONT</td>
<td>Nosocomial infections</td>
</tr>
<tr>
<td>Morganella Morganii</td>
<td>PATHOBIONT</td>
<td>Nosocomial infections</td>
</tr>
<tr>
<td>Oxalobacter formigenes</td>
<td>COMMENSAL BACTERIA</td>
<td>Oxalate homeostasis</td>
</tr>
<tr>
<td>Prevotella spp.</td>
<td>COMMENSAL BACTERIA</td>
<td>Predominant in fibre-diet, involved in inflammation</td>
</tr>
</tbody>
</table>

**Fusobacterium nucleatum** has been observed in subject arthritis, metabolic disorders, low-grade systemic inflammation, bone diseases and HIV176-178. Reduced presence of bacteria belonging to this genus has been observed in subjects affected by Parkinson’s diseases and autism179,180.

**Hafnia alvei** plays an active role in fermented foods, such as cheeses, kimchi and other traditional fermented dishes. Increased serum IgA and IgM have been found in chronic fatigue patients with increased intestinal permeability to Hafnia alvei, Pseudomonas aeruginosa, Morganella morgani, Proteus mirabilis, Pseudomonas putida, Citrobacter koseri, and Klebsiella pneumoniae157. *Hafnia alvei* has the potential to decarboxylate histidine to histamine.

**Klebsiella oxytoca** is a gram-negative bacterium belonging to the Enterobacteriaceae family. As for the Enterobacter genus, this bacterium is mainly related to nosocomial infections158. It usually causes colitis and sepsis and it is antibiotic resistant159. A recent study in animals correlated *K. oxytoca* to cancer cachexia by its capacity to alter gut barrier function160.

**Klebsiella pneumoniae** is a facultative anaerobe, gram-negative bacterium161. Similar to *K. oxytoca*, it is an antibiotic resistant strain usually causing extra-intestinal nosocomial infections like liver, urinary infections and, above all, pneumonia162. Klebsiella pneumoniae has been linked with development of Ankylosing spondylitis163. High alcohol producing strains of *K. pneumoniae* have been linked with increased occurrence of fatty liver disease (non-alcoholic steatohepatitis, or NASH)164. *Klebsiella pneumoniae* has the potential to decarboxylate histidine to histamine.

**Morganella Morganii** belongs to the Enterobacteriaceae family and - as with the other described bacteria of this family - it is an opportunistic pathogen involved in nosocomial infection. Occurrence rate of this bacterium in post-surgery infection is not as high as *K. pneumoniae*, *C. koseri* and *P. mirabilis*153. Nevertheless, mortality rate by its infections is high due to the increased resistance to antibiotic treatment and virulence157. *Morganella Morganii* has the potential to decarboxylate histidine to histamine.

**Oxalobacter formigenes** is an obligate anaerobe and it uses oxalic acid as its sole carbon source165. Accumulation of oxalate leads to the formation of kidney stones, leading to chronic kidney disease, and promotes arthralgias and breast cancer166-167. Its use as a probiotic did not have any significant results, probably due to the used models or small study sizes, but its association to reducing oxalate concentration is widely recognised168.

**Prevotella** is a genus of gram-negative bacteria belonging to the Bacteroidetes phylum172. Subjects consuming a fibre-rich diet have a higher abundance of *Prevotella* than Bacteroides, suggesting a negative correlation between the two genera154,173. Increased abundance of *Prevotella* has been associated with periodontitis, bacterial vaginosis, rheumatoid arthritis, metabolic disorders, low-grade systemic inflammation, bone diseases and HIV176-178. Reduced presence of bacteria belonging to this genus has been observed in subjects affected by Parkinson’s diseases and autism179,180.
Prevotella copri, alongside Bacteroides vulgatus are identified as the main species driving the association between biosynthesis of branched chain amino acids (BCAAs) and insulin resistance. Conversely, research links P. copri to improved insulin utilisation, as long as the diet is high in fibre, which suggests a diet-dependent symbiosis/dysbiosis interaction. P. copri is found in a higher abundance and diversity of clades in non-westernised diets.

<table>
<thead>
<tr>
<th>Pathobiont</th>
<th>Nosocomial infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteus mirabilis</td>
<td></td>
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</tbody>
</table>

Proteus mirabilis is a gram-negative and facultative anaerobe which produces high levels of urease. For this reason, it is commonly associated to kidney stone formation and urinary tract infections. P. mirabilis can also cause sepsis and brain infections and it was found in increased abundance in urine of subjects affected by Parkinson’s disease. Animal studies have shown the administration of P. mirabilis causes dopaminergic neuronal damage and inflammation, associating the bacterium to Parkinson’s disease pathogenesis.

Early microbiome research found an additive effect of the immune response to two P. mirabilis antigens – haemolysin and urease – in the disease development of rheumatoid arthritis (RA), and molecular mimicry from a P. mirabilis infection is still thought to a contributing factor in some RA development.

<table>
<thead>
<tr>
<th>Pathobiont</th>
<th>Nosocomial infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa</td>
<td></td>
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</tbody>
</table>

Pseudomonas aeruginosa is an encapsulated, gram-negative bacterium responsible for 10–15% of the nosocomial infections worldwide. It can cause diarrhoeal diseases and systemic infections, but it is mainly associated with urinary and respiratory infections, particularly with cystic fibrosis lung infections. It is naturally antibiotic resistant, so when infections are set up outside of the GIT tract, they are often hard to treat.

<table>
<thead>
<tr>
<th>Pathobiont</th>
<th>Nosocomial infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veillonella spp</td>
<td></td>
</tr>
</tbody>
</table>

Veillonella spp are non-fermentative, strictly anaerobic, Gram-negative cocc i that form part of the human gastrointestinal tract, mouth and vaginal flora. Abundance of Veillonella parvula has shown associations with liver disease severity and also significantly increased in patients with long-term PPI therapy.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Food poisoning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yersinia enterocolitica</td>
<td></td>
</tr>
</tbody>
</table>

Yersinia enterocolitica is a gram-negative bacterium. As with salmonella and shigella, it is a foodborne pathogen – mainly found in pork – and it causes bloody/watery diarrhoea and fever. When it invades the mesenteric lymph nodes, it causes lymphadenopathy, also named pseudoappendicitis. Reactive arthritis or erythema nodosum are often developed post-infection. Yersinia enterocolitica antibodies in the serum have been found in higher numbers in autoimmune thyroid diseases such as Grave’s disease and Hashimoto’s.

<table>
<thead>
<tr>
<th>Pathobiont</th>
<th>Nosocomial infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serratia marcescens</td>
<td></td>
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</table>

Serratia marcescens is a facultative anaerobe belonging to the Enterobacteriaceae family. It is a multidrug-resistant bacterium typically associated with hospital-acquired infections, especially of the urinary tract.

<table>
<thead>
<tr>
<th>Pathobiont</th>
<th>Nosocomial infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus faecalis</td>
<td></td>
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</tbody>
</table>

Enterococcus faecalis is a gram-positive bacterium commonly associated with nosocomial infections. Frequently associated with failure of endodontic treatments, it can also cause sepsis, meningitis and urinary tract infections.

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E. faecalis metalloprotease increases intestinal inflammation by compromising the gut epithelial barrier\textsuperscript{203}. Increased abundance of E. faecalis was found in colorectal cancer patients\textsuperscript{207}. Interestingly, the interaction with C. albicans promotes a non-pathogenic association with the host\textsuperscript{204}.

<table>
<thead>
<tr>
<th>Enterococcus faecium</th>
<th>PATHOBIONT</th>
<th>Nosocomial infections</th>
</tr>
</thead>
</table>

Enterococcus faecium is a gram-positive bacterium that can cause vancomycin-resistant infections\textsuperscript{209}. For this reason, it is usually associated with nosocomial infections. A specific strain susceptible to vancomycin has been investigated as a possible probiotic\textsuperscript{206}. Enterococci are members of the lactic acid bacteria and can improve intestinal health and reduce serum cholesterol levels\textsuperscript{207,208}. Enterococci can also produce enterocins, a class of bacteriocins that inhibit the growth of pathogens\textsuperscript{207}.

<table>
<thead>
<tr>
<th>Enterococcus gallinarum</th>
<th>PATHOBIONT</th>
<th>Nosocomial infections</th>
</tr>
</thead>
</table>

Enterococcus gallinarum is a gram-positive bacterium. It has low-level resistance to vancomycin compared to E. faecalis and E. faecium due to the presence of a different gene\textsuperscript{206}. For this reason, it rarely causes nosocomial infections\textsuperscript{210}. A recent study showed that the translocation of this bacterium to the liver, or other systemic tissues, can trigger autoimmune responses\textsuperscript{212}.

<table>
<thead>
<tr>
<th>Methanobrevibacter smithii</th>
<th>COMMENSAL ARCHAEA</th>
<th>Hydrogen homeostasis and methanogenic</th>
</tr>
</thead>
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Methanobrevibacter smithii is a methanogenic archaean. It balances gut H\textsubscript{2} levels by converting carbon dioxide (CO\textsubscript{2}) and hydrogen (H\textsubscript{2}) into methane (CH\textsubscript{4})\textsuperscript{211}. Literature reports there is an increased presence of M. smithii in subjects affected by IBS-Constipation\textsuperscript{206,207}. On the other hand, reduced abundance of M. smithii has been observed in IBD patients with a concomitant increment in subject on clinical remission\textsuperscript{212}. Lower concentrations of M. smithii are also considered a risk factor for IBS, colorectal cancer, diverticulosis and obesity\textsuperscript{211,212}.

<table>
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<th>Mycobacterium avium</th>
<th>PATHOGEN</th>
<th>Linked with Crohn’s disease</th>
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M. avium is a slow-growing mycobacterium which can be an opportunistic intracellular pathogen. It is found worldwide and can cause lung and gastrointestinal infections, especially in the immunocompromised\textsuperscript{200}. Infection of M. avium has been linked with the development of Crohn’s disease, although not everyone carrying it necessarily has symptoms. It is thought that is a dual combination of immune dysregulation plus infection with M. avium which is thought to bring on the progression of the disease\textsuperscript{221}.

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<th>Ruminococcus gnavus</th>
<th>PATHOBIONT</th>
<th>IBD</th>
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Ruminococcus gnavus is a gram-positive bacterium belonging to the Clostridia class. Increased abundance of this bacterium has been observed in IBD patients, particularly in the ones affected by Crohn’s disease\textsuperscript{222,223}. It produces an inflammatory molecule which induces the production of inflammatory cytokines, including TNF\textsubscript{222,223}.

Specific strains have found to degrade mucin and to encode for beta-glucuronidase activity\textsuperscript{224,225}. R. gnavus positively correlates with the development of respiratory allergic diseases in infants and causes airway inflammation in mice through T\textsubscript{H}2 cells stimulation\textsuperscript{228}.

In the mucus of inflammatory bowel disease patients, an increased number of the mucus-degrading bacterium Ruminococcus gnavus has been observed, producing a defective mucus layer which may lead to increased translocation of bacterial lipopolysaccharide, thereby contributing to metabolic diseases\textsuperscript{224}.

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<tr>
<th>Ruminococcus torques</th>
<th>PATHOBIONT</th>
<th>IBD and ASD</th>
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Ruminococcus torques is a mucolytic gram-positive bacterium associated with IBD\textsuperscript{223,227,210}. The ability to degrade mucin and adhere to the gut epithelium is believed to alter the permeability of the intestine, inducing inflammatory responses. R. torques has been found in higher concentrations in children with autism spectrum disorders\textsuperscript{227}. Ruminococcus torques has been found to be in higher levels in humans with circadian rhythm disturbances\textsuperscript{232}.

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Staphylococcus aureus | PATHOBIONT | Nosocomial infections

*Staphylococcus aureus* is a gram-positive bacterium frequently found on the skin and the upper respiratory tract. It is a facultative anaerobe, allowing it to colonise the intestine and the reproductive tract in women. It commonly causes skin infections but it is also responsible for pneumonia, bone infections, food poisoning and bloodstream infection after surgery. Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are rapidly rising worldwide, making this bacterium one of the principal nosocomial pathogens.

Streptococcus agalactiae | PATHOBIONT | Neonates infections

*Streptococcus agalactiae*, also known as Lancefield’s group B streptococcus (GBS), is a gram-positive facultative anaerobe. Found in around 30% of healthy adult gastrointestinal tracts and vaginas, it can cause severe infections. The bacterium is the leading cause of sepsicaemia, pneumonia and meningitis in neonates. Additionally, a recent study showed that neonates of GBS+ women have a different microbiota composition compared to GBS-, possibly leading to disease development later in life.

Streptococcus pneumoniae | PATHOBIONT | Respiratory infections

*Streptococcus pneumoniae* is a gram-positive bacterium commonly found in the respiratory tract. It can cause pneumonia, meningitis and sepsis in immunocompromised patients. Penicillin-resistant *Streptococcus pneumoniae* (PRSP) infections are rapidly rising worldwide. A recent animal study suggested an active role of the gut microbiota as a protective mediator during pneumococcal pneumonia.

Streptococcus pyogenes | PATHOBIONT | Throat infections

*Streptococcus pyogenes* is an aerotolerant, gram-positive bacterium. It is commonly known to cause strep throat, but it can also be found on the skin, rectum and genital mucosa. It is human-specific and it is transmitted by contact. *S. pyogenes* can sometimes cause autoimmune diseases, such as rheumatic fever. This bacterium stimulates a Th1-type cytokine response in monocyte-derived dendritic cells.

### Mycology

Aspergillus fumigatus | PATHOGEN | Infections

*Aspergillus fumigatus* is a ubiquitous fungus found in the environment. Inhaled spores are constantly destroyed by the immune system but it can cause infections in immunocompromised subjects under cancer therapies or organ transplants and AIDS patients. It is estimated that *A. fumigatus* causes over 600,000 deaths a year, mainly due to the lung infection aspergillosis.

Candida albicans | PATHOBIONT | Infections & SIFO

*Candida albicans* is a filamentous yeast that colonises the mouth and gastrointestinal tract of more than 60% of healthy adults. In immunocompromised subjects (cancer, organ transplants, AIDS) it often causes the infection candidiasis. Superficial infections commonly affect the mouth and vagina, while systemic infections, often together with *S. aureus*, have a 40-60% mortality rate. *C. albicans* is associated with small intestinal fungal overgrowth (SIFO), causing bloating, diarrhoea and nausea. *C. albicans* is also associated with Crohn’s disease.

Candida krusei | PATHOBIONT | Infections

*Candida krusei* is an emerging nosocomial pathogen causing fungemia in immunocompromised subjects. As with the other Candida species, it is responsible for superficial candidiasis, infecting the mouth and vagina, as well as systemic infections. It is less abundant and virulent compared to *C. albicans.*
**Candida tropicalis** PATHOBIONT Infections

*Candida tropicalis* is the second most virulent *Candida* species affecting humans and can cause oral and vaginal candidiasis275. *C. tropicalis* infections are usually associated with patients suffering from leukaemia or neutropenia (low concentrations of neutrophils)276.

**Malassezia restricta** PATHOBIONT Associated with IBD

*Malassezia restricta* is a common fungi resident in the skin. *M. restricta* has been found in the mucosal lining of patients with Crohn’s disease. It has been shown to elicit innate inflammatory responses, especially in patients with a polymorphism with CARD9, a signalling adaptor important for anti-fungal defense253. Higher levels of *Malassezia restricta* and other fungi such as, *Candida albicans*, *Candida glabrata*, and *Aspergillus gracilis* have been found in patients with uveitis254.

**Helicobacter pylori**

*Helicobacter pylori* is a gram-negative bacterium usually found in the stomach. It is believed to be a stable member of the human microbiota and it is asymptomatic in 90% of the population255. This bacterium can cause gastroesophageal reflux disease (GERD), chronic gastritis, gastric or duodenal ulcers and oesophageal or stomach adenocarcinomas256,257. Reduced colonisation of *H. pylori* has been correlated to an increased incidence of asthma and allergy development258. *H. pylori* antibodies in the serum have been correlated with autoimmune thyroid diseases259. If *Helicobacter pylori* is detected by PCR, reflexively there will be the HP antigen test performed, which is the same process used by the NHS in the UK at present. It is possible that there will be positive on PCR due to sensitivity, but negative on the antigen testing. We always advise correlation with clinical symptoms before approaching any treatment regime.

**Parasitology**

*Dientamoeba fragilis* PATHOBIONTGI pain, diarrhoea, IBD/IBS

*Dientamoeba fragilis* is a protozoan parasite that colonises the human gastrointestinal tract, especially in developed countries259. Infections are often asymptomatic, but symptoms can include abdominal pain, nausea and acute/chronic diarrhoea. Research studies have associated this protozoa to the development of colitis, IBD and IBS260.

**Blastocystis hominis** PATHOBIONT Diarrhoea and IBS

*Blastocystis hominis* is a common human intestinal parasite. Mostly asymptomatic, it can cause abdominal pain and diarrhoea. It has been associated with IBS, in particular when in a co-infection with *Dientamoeba fragilis*261,262. It has been reported that *B. hominis* infection can cause acute urticaria263,264.

**Entamoeba histolytica** PATHOGEN Amoebiasis

Entamoeba histolytica is a protozoan parasite causing amoebiasis, a major health problem in developing countries outpaced only by malaria and schistosomiasis. In 90% of cases, amoebic infections are asymptomatic, but the pathogenic phenotype can cause deadly dysentery, colitis and liver abscess265,266.

**Giardia lamblia** PATHOGEN Giardiasis

*Giardia lamblia*, also known as *Giardia intestinalis*, is a single-cell parasite that colonises the human small intestine267. It causes giardiasis, characterised by diarrhoea, abdominal pain and weight loss. It is commonly contracted by travellers in developing countries268.

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SUMMARY

The Phylobioscience GI Ecologix™ Gastrointestinal Health & Microbiome Profile is an innovative tool for analysis of the gastrointestinal microbiota composition and host immune responses. Phylobioscience recommend the profile is used on an annual basis to monitor changes in gut health. The gut health and microbiome profile may also be used as a diagnostic tool in symptomatic patients. For effective analysis of results, the interpretive guide should be used in combination with Invivo Healthcare’s Gut Microbiome Clinical Considerations, the patient’s symptomology and results. The Gut Microbiome Clinical Considerations provides further information on lifestyle and environmental factors that may influence the gut microbiota. The Gut Microbiome Clinical Considerations also provides recommendations for treatment and management plans for results indicative of dysbiosis, inflammation, and infection. If you have any queries on patient results or clinical considerations, please contact the Clinical Education team at support@invivohealthcare.com

RECOMMENDED READING

Functional interactions between the gut microbiota and host metabolism

The gut microbiota shapes intestinal immune responses during health and disease

Normal gut microbiota modulates brain development and behaviour

The Impact of the Gut Microbiota on Human Health: An Integrative View

The gut microbiota in IBD

Gut microbiota metabolism of dietary fibre influences allergic airway disease and haematopoiesis

Probiotics and the gut microbiota in intestinal health and disease

The impact of the gut microbiota on brain development and behavior

Dysbiotic microbiota modulates brain development and behavior

Interpretive Guide

REFERENCES

2. Kamada, N., Chen, G., Inohara, N., Immunology, G. N.–N. & 2013, undefined. Control of pathogens and pathobionts by the gut microbiota. nature.com
10. Sender, R., Fuchs, S. & Milo, R. Are We Really Vastly Outnumbered? Revisiting the Ratio of Bacterial to Host Cells in...
36. NICE. Adoption support resource – insights from the NHS. *20, 1–13 (2019).*
37. Manceau, H., Chicha–Cattoir, V., Puy, H. & Peoch, K. Fecal calprotectin in inflammatory bowel diseases: Update...
70. Remely, M., Tesar, I., Hippe, B., … S. G.-B. & 2015, undefined. Gut microbiota composition correlates with changes in body fat content due to weight loss. wageningenacademic.com
72. Teixeira, T., Grześkowiak, Ł., Salminen, S., nutrition, K. L.-C. & 2013, undefined. Faecal levels of Bifidobacterium and Clostridium cocoides but not plasma lipopolysaccharide are inversely related to insulin and HOMA index in women. Elsevier
75. Zhang, X. et al Human gut microbiota changes reveal the progression of glucose intolerance. journals.plos.org


Schroeder, B. O. Fight them or feed them: how the intestinal mucus layer manages the gut microbiota. doi:10.1093/gastro/goy052 2019.


113. Wang, T. et al. Structural segregation of gut microbiota between colorectal cancer patients and healthy volunteers. *nature.com*


133. Schneeberger, M., Everard, A., reports, A. G.–V.–S. & 2015, undefined. Akkermansia muciniphila inversely correlates with the onset of inflammation, altered adipose tissue metabolism and metabolic disorders during obesity. *nature.com*


Shih, C., Chen, Y., Chang, S., ... K. L.-C. infectious & 1996, undefined. Bacteremia Due to Citrobacter Species: Significance of Primary Intraabdominal Infection. academic.oup.com


Maes, M., Mihaylova, I. & Leunis, J.-C. Increased serum IgA and IgM against LPS of enterobacteria in chronic fatigue syndrome (CFS): Indication for the involvement of gram-negative enterobacteria in the etiology of CFS and for the presence of an increased gut-intestinal permeability. (2006). doi:10.1016/j.jad.2006.08.021


Sundqvist, G. *et al.* Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. *Elsevier*


224. Png, C., Lindén, S., ... K. G.-T. A. journal of & 2010, undefined. Mucolytic Bacteria With Increased Prevalence in IBD patients with Crohn’s disease and their unaffected relatives. *gut.bmj.com*


