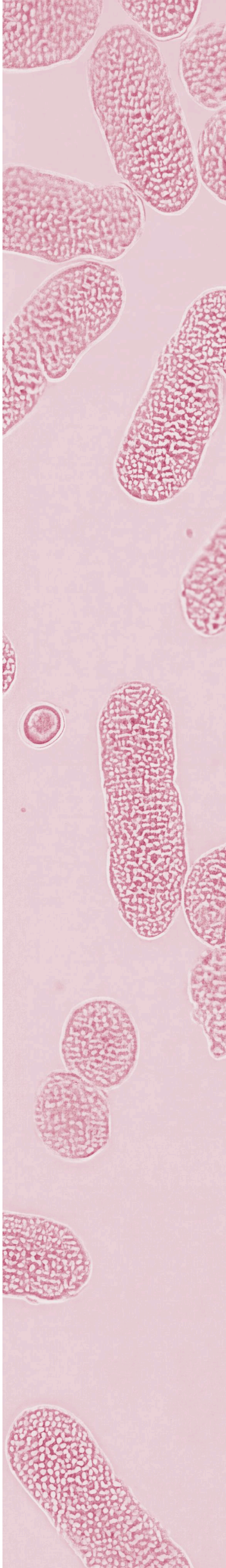
microba

Deep Dive:
The science behind Microba's gut health testing

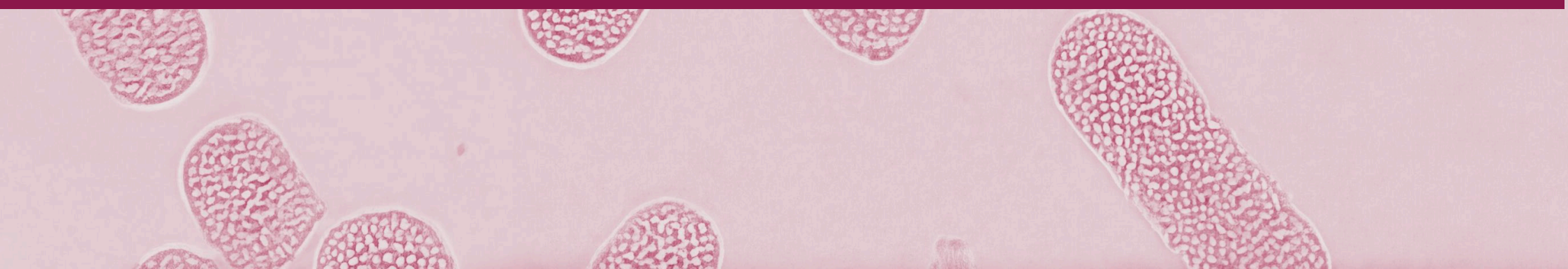


How Microba's science and technology enable clinically useful microbiome and gut health testing — the complete scientific and technological foundation.

001

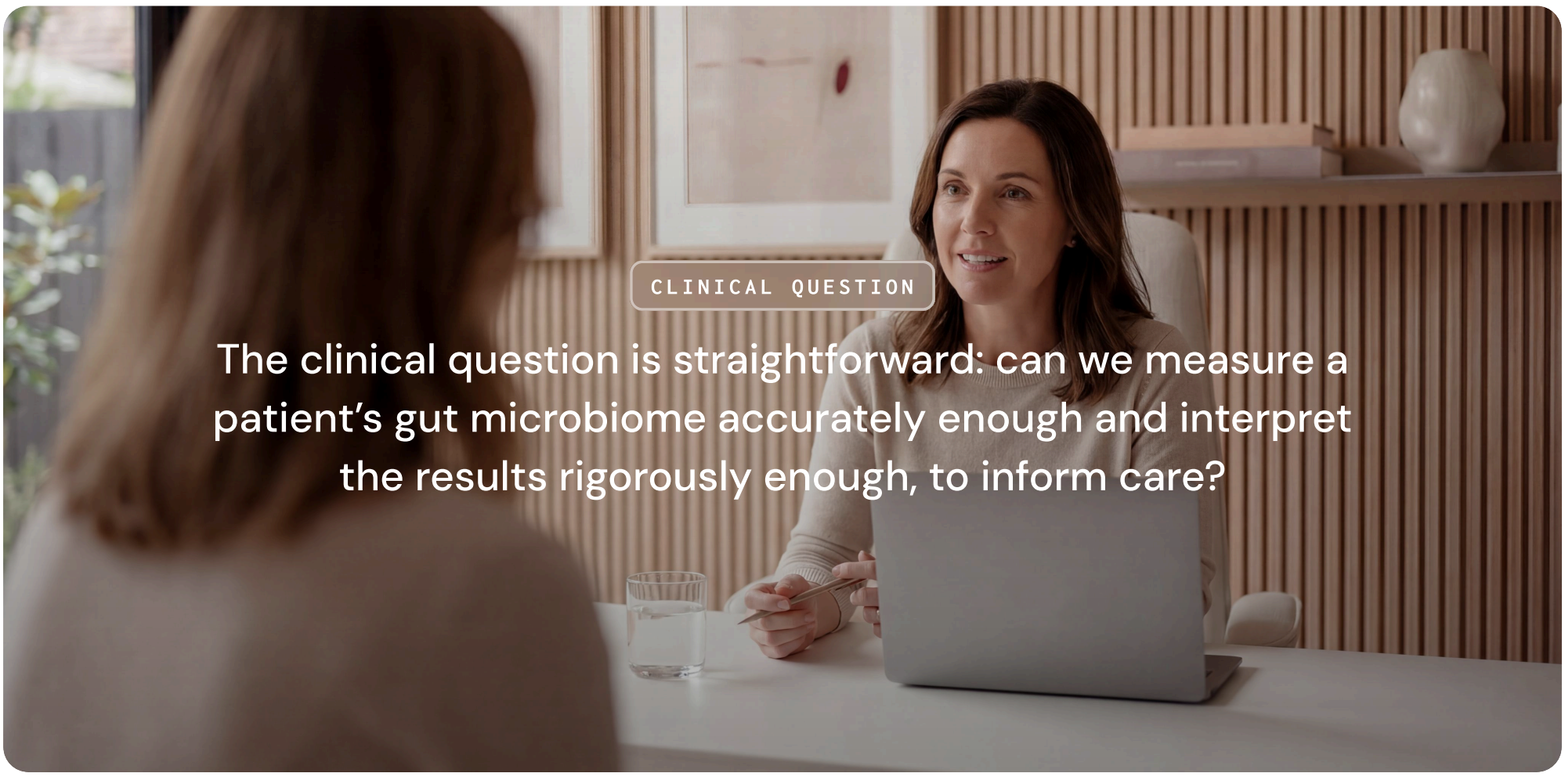
Measuring the microbiome

THE SCIENCE BEHIND MICROBA'S GUT HEALTH TESTING



The gut microbiome

The human gut harbours trillions of microorganisms whose collective genome — the microbiome — encodes metabolic capabilities far exceeding those of the human genome alone.[1] Over the past two decades, large-scale sequencing studies have demonstrated that the composition and function of this microbial community are associated with a wide range of health outcomes.[2,3] Disruption of a healthy gut microbiome, often called dysbiosis, has been linked to gastrointestinal disorders, autoimmune conditions, cardiometabolic diseases, and neurological conditions.[4]



Testing has evolved. Not all tests have.

Many gut health tests available today rely on older methods — culture-based assays, quantitative PCR (qPCR), or 16S ribosomal RNA (rRNA) gene sequencing that provide only a partial view of the microbiome. These approaches can be good in specific circumstances (e.g. identification of pathogens), but have well-documented limitations in their ability to identify microbes at species level, coverage of the entire microbiome, and the ability to assess microbial function.[6,7]

A step change in capability

The emergence of shotgun metagenomics — sequencing all DNA in a sample rather than a single gene has fundamentally changed what is possible, enabling species and even strain-level identification alongside functional pathway analysis.[8]

Technology alone isn't enough

The quality of the reference data results are compared against, and the rigour of the evidence framework used to interpret them, determine whether that technology translates into something a practitioner can actually rely on. This paper documents how Microba has built to that standard at every layer — because the quality of the science behind a test determines the reliability of the result.

Accurate gut testing starts with the fundamentals

01

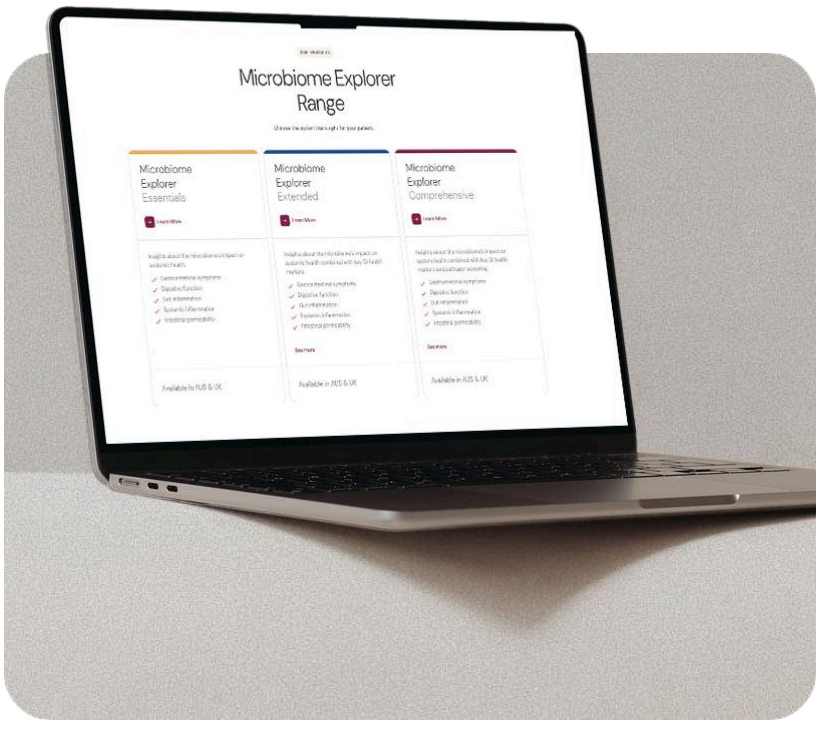
Measurement technology
The sequencing method used to capture the microbial community and its resolution, coverage, and accuracy

02

Reference data quality
The reference cohort against which results are compared and whether it eliminates bias

03

Scientific framework
The evidence framework that turns microbiome data into something clinically useful



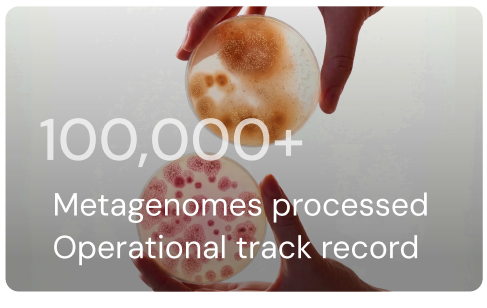
Microba Microbiome Explorer

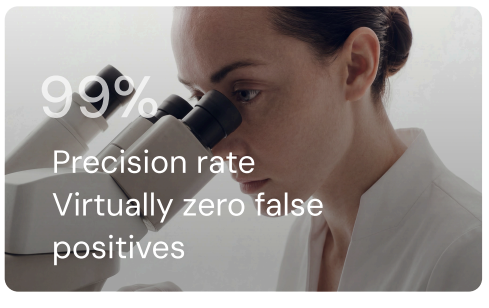
The quality of a result comes from everything behind it

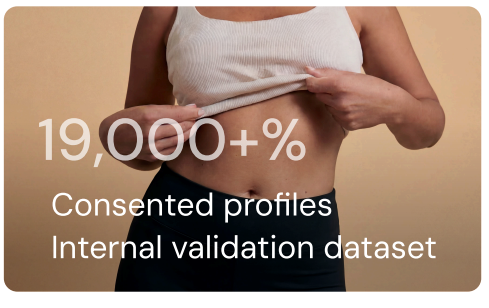
Microba Microbiome Explorer combines accredited gastrointestinal diagnostics with high-resolution shotgun metagenomic microbiome profiling. Pathogen detection and gastrointestinal markers are CE-certified and run within an ISO 15189 NATA-accredited medical laboratory. This deep dive covers the complete scientific and technological foundation — from how a sample is preserved, through to the clinical insights a practitioner reads in the report.

Many gut health tests available today rely on older methods — culture-based assays, quantitative PCR (qPCR), or 16S ribosomal RNA (rRNA) gene sequencing — that provide only a partial view of the microbiome. These approaches can be effective in specific circumstances (e.g. identification of pathogens), but have well-documented limitations in taxonomic resolution, coverage of the entire microbiome, and the ability to assess microbial function.

The answer to the clinical question — can we measure and interpret the microbiome rigorously enough to inform care — depends entirely on the technology, reference data, and scientific framework behind the test. The sections that follow describe how Microba's laboratory processes, bioinformatic technology, scientific curation practices, and quality systems work together to deliver that answer.







002

How accuracy is built

THE SCIENCE BEHIND MICROBA'S GUT HEALTH TESTING

Validated sample preservation protects the accuracy of every result

The moment a sample is collected, the clock starts. Microbial composition shifts fast if preservation isn't handled correctly — and most collection methods weren't designed with that in mind.

Microba's FLOQSwab-ADT was benchmarked head-to-head against the most widely used alternatives.[9] It came out on top. Practitioners can be confident the sample that leaves the patient's home is the sample that gets analysed. No degradation. No compromise.

Peer-reviewed validation

In a peer-reviewed evaluation, Microba's swab demonstrated the best performance for both technical (between-replicate) reproducibility and compositional stability relative to flash-frozen controls. Additionally, a second experiment in the same study demonstrated that the FLOQSwab-ADT maintained its performance across storage at -20°C, room temperature, and 50°C for four weeks, making it suitable for postal collection in a wide range of climatic conditions.[9]

Best reproducibility

Highest technical (between-replicate) reproducibility and compositional stability relative to flash-frozen controls in a peer-reviewed evaluation

Climate resilient

Stable across -20°C, room temperature, and 50°C for four weeks — suitable for postal collection Australia-wide

Practitioners can be confident that the microbial profile generated from a swab collected at home closely reflects the true composition of the patient's sample at the time of collection.

1 **COLLECT**
Patient collects at home

2 **PRESERVE**
FLOQSwab-ADT preserves sample

3 **SHIP**
Postal delivery in any climate

4 **ANALYSE**
Microba lab receives intact sample

Did you know?

Microbial markers are scientifically curated using a rigorous three-tier evidence framework, and results are compared against a carefully defined healthy reference group of more than 450 individuals.

100,000 metagenomes processed. One accredited laboratory. Zero compromises

Accreditation sets the floor. What happens inside the laboratory is what raises it. Microba operates an ISO 15189 NATA-accredited medical testing laboratory, the internationally recognised standard for medical laboratories — running the latest DNA sequencing technology at a high level of automation from sample receipt through to data generation.

Every sample is monitored by an automated quality control pipeline. Anything that falls outside predefined quality thresholds gets flagged before it goes further. More than 100,000 metagenomes processed. That's not a claim — it's a track record.

Clinical-grade quality doesn't stop at the bench

The software systems used to analyse and interpret metagenomic data are developed under an ISO 13485 quality management system for software as a medical device.

Most providers stop at laboratory accreditation. Dual accreditation — ISO 15189 for laboratory processes and ISO 13485 for the bioinformatic pipeline, means the same rigour applies to how data is analysed, not just how it's collected

001 SAMPLE RECEIPT

002 DNA EXTRACTION

003 SEQUENCING

004 DATA GENERATION

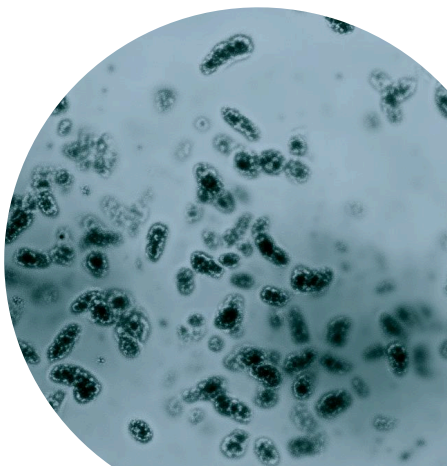
DUAL ACCREDITATION

ISO 15189

Internationally recognised standard for medical laboratory processes — covering sample receipt, sequencing, and data generation

ISO 13485

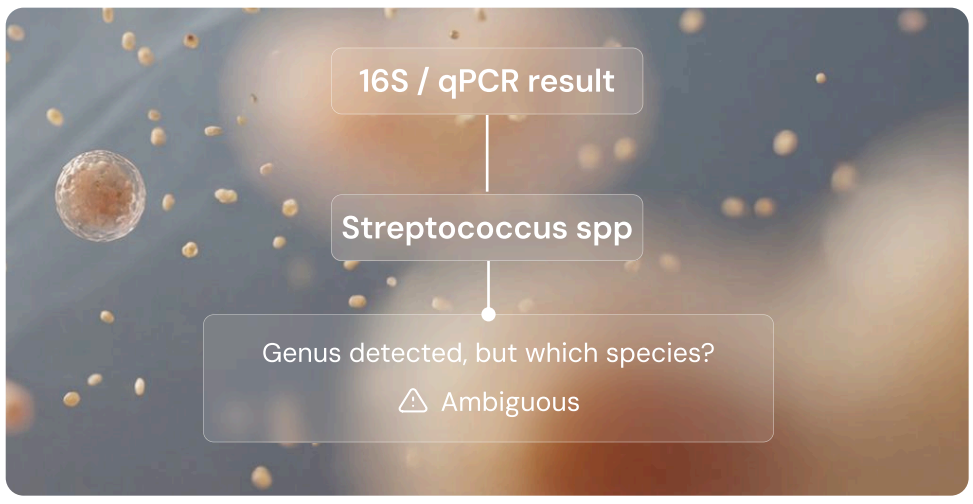
Quality management system for software as a medical device — covering the bioinformatic analysis and interpretation pipeline

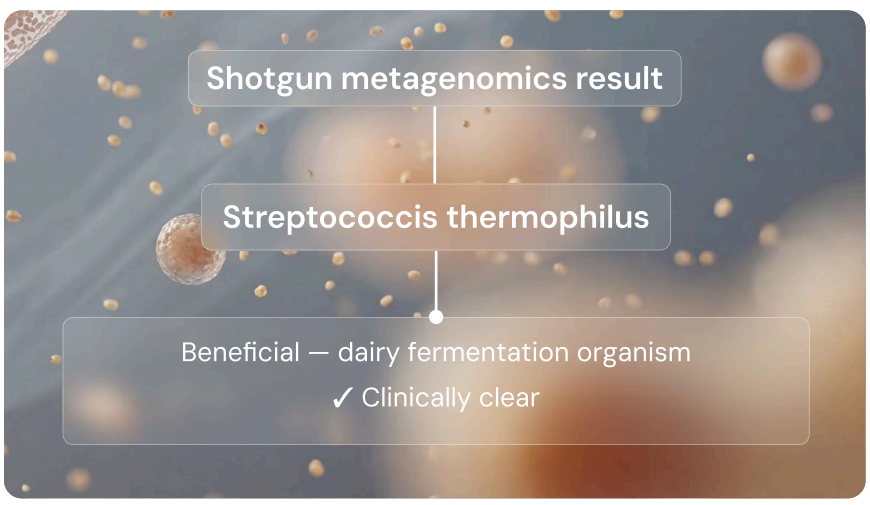


Shotgun metagenomics identifies species that other methods miss entirely

Shotgun metagenomics sequences all DNA extracted from a faecal sample — not a single gene, not a predefined panel. The result is a comprehensive, relatively unbiased view of the entire microbial community at species level.[8] That distinction matters more than it sounds. Different species within the same genus can have completely different roles in health. Without species-level resolution, you're working with an incomplete picture. Take Streptococcus. Streptococcus thermophilus is beneficial — widely used in dairy fermentation. Sreptococcus anginosus is an opportunistic pathogen linked to abscess formation. Same genus. Completely different clinical implications. A test that can only tell you "Streptococcus" isn't giving you enough to act on.

The Streptococcus example





A comparison of technologies

	16S RRNA	QPCR / CULTURE	SHOTGUN METAGENOMICS
Taxonomic resolution	Genus level	Predefined targets only	Species and strain level
Coverage	Bacteria and Archaea only	Limited panel	Bacteria, Archaea, eukaryotes
Functional profiling	Not possible	Limited panel	Gene and pathway level
Novel species detection	No	No	Yes
PCR bias	Yes	Yes	Minimal

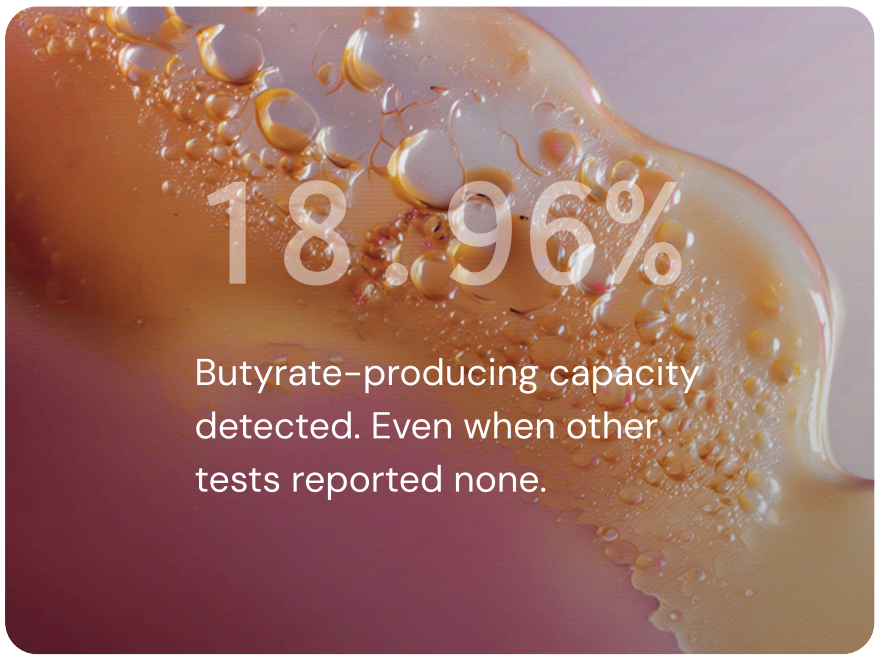
632 species vs 57

In a direct comparison, shotgun metagenomics identified 632 species in a sample where 16S rRNA gene sequencing detected only 57 — an order-of-magnitude difference in resolution that directly affects clinical utility.

Species tell you who's present Function tells you what they're doing

Identifying what's in the microbiome is the starting point, not the finish line. Shotgun metagenomics goes further, identifying the metabolic genes and pathways present across the entire microbial community. That means assessing functional capacity — what that community is actually capable of doing.

Can this community produce butyrate? Is it degrading the protective mucus layer? These are the questions that move a result from interesting to actionable. Other sequencing methods can't get you there.



Functional capacity tells the real story

Some tests reported low short-chain fatty acid metabolites and stopped there. That's the problem with measuring outputs alone. It tells you what's happening right now, not what the community is capable of. Microba's assessment of functional capacity told a different story. 18.96% butyrate-producing capacity present across the microbial community. The functional machinery was present.[8]

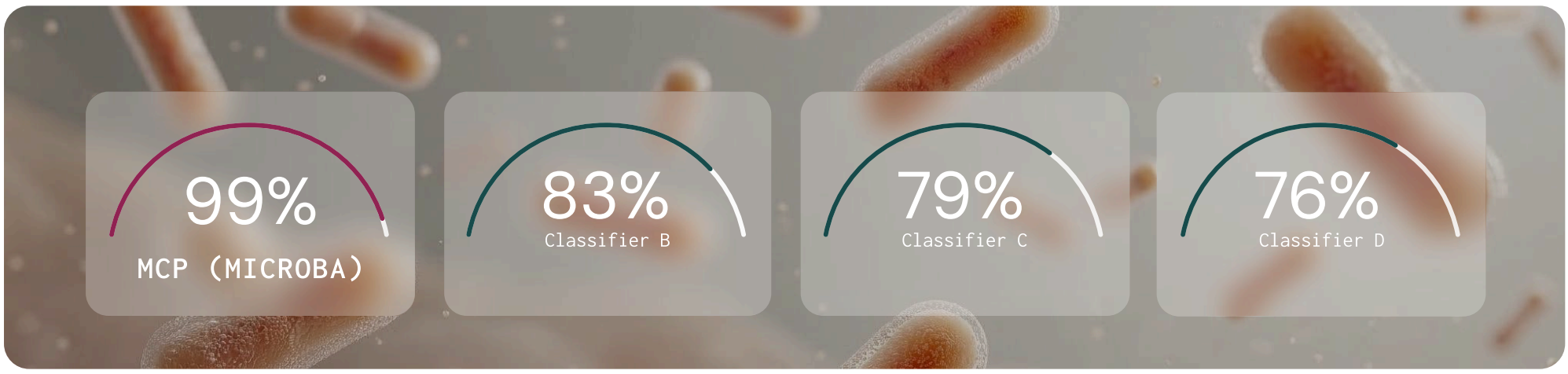
The real question wasn't whether the microbiome could produce butyrate, it was whether the right dietary substrates were available to support it. That's a completely different clinical conversation. One that other sequencing methods can't have.

The Microba Community Profiler achieves 99% precision — peer-reviewed and benchmarked

Generating sequence data is only the first step. What you do with it is where most tools fall short. The accuracy of a microbiome profile depends entirely on the bioinformatic tools used to classify millions of short DNA sequences and not all classifiers are built to the same standard.

The Microba Community Profiler (MCP) is a proprietary whole-genome alignment tool purpose-built to produce species-level community profiles with the highest possible accuracy. Its performance has been formally benchmarked in a peer-reviewed study in *Frontiers in Microbiology* — evaluated against nine widely used academic classifiers across 140 simulated microbial communities of varying complexity.[5] The numbers tell the rest of the story.

Precision (% — Higher better)



Tested against nine classifiers. MCP outperformed every one

Accurate abundance estimates

MCP reports how much of each species is present — not just whether it's there. Those estimates closely match what's actually in the sample, with a level of accuracy that matches or exceeds every other leading classifier tested.[5]

Highest accuracy

MCP achieved the highest combined precision and recall across all tested conditions — outperforming every other evaluated classifier by five to 20 percentage points. Not one. All nine.[5]

Fewest false positives

Virtually every species MCP reports is genuinely present in the sample. It produced four to 16 times fewer false positive predictions than other classifiers — because false positives don't just inflate a report, they can change a clinical conversation in the wrong direction[5]

Lowest detection limit

MCP can reliably detect species at abundances 20 to 60 times lower than competing tools, with a detection limit as low as 0.007% at a false discovery rate of 0.1%. Clinically relevant organisms at low abundance don't get missed.[5]

The MCP automatically filters species predictions to report only those with high confidence — no manual threshold-setting required. Most other classifiers report everything and let you figure it out. MCP only reports what's genuinely there.

Three integrated layers translate microbial presence into clinical meaning

No single marker tells the whole story. Microba Microbiome Explorer integrates three layers of information to connect what's present in the microbiome with measurable clinical markers of gut function and inflammation.

Pathogen detection

A panel of 13 common bacterial pathogens and five parasites detected via CE-certified multiplex PCR assays.

Human stool markers

Six GI health markers including calprotectin, lactoferrin, faecal occult blood, secretory IgA, pancreatic elastase, and zonulin — assessed using CE certified immunohistochemistry assays. Faecal pH is also measured as an investigative marker for research use only.

Microbiome profiling

Species-level profiling of 28,000+ species including microbial diversity score, microbial richness, and 16 health-associated functional markers — such as butyrate production, trimethylamine, hexa-acylated lipopolysaccharides, mucin degradation, and oxalate consumption.

Separately, each layer is informative. Together they deliver something no single method can.
By combining diagnostic gastrointestinal markers with microbiome profiling, the test moves beyond simple species detection to provide a functional picture of gut health, linking what is present in the microbiome with measurable clinical markers of gut function and inflammation.

Bring confident care to your practice

Start Referring

Every marker reported is clinically relevant and evidence-backed

Not every microbial signal is clinically meaningful. To ensure that the microbiome markers reported in the test are supported by robust evidence, Microba applies a rigorous three-tier scientific curation framework. Only markers that satisfy all three tiers (with limited, clearly disclosed exceptions) are included in the report.* Every marker a practitioner sees has cleared a high evidence bar, nothing is included on association alone.

**A small number of markers are included with clearly disclosed exceptions — acetate and intestinal inflammation, beta-glucuronidase and impaired detoxification (both mechanism only), and Emerging Markers. Where exceptions exist, they are explicitly flagged in the report.*

ALL POTENTIAL MICROBIAL SIGNALS	TIER 1 + 2: MECHANISTIC AND HUMAN EVIDENCE	TIER 3 - INTERNAL SCIENTIFIC VALIDATION
Thousands of possible associations from the literature	Filtered for plausible mechanism and reproducible human associations	Only markers validated in Microba's 19,000+ profile dataset (FDR < 0.05)

Tier 1

Plausible mechanism of action

There must be in-vitro or in-vivo data demonstrating why the microbial marker is biologically connected to the relevant health category.

Example

The link between mucin-degrading species and intestinal inflammation is supported by mechanistic studies showing that depletion of the mucus barrier increases microbial proximity to the epithelium, driving immune activation.[10-13]

Tier 2

Reproducible human associations

At least two peer-reviewed human studies must show a direct or indirect link between the marker and the health outcome.

Example

Direct evidence includes correlation of the marker with a clinical measure (e.g. a positive correlation between oral species abundance and faecal calprotectin). Indirect evidence includes correlation with relevant diseases, or plasma levels of the microbial marker with a clinical measure.[14,15]

Tier 3

Significant associations in Microba's dataset

The marker must show a statistically significant association in Microba's own database of 19,000+ consented patient profiles, controlled for age, sex, BMI, and bowel habits.

Example

This internal validation step ensures that markers are not only supported by published literature but are also reproducible in a large, independent cohort processed through Microba's own laboratory and analysis pipeline.

The reference group determines the quality of every result

To determine whether a patient's microbiome markers are within a healthy range, results must be compared against an appropriate reference group. Many commercially available tests either provide no details about their reference cohort, use publicly available microbiome data (which introduces significant variability due to differences in sample collection, processing, and analysis methods), or compare samples against their entire database regardless of health status. A rigorously selected healthy cohort means a patient's result reflects what's actually happening, not the limitations of a poorly defined baseline.

The Microba's healthy reference group

Carefully selected to include more than 450 individuals meeting strict inclusion criteria. Critically, all reference samples were collected and processed using exactly the same workflow as patient test samples, eliminating a common source of technical bias.

Selected inclusion criteria

Selected participants met key lifestyle criteria consistent with general health, including:

- BMI above 30
- Regular daily intake of fruits and vegetables
- Low to moderate alcohol consumption.
- Individuals also reported no or minimal gastrointestinal symptoms.
- No major medical conditions
- Mild or lower stress, anxiety, and depression

The report tells you what's there, evidence-graded actions tell you what to do about it.

Generating accurate microbiome data is necessary but not sufficient. For gut health testing to be clinically useful, the data must be translated into interpretable insights that healthcare practitioners can act upon. In Microba's Microbiome Explorer, microbial markers and gastrointestinal markers are organised into health categories that correspond to recognisable clinical concepts applicable, links to the relevant diagnostic GI markers for clinical correlation. Each health category provides a clear interpretation of whether the patient's results fall within or outside the healthy reference range, and, where applicable, links to the relevant diagnostic GI markers for clinical correlation.

- Intestinal inflammation
- Systemic inflammation
- Metabolic health
- Gut motility
- Pathogen presence
- Gut barrier function

When a marker is identified as out of range, the report provides evidence-graded possible actions. Microba's science team has undertaken a rigorous review of the available scientific evidence for different dietary, supplement, or lifestyle interventions to modulate microbial functions and graded these using the NHMRC evidence grading framework. Each listed action is accompanied by the evidence grade and hyperlinks to the references that informed the grade.

Worked example: Mucin degradation and intestinal inflammation

The proportion of mucin-degrading species in the microbiome is one of the markers assessed in the test. The mechanistic basis for this marker is well established: when dietary fibre is insufficient, mucin- degrading microbes can consume the protective mucus layer lining the gut, increasing microbial contact with the intestinal epithelium and triggering immune activation. A large cross-sectional study of more than 1,000 individuals found a significant positive association between mucin-degrading pathway abundance and faecal calprotectin (a clinical marker of intestinal inflammation).[14] Elevated mucin degrading pathways have also been observed in colorectal cancer cohorts.[15,16] In Microba's own dataset, the relative abundance of mucin-degrading species is significantly increased in several health conditions related to intestinal inflammation compared to healthy controls, after controlling for confounders.

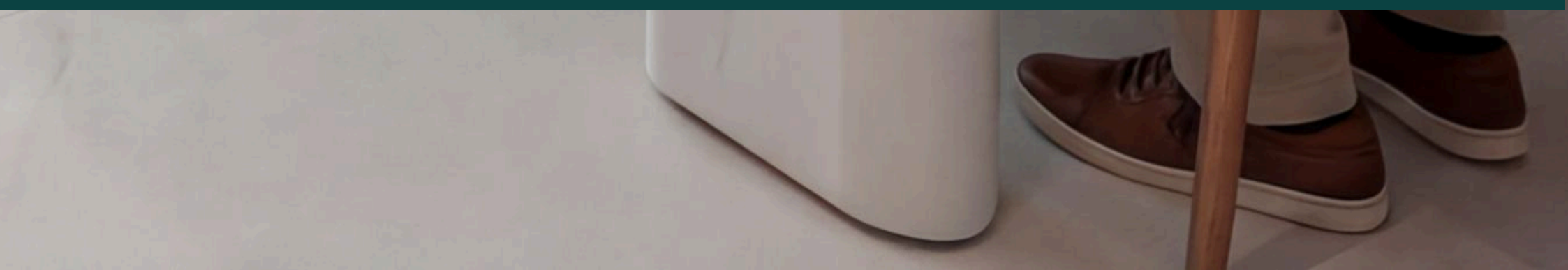
Built for clinical confidence

This layered approach — mechanistic plausibility, published human evidence, and internal validation, gives practitioners a clear rationale for each marker and a basis for evidence-informed clinical conversations with patients. Microba Microbiome Explorer is exclusively offered through healthcare professionals. This distribution model ensures that results are interpreted accurately, responsibly, and in the best interests of patient care.

003

What sets Microba apart

THE SCIENCE BEHIND MICROBA'S GUT HEALTH TESTING



This is what sets Microba's approach apart

The gut health testing market includes a range of product built on different technologies, evidence standards, and quality systems. Several features distinguish Microba's approach from legacy and competing tests.

High-resolution metagenomics, not 16S or qPCR

While many commercial gut tests rely on 16S rRNA gene sequencing (which typically resolves only to genus level) or targeted qPCR panels (which assess only a predefined list of organisms), Microba uses shotgun metagenomics to deliver species-level resolution across the entire microbial community. In a direct comparison, shotgun metagenomics identified 632 species in a sample where 16S rRNA gene sequencing detected only [57].

Whole-microbiome functional assessment

Some competing tests claim to provide functional information but assess only a handful of known species associated with a particular function. Microba's functional markers assess the capacity of the entire microbial community to perform a given metabolic function — for example, butyrate production across all species that carry the relevant genes.

Peer-reviewed, benchmarked bioinformatics

The Microba Community Profiler has been formally benchmarked against nine other classifiers in a peer-reviewed study and demonstrated the highest accuracy and lowest false-positive rate. Many competing tests do not disclose their bioinformatic methods or provide evidence of benchmarking.

Rigorous three-tier evidence curation

Microba's three-tier marker curation framework — requiring mechanistic plausibility, reproducible human associations, and internal dataset validation — stands in contrast to many tests that include numerous "microbiome scores" or "gut–axis" risk associations with limited or no published scientific support.

Like-for-like healthy reference group

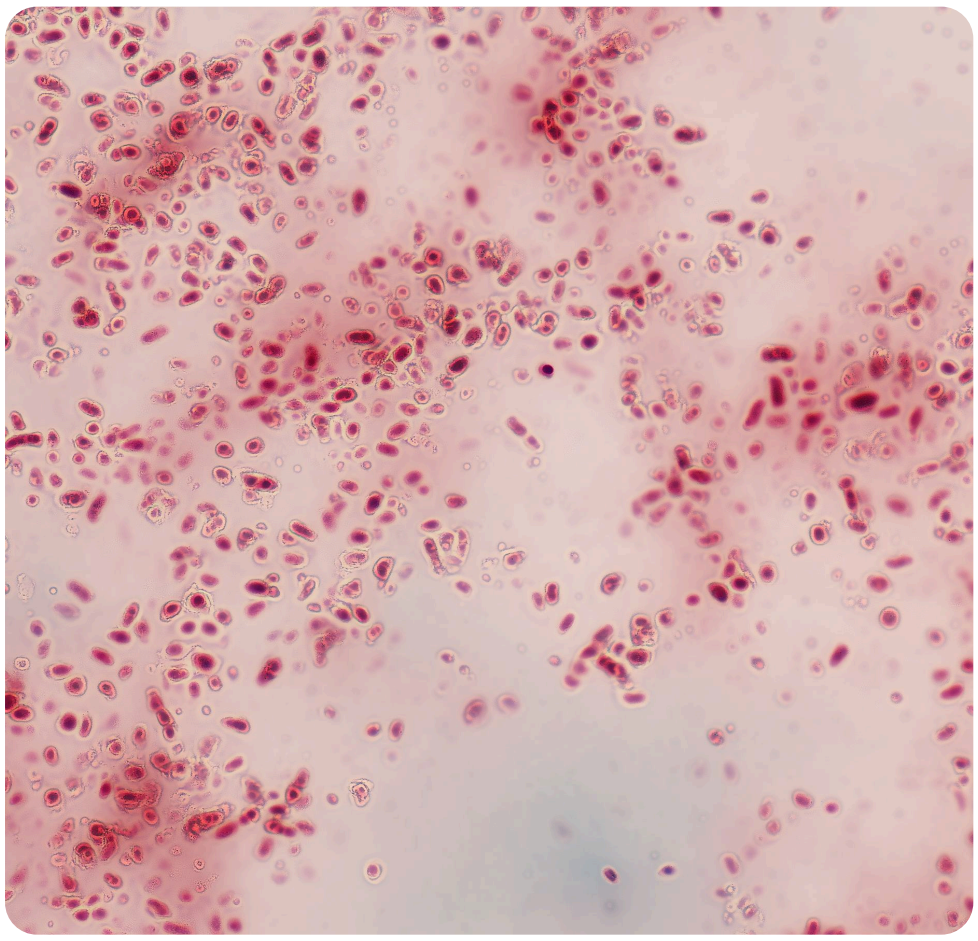
By defining a reference group with strict health criteria and processing all reference samples through the same workflow as patient samples, Microba eliminates a significant source of technical and biological confounding that affects tests relying on public databases or heterogeneous internal cohorts.

Dual accreditation

The combination of ISO 15189 medical laboratory accreditation and ISO 13485 software-as-a-medical- device certification reflects a level of quality assurance that is uncommon among commercial microbiome test providers.

Science, evidence, and quality integrated to deliver what no legacy test can

Microba's Microbiome Explorer represents a considered integration of advanced metagenomic science, rigorous evidence curation, and accredited laboratory quality. By combining CE-certified gastrointestinal measures with high-resolution microbiome profiling, the test provides healthcare practitioners with a comprehensive view of gut health that goes well beyond what legacy testing methods can offer. Every layer of the technology stack — from validated sample preservation and automated laboratory processing, through to the peer-reviewed MCP — has been designed to maximise accuracy and minimise the risk of misleading results. The three-tier marker curation framework ensures that only microbial markers with robust supporting evidence are included, and results are compared against a carefully curated healthy reference group.



MICROBA COMMITMENT

Microba is committed to translating advances in microbiome science into testing that practitioners can trust. Every layer of the Microbiome Explorer, from sample preservation through to clinical insights, has been built to the highest scientific and quality standards, and that commitment extends to how the science evolves.

As new evidence emerges, markers are refined, the reference group expanded, and validation studies continued, so the test remains at the leading edge of what microbiome science can reliably deliver for practitioners and their patients.

1

SAMPLE COLLECTION
Validated FLOQSwab–ADT
Climate-resilient postal collection

2

LAB + DATA GENERATION
ISO 15189 accredited lab
100,000+ metagenomes
Automated QC pipeline

3

CORE ANALYSIS
Shotgun metagenomics
Microba Community Profiler (99% precision)

4

INTERPRETATION LAYER
Three-tier evidence curation
450+ healthy reference group
NHMRC-graded actions

*The microbiome component of Microba Microbiome Explorer is for research use only and is not a diagnostic tool. Microbiome results should be interpreted by qualified healthcare practitioners in the context of a patient's clinical history, symptoms, and other diagnostic findings.

Transparency about limitations is essential for responsible clinical use

The test does not diagnose, treat, or prevent any disease. Like all sequencing-based microbiome tests, Microba's metagenomic profiling produces compositional data — it measures the relative proportions of different species rather than their absolute quantities.
This is an inherent characteristic of current sequencing technology and means that changes in the abundance of one organism can influence the apparent abundance of others. On average, Microba will be able to assign a species ID to 82.4% of sequencing reads. Microba continues to expand the database with newly identified species and strains, and ongoing updates to the reference database aim to increase the proportion of sequencing reads assigned a species ID and lower detection limits further. The marker curation framework is also a living process: as new evidence emerges, markers may be added, refined, or retired. Future directions include further validation studies, expansion of the healthy reference group to include additional populations, and continued development of evidence-graded suggested actions informed by the latest intervention research.

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