

# TESTING SNAPSHOT

## PROTEOMICS: A Revolution in the evaluation of Gastrointestinal Microflora



### MATRIX ASSISTED LASER DESORPTION/ IONISATION - TIME OF FLIGHT MS

#### High complexity clinical microbiology, as performed at DDI

Upon receipt the stool specimens in the transport media are mixed and one gram of stool is streaked onto a quadrant of ten different agar plates (5 aerobic, 3 anaerobic, 1 microaerophilic, and 1 yeast plate that contains chromogenic media). After plate-specific variable incubation times growth for each and every colony that grows is scored (0-4+), and individual colony forming units (CFU) are subcultured (selective agars and media) guided by the results of gram staining and basic biochemical discrimination. After the second incubation the pure isolated bacteria and yeast are ready for identification.

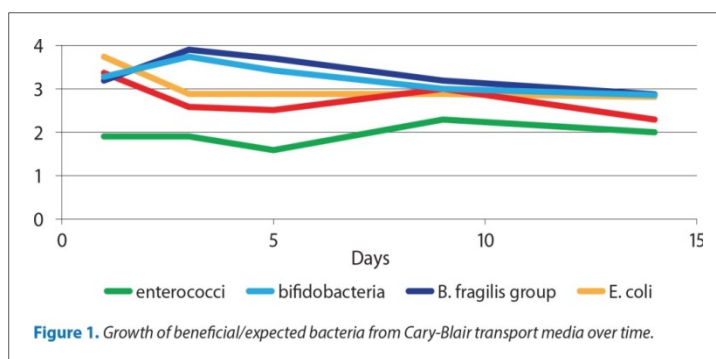
*Culture remains the current standard of practice in high complexity clinical microbiology testing.*

### Technology Timeline

#### Identification by Phenotype

- Manually by a vast array of time consuming biochemical reactions (phenotypic identification).
- Automated systems (e.g. vitek-2™) to perform the phenotypic identification processes.
- Markedly reduced manual labor, human error/judgment and time to identification.
- Limited with respect to the number of microorganisms that can be confidently identified.
- Can take as long as 6-24 hours and prolongs the time for subsequent antimicrobial susceptibility testing

*Doctor's Data utilizes a revolutionary state-of-the-science proteomic methodology for rapid, accurate and reliable identification of thousands of gastrointestinal bacterial and yeast species, and even sub-species.*



## Identification by Proteomic Analysis

Microorganisms have unique ribosomal protein “fingerprints.” Doctor’s Data utilizes a revolutionary state-of-the-science proteomic methodology for rapid, accurate and reliable identification of thousands of gastrointestinal bacterial and yeast species, and even subspecies. MALDI-TOF MS enables rapid and accurate identification of normal and pathogenic gastrointestinal microorganisms based upon their signature high-abundance proteins.

### Five Steps:

1. Ionisation of proteins from a pure isolate
2. separation of the vaporised, ionised proteins that have different masses
3. Detection of the number of the different ionised proteins, and
4. Collection of the data to generate the mass spectrum
5. Comparison of the unknown’s spectrum to a huge data base

PCR has proven utility for clinical microbiology use in hospital settings for rapid (<3 hours) identification of causative enteric bacteria associated with serious diarrheal diseases.

### **Sensitivity issue - Main issue with DNA:**

- Limited probes that would be very expensive
- Detection limit – they’re not very sensitive. The ability for a DNA sequence probe to fit lock and key with another DNA means it has to have access so they try emulsion technologies; they try and spread the surface area to access more of the stool sample. Still with this technology you need thousands and thousands of the organism to be able to pick it up.

## Matrix-Assisted Laser Desorption- Time of Flight

Laser based technology

- TOF – they use a laser to mobilize these proteins within these organisms and take a snapshot of it in that moment

DDI: Accuracy and sensitivity (sensitivity in that it can pick up even one single organism and culture it): With 600 stool tests – they had 100% accuracy and positive ID. Reproducibility: 99% using MALDI-TOF technology between results.

PCR (Polymerase Chain reaction) which is what DNA testing is (only allows for detection of general families – not sub-species)

To date using MALDI-TOF MS, DDI has been able to identify over 400 bacterial species and eight yeast species that were not identifiable using the previously employed automated phenotypic instruments.

Identification of yeast at the species level at a ranking score of just 1.800

### Culture growth testing

Detecting what is present (live bacteria). When we do that we are not only seeing the genotype but also the phenotype (what metabolic mode it’s in and therefore what it’s susceptible to and because if it’s a pathogen we want to know how to eradicate it so the susceptibility testing is the big ‘stand out’ in comparing the two methods).

### **Live culture growth – how is the organism kept stable in transit?**

- Cary-blair transport media
- Anaerobes are all stabilized
- The fast growers/ fast metabolism organisms are also stabilized
- refer to graph that tests stability of organisms

## Evaluation of a Commercial DNA stool Test

The results of the proficiency study indicate that the proprietary DNA methodology employed by the subject Laboratory was not able to detect pathogenic bacteria at levels that are known to be clinically significant, and grossly higher.

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*An unanticipated finding was that the proprietary DNA methodology yielded random and non-specific results for parasites and inconsistent results for yeast as well.*

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## Extremely Comprehensive stool Testing – More than pathogen detection

A Comprehensive stool analysis (CSA) will provide biomarkers for digestion and absorption, inflammation, and immune status. A functional assessment of microbiome functions is provided by measurement of short chain fatty acid (SCFA) production. Biomarkers of overall health include testing for stool pH, red blood cells, white blood cells, mucus and occult blood in the stool and a visual inspection of the stool is performed to assess color and consistency

### Microbiology

Culture and sensitivity testing remains the international standard in microbiology.

The rate of false positives and negatives from PCR testing, when compared side by side with conventional culture, may still be unacceptably high. Samples used for PCR testing cannot be cultured to provide microbial sensitivities to guide treatment with pharmaceuticals or natural anti-microbials.

Doctor's Data uses MALDI-TOF MS for the most accurate identification of species and even sub-species of bacteria and yeast.

### Inflammation Biomarkers

Inflammation is an integral part of disease processes that may be associated with Irritable Bowel Disease (IBD), gluten enteropathy, infections, or pharmaceuticals.

Lysozyme is a general marker of inflammation. It may be elevated from a variety of causes. Lactoferrin is a FDa-cleared specific biomarker for Inflammatory Bowel Disease.

### Parasitology

Slide microscopy and immunoassay testing continue to be valuable components of parasitology. MALDI-TOF is able to identify proteins specific to parasites. The blending of new and proven technologies will improve the sensitivity of parasite detection.

### Digestion and Absorption Biomarkers

Indication of protein, fat and carbohydrate digestion and use of specific digestive enzymes accordingly.

### Immunology

Secretory IgA (sIgA) is secreted by the gut mucosa and is an important component of the immune barrier. An elevated sIgA indicates an ongoing or acute immune challenge. A diminished level of sIgA may indicate either a chronic challenge that depletes sIgA or an inability to manufacture sIgA (primary sIgA deficiency).

## Short Chain Fatty Acids

Short chain fatty acids (SCFAs) are important biomarkers and protectors of colon health. The presence of specific beneficial bacteria (*Bifidobacterium* and *Lactobacillus* spp.) and SCFAs in the colon may improve cellular health and moderate cancer risks.

- Acetate – metabolized by peripheral tissues
- Propionate – used primarily by the liver
- Butyrate - primary fuel source for colonocytes

High levels of SCFAs may indicate simple carbohydrate malabsorption, decreased transit times or dysbiosis. Low levels usually indicate low populations of beneficial bacteria and/or insufficient intake of soluble fiber.

### Intestinal Health Markers

The presence of red blood cells (RBCs) may confirm infection or inflammation indicated by other biomarkers of the CSA. Local problems such as diverticulitis, hemorrhoids or anal fistula. The occult blood test indicates the presence of hemoglobin released from RBCs elsewhere in the GI tract.

### Macroscopic Appearances

Stool color and consistency may provide information that leads to further assessment and treatment. Stool consistency reflects the water content and transit time of the stool. Stool color may indicate various clinical disorders, once dietary color inputs (beets, greens, food colorings, etc.) have been accounted for.

## Why did DDI choose MALDI-TOF mass spectroscopy instead of a PCR-based technology?

The Cary-Blair transport media used by DDI is specifically designed with low levels of nutrients to suspend the growth of stool bacteria during transport, and inhibit the growth of other bacteria. The media is buffered to prevent shifts in pH during transport, and contains a reducing agent designed to markedly decrease oxygen levels to favor the facultative and strict anaerobic species known to inhabit the GI tract. When properly collected and shipped, beneficial and pathogenic species may be successfully recovered quantitatively from the transport media for up to 14 days.

## Microbiology: Results

**Growth quantified as colony forming units per gram stool (CFU/gm)**

- NG—no growth
- 1+ or rare (<103)
- 2+ or few (103-104)
- 3+ or Moderate (105-106)
- 4+ or Many (>107)



*First in Nutritional and Environmental Medicine*