GET THE BEST CLINICAL RESULTS WITH THE INDUSTRY'S LEADING STOOL TEST

GI-MAP® gives you the highest-quality and most clinically actionable test results with:

- The most clinically-relevant markers
- The most accurate quantitative methodology
- The most comprehensive interpretive and educational support

***GI-MAP®* – Unparalleled DNA Based Stool Testing**

Our mission: to deliver innovative, accurate and clinically relevant diagnostic testing in a timely and cost-effective manner

Diagnostic Solutions laboratory

RESEARCH. TECHNOLOGY. RESULTS.

GI-MAP markers are based on the latest scientific research and established clinical applicability. The stool test measures pathogens, commensals, opportunistic bacteria, protozoa, fungi, viruses, and worms, as well as digestive function, immune responses, and intestinal barrier integrity to help you detect:

- Gastroenteritis
- GI inflammation
- Hypochlorhydria
- Maldigestion/malabsorption
- Autoimmune triggers
- Gliadin reactivity
- Leaky Gut
- Dysbiosis
- Immune function
- Microbial balance and the gut ecosystem
- Overall GI physiology
- And more...

The Most Accurate Quantitative Technology

When assessing a patient, do you want only a positive/negative result, or do you want a truly quantitative result?

GI-MAP's qPCR technology provides you with true quantitative values. It helps differentiate trace levels of an organism from frank elevations indicative of active infection. The GI-MAP provides absolute values — *not relative levels* — of each microbe. This information gives you valuable clinical insight that allows you to create personalized treatment plans for your toughest cases.

Molecular Methods Revolutionize Diagnostic Testing

In 1885, the first microbe was cultured. In 1969, breakthroughs in anaerobic microbial culture helped further define the GI ecosystem. But in 2000, molecular techniques, or DNA analysis, sparked what was initially described as a Renaissance and later called, the *"molecular revolution."*[1] These molecular methods, also used in the landmark Human Microbiome Project, made it possible to see 50 percent more microbes than had ever been seen before.[2-4]

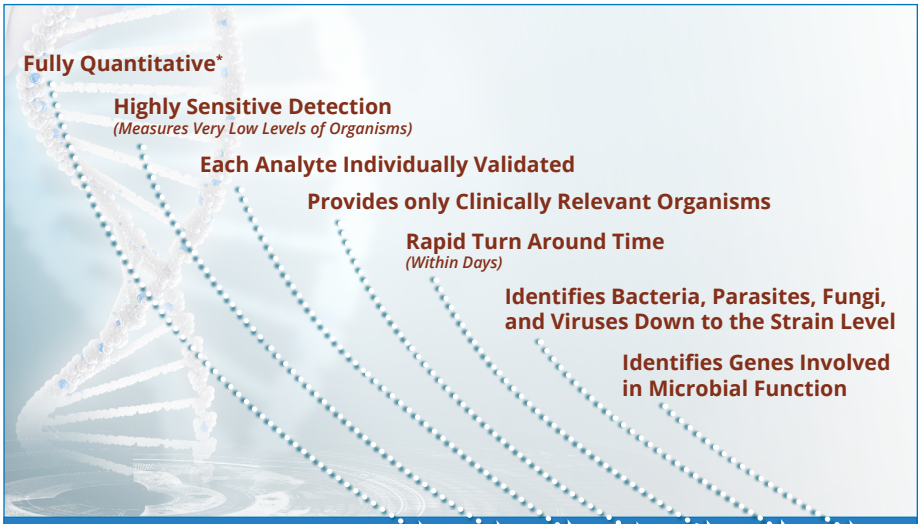
"qPCR is one of the most powerful and sensitive gene analysis techniques available."

qPCR is quickly becoming the standard for diagnostics due to the increased specificity, sensitivity, and reproducibility of PCR techniques. qPCR panels are able to rapidly detect viruses, parasites, and anaerobic bacteria, which can be missed by traditional methods.[5,6]

Government and private institutions around the world are incorporating it into patient care. The FDA had cleared three molecular-based gastroenteritis testing panels and was considering an additional four as of a 2015 article.[6] While there are many PCR diagnostic tests for gastroenteritis, the GI-MAP stands alone as the largest panel that quantifies each pathogen and non-pathogen, making the GI-MAP the best tool to assess both acute and chronic GI complaints.

GI-MAP®
GI Microbial Assay Plus

Comparison of Microbial Detection Methods



METHOD							
*GI-MAP® – DNA Stool Testing by qPCR**	+++	+++	+++	++	+++	+++	++
Standard PCR	–	++	++	++	+++	++	++
Shotgun Metagenomic Sequencing	–	+	–	–	–	++	+++
Metatranscriptomic Sequencing	–	+	–	–	–	++	+++
16S Sequencing	–	+	–	–	–	–	–
Culture + MALDI-TOF MS	–	–	+	+	+	–	–
Microscopy	–	–	+	++	+	–	–

** Only GI-MAP is Fully Quantitative!*

Definitions

Polymerase Chain Reaction (PCR) Methodologies are DNA replication methods that make numerous copies of a target sequence of DNA in the presence of primers (short, single-stranded sequences of nucleic acids) and DNA polymerase (the DNA-replicating enzyme). PCR methods are targeted approaches for rapidly detecting, identifying, differentiating, and quantitating specific microbes and genes of clinical relevance.

- ***Quantitative PCR (qPCR)*** or real-time polymerase chain reaction (RT-PCR) is widely used in biomedical research as well as in clinical diagnostics to accurately identify and quantitate specific organisms or genes present in a sample. In qPCR, segments of DNA that are highly specific for selected targets are amplified (replicated many times). Colorimetrically labeled DNA probes (single-strand nucleic acid sequences designed to bind the amplified target gene) make it possible to quantitate the amplification process as it occurs, in real time, yielding a truly quantitative DNA result.

- ***Standard PCR*** is similar to qPCR, except that it is not regarded as truly quantitative, because the amplified DNA is quantified only at the final stage of the PCR process, making it impossible to determine the true quantity of target DNA. It may instead be estimated by comparing it to a standard curve or by analyzing the quality and yield of PCR-products with gel electrophoresis.[7]

DNA or RNA Sequencing Methodologies determine the order of nucleic acids (adenine, cytosine, guanin, thymine) in a DNA molecule and count them.[8] They are untargeted approaches for obtaining a general, high-level profile of the microbes or genes present in the microbiome.

- ***Metagenomic and metatranscriptomic*** sequencing-based approaches provide a general profile of the microbiome. Both approaches involve high-throughput methods for sequencing nucleic acids, followed by advanced computational analyses on the resulting data to identify microbes, and either individual genes (DNA / metagenomic sequencing) or transcripts (RNA / metatranscriptomic sequencing). Metagenomic approaches can be used to identify potential microbial functions,

whereas metatranscriptomic approaches can be used to analyze gene expression patterns. These approaches can provide approximate, relative levels of organisms, genes or transcripts, but are not considered sufficiently accurate for true quantitation. The accuracy of any given sequencing technology may depend on the numbers of reads used. Read length is the number of base pairs sequenced from a DNA fragment. Sequencing depth refers to the number of times a given sequence has been read. Higher numbers of reads, often found in research settings, produce more accurate results. Lower numbers of reads, which helps minimize costs in commercial settings, produce less accurate results.

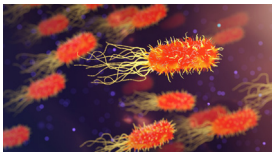
- ***16S Sequencing*** is similar to other sequencing methods, but only a single gene, 16S ribosomal RNA, is sequenced. The 16S rRNA gene is common to almost all bacteria and archaea, which are bacteria-like organisms. Determining an organism's abundance when using the 16S gene is not as accurate as other sequencing methods because the 16S gene varies widely in copy numbers.

Culture and Microscopic Methodologies

- ***Culture + MALDI-TOF (Matrix Assisted Laser Desorption/Ionization Time-of-Flight) Mass Spectrometry*** relies on bacterial culture of the fecal specimen. The fecal specimen is plated with at least four growth medias under specific growth conditions to optimize microbial growth. Isolated microbial colonies are recovered and may be examined for phenotypic properties or screened with biochemical tests for precise identification. Isolated organisms are then identified using the MALDI-TOF MS, which is a proteomic method that measures ribosomal protein fingerprints of microorganisms, compared to a reference database.

- ***Ova and Parasite Examination (O&P, Microscopy).*** A routine O&P detects parasites and ova in fecal specimens using macroscopic and microscopic characteristics. Microscopic evaluation consists of a direct wet mount, concentration, and permanent-stain smear. Fecal specimens are analyzed by a lab technician using a bright-field microscope. Accuracy is highly dependent on the expertise of the technician. Concentration methods, which are intended to increase the likelihood of finding ova, cysts, and larvae, can inadvertently reduce the numbers of cysts and ova in the sample. This may lead to underestimation of parasites in a stool specimen.[9,10]

Results You Can Trust



Each analyte on the GI-MAP is individually validated and meets or exceeds federal Laboratory Developed Test (LDT) and CLIA requirements. Before adding any organism to the GI-MAP report, the following analyses must be completed successfully:

- ***Assay specificity*** — the assay detects the intended organism and nothing else
- ***Assay sensitivity*** — the assay can measure accurately within a certain range of detection *(e.g. how low and how high the organism can be quantified)*
- ***Assay variation*** — if the same sample is tested multiple times, in different batches, on different days, the variation *(coefficient of variation, CV)* must be below 10%
- Reference range development
- Cross-assay comparison, when available

All organisms quantified by qPCR on the GI-MAP have less than 10% CV or variation, even though CLIA allows for 15% CV. This means that two identical samples, tested on different days, can only vary 10% from each other.

Validation reports are published internally, approved by company leadership before adding any organism to the test panel, and reviewed during CLIA inspections. We use validated DNA positive controls from vendors such as ATCC to test our molecular targets during assay validation.

All of our assays and laboratory personnel undergo proficiency testing, as required by CLIA. Proficiency testing is the analysis of unknown samples submitted by an authorized provider as a measure of external quality control.

All patient samples are tested alongside control samples, standard samples, and endogenous controls to meet quality control requirements.

- Negative controls contain no target DNA.
- Positive controls contain a known amount of target DNA.
- Standard samples contain known concentrations of each target organism at serial dilutions. They are run on a routine basis and are used to establish a calibration curve with a coefficient of determination (R2) > 0.95.
- Endogenous controls are target organisms detected in most clinical samples.

These quality control measures allow multiple checkpoints in the assay to verify adequate nucleic acid extraction and proper amplification. If results for positive or negative controls, standard samples, or endogenous controls are abnormal, or if results are questionable for any other reason, DNA is re-extracted and the assay is repeated. All patient results are reviewed at multiple levels of management.

Sample Reproducibility Data. One patient specimen was extracted for DNA and run eight different times by qPCR. All CVs were below 6.5%, indicating that assay variation was low. Cq is the quantitation cycle, or the result, from qPCR. Lower Cq values indicate higher starting copy numbers of the target DNA.

GI-MAP®
GI Microbial Assay Plus

GI-MAP Target Organism	CV	Quantitation Cycle (Cq)							
Blastocystis	**6.31**	10.76	10.22	12.4	10.78	10.72	10.95	10.27	10.54
Bacillus	**1.09**	13.53	13.52	13.51	13.3	13.31	13.53	13.76	13.41
Faecalibacterium	**1.01**	23.82	23.79	23.97	23.95	24.11	24.45	24.03	24.39
EHEC (eae)	**1.98**	24.56	24.51	25.79	25.53	24.75	25.33	24.74	25.46
Enterococcus faecium	**0.70**	15.45	15.5	15.43	15.34	15.15	15.3	15.37	15.36
Morganella	**2.01**	22.83	22.33	23.27	23.54	23.8	23.33	23.29	22.84
***Proteus* spp.**	**1.57**	25.47	25.42	25.15	24.44	25.01	25.39	24.83	24.58
Proteus mirabilis	**2.56**	23.79	25.47	24.81	24.07	23.61	23.97	24.15	24.72
***Pseudomonas* spp.**	**4.08**	18.86	19.81	18.87	20.06	20.66	19.06	20.99	19.99
Pseudomonas aeruginosa	**1.87**	25.13	24.84	25.6	24.72	25.5	25.35	24.31	25.6
Salmonella enterica	**1.54**	25.32	25.45	25.33	24.42	25.41	25.58	25.52	24.92
Staphylococcus aureus	**3.84**	25.84	25.62	24.17	25.62	23.19	25.12	24.28	25.69
***Streptococcus* spp.**	**3.07**	30.89	29.9	30.28	31.37	29.42	30.28	31.83	32.07

Leadership and Research Teams with a Proven Track-Record

Diagnostic Solutions Laboratory has a scientific team with elite, experienced molecular scientists with over 900 assays developed for publicly traded contract research organizations and clinical labs. Our medical director has over 200 scientific papers to her credit.

Our CEO was at the forefront of the planning, management, and launch of four successful stool analysis laboratories, prior to founding Diagnostic Solutions Laboratory. He is an expert in the ecology of the gastrointestinal microbiome, as measured by both traditional culture techniques and next-generation DNA analysis.

The Best and Most Comprehensive Interpretive and Educational Support

Our highly trained and knowledgeable research and medical education team helps you get the most clinically-relevant insights from the GI-MAP. We offer:

- Complimentary, 30-minute, one-on-one, consultations with clinical experts
- In-depth webinars by leading healthcare practitioners, covering the science and giving clinical examples
- A fully referenced 44-page white paper about the GI-MAP targets
- A comprehensive interpretive guide with treatment recommendations

GI-MAP®
GI Microbial Assay Plus

RESULTS YOU CAN RELY ON

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Diagnostic Solutions laboratory

Phone: 877-485-5336

Email: cs@diagnosticsolutionslab.com

Web: diagnosticsolutionslab.com