CLINICAL APPLICATION GUIDE

ARRAY 22

ARRAY 22 – Antibody

IRRITABLE BOWEL/SIBO SCREEN™

CYREX® Laboratories

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CLINICAL APPLICATION GUIDE TO IRRITABLE BOWEL / SIBO SCREEN™

OVERVIEW

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder. Despite this, as many as about 75% of affected individuals do not seek medical care.[4] This is partially due to the lack of sensitive and specific evaluation strategies that has resulted in frustration for both patients and healthcare providers.

A large portion of IBS occurs exclusively after infection with bacteria such as *E. coli*, *Samonella*, *Shigella* and *Campylobacter jejuni*.[1 2] All these organisms produce in common cytolethal distending toxins, which have a major role in the disruption of both tight junction and cytoskeletal proteins such as vinculin, talin and α-actinin, thus weakening the epithelial cell structures. Dysregulated immunity, low-grade inflammation and altered gastrointestinal (GI) permeability, the presence of mood disorders, fatigue, fibromyalgia and migraine found in up to 70% of patients add complexity to the accurately diagnosing this disorder.[5] Currently, diagnosis is made by exclusion of disorders that mimic IBS and by focusing on IBS common symptoms.

Furthermore, it is common for patients with small intestinal bacterial overgrowth (SIBO) have symptomatologies that overlap with IBS.[6] SIBO is defined as the presence in the small intestine of excessive gut bacteria that originates from the colon. Similar to IBS there is currently no definite test for the diagnosis of SIBO.[3] The jejunal aspirate and bacterial culture test as well as the breath test after administration of fermentable substrate are both problematic and suffer from many false positives and some false negative results. Therefore, there is a need for a blood test for the determination of IBS/SIBO.

In IBS and SIBO patients, the goals of lab testing are to determine the cause and to establish the diagnosis as early as possible in order to initiate successful treatment regimens. One such lab test that was established based on animal models and in human subjects with IBS/SIBO is the measurement of antibodies against bacterial cytotoxins and cytoskeletal proteins in human blood.[7 8] This is based on the scientific fact that bacteria from the colon move up to the upper gut, and their concentrations become much higher than 10^5/mL. By producing cytotoxins, these bacteria affect the delicate environment of the small intestine, and then gain entry into the cell, where, by binding to the cellular DNA, they induce apoptosis (cell death). They then find their way to the submucosa, regional lymph nodes, and into the circulation. Immune response against the bacterial cytotoxins results in the production of IgA, IgM and IgG antibodies against them.

Due to the antigenic similarity between *E. coli*, *Salmonella*, *Shigella toxins*, *Campylobacter jejuni* and human cytoskeletal proteins such as vinculin and talin, these antibodies cross-react with the cytoskeletal proteins, causing depolymerization of and release of these proteins from the epithelial cells, with subsequent antibody production against the cytoskeletal proteins vinculin, talin, and actinin. These IgA, IgM and IgG antibodies against bacterial cytotoxins and cytoskeletal proteins are detected in the blood with high accuracy and reproducibility. Thus, bacterial over-growth can be an instigator of extra-intestinal autoimmunity. See **Figure 1**.

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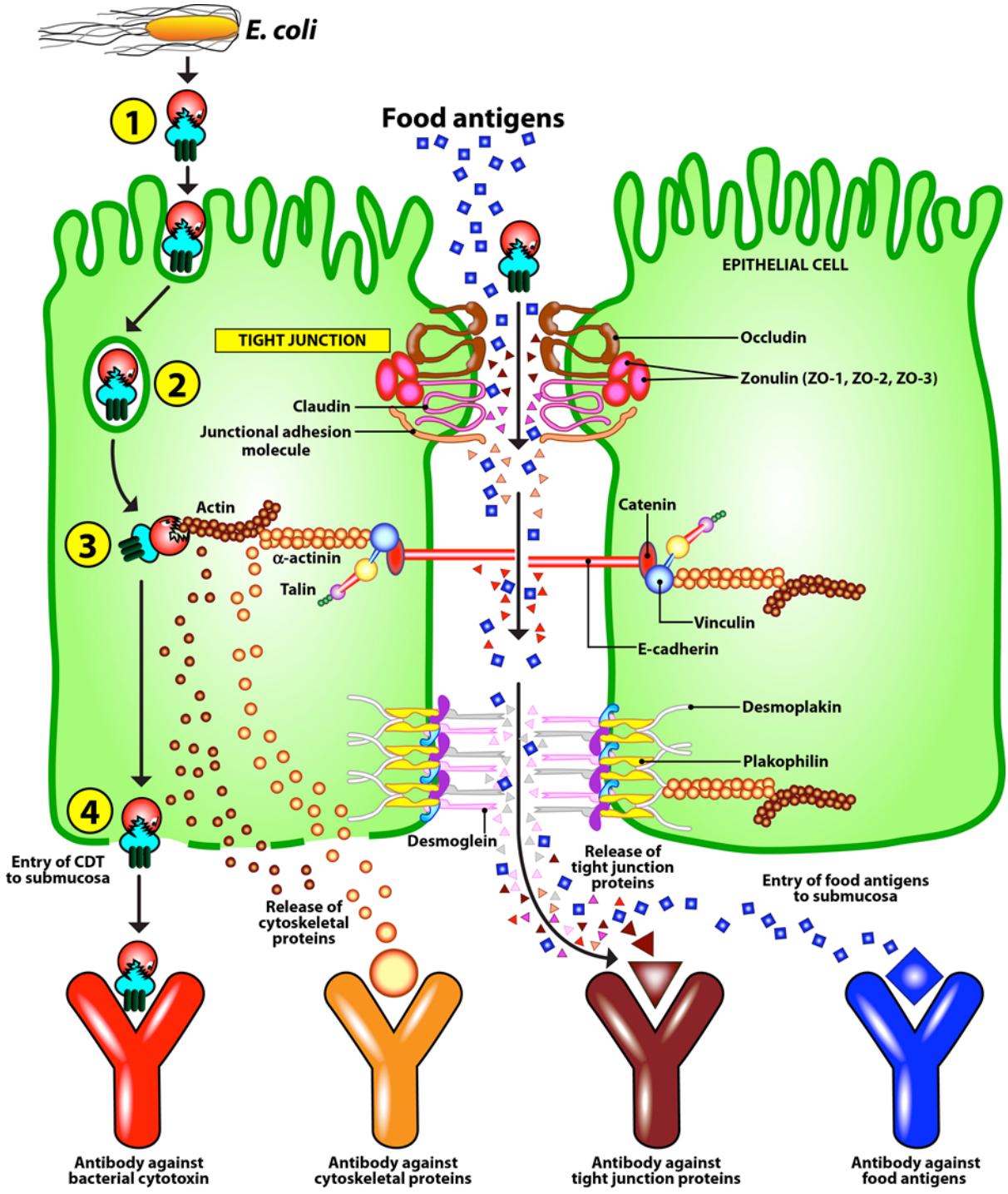

Figure 1. The Production of Antibodies Due to Bacterial CDT and Breakdown of Intestinal Cells. CDT released by *E. coli* (1) undergoes endocytosis (2), attacks cytoskeletal proteins (3), then enters the submucosa (4). CDT-assisted breakdown of the tight junctions results in the release of bacterial cytotoxins and cytoskeletal proteins, and the penetration of food antigens. Antibody production against these molecules can result in immunological activity within the gut and beyond.

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Irritable Bowels and Small Intestinal Bacterial Overgrowth

In North America, IBS prevalence is 5% to 10%, affecting any age, and is 3 to 4 times more common in women.[9]

According to Rome III criteria, set by a committee of leading gastroenterologists:

> patients experiencing abdominal discomfort or pain during the last 3 months for at least 3 days during a month and have at least 2 of the following: abdominal pain with change in stool appearance, abdominal pain with change in stool frequency, or improved abdominal pain with defecation,

can be diagnosed with irritable bowel syndrome (IBS). IBS is further subcategorized depending on bowel movements: diarrhea-predominant IBS (D-IBS), constipation predominant IBS, and alternating diarrhea/constipation IBS. If diarrhea-predominant IBS is preceded by a gastrointestinal infection, it is designated as post-infectious IBS (PI-IBS). Criteria for D-IBS is the presence of loose (mushy) or watery stools without the use of antidiarrheals or laxatives, ≥25% and hard or lumpy stool ≤25% of bowel movements.[9]

When diagnosing irritable bowel disorders, organic causes for symptoms must be excluded. Clinical presentations that prompt further investigation include:[reviewed in 9]

- weight loss
- fever
- hematochezia
- older age
- family history of gastrointestinal malignancy.

Physical examination should include:[reviewed in 9]

- peritoneal signs
- ascites
- masses
- hemoccult positive stool also constitute alarm signs.

Fibromyalgia, migraine and other non-gastrointestinal functional pain syndromes, as well as, mood disorders (symptoms worsen with stress)[10] are present in up to two thirds of patients with irritable bowel syndrome, whereas somatization (perceptive symptoms across multiple systems) occurs in 40%.[reviewed in 9] To compound IBS, SIBO can develop in IBS patients. Oder age and female sex are predictors of SIBO in IBS patients.[11]

SIBO is seen as a quantitative change in luminal bacteria of the small intestine disrupts digestion and absorption. According to Bohm et al, SIBO can result from the failure of the gastric acid barrier, failure of small bowel clearance, small bowel anatomic alteration, local and systemic immune deficiency and systemic disease associations.[12] Once coliform bacteria begin to thrive in the small intestine, a series of events can occur, which leads to SIBO. See **Figure 2.**

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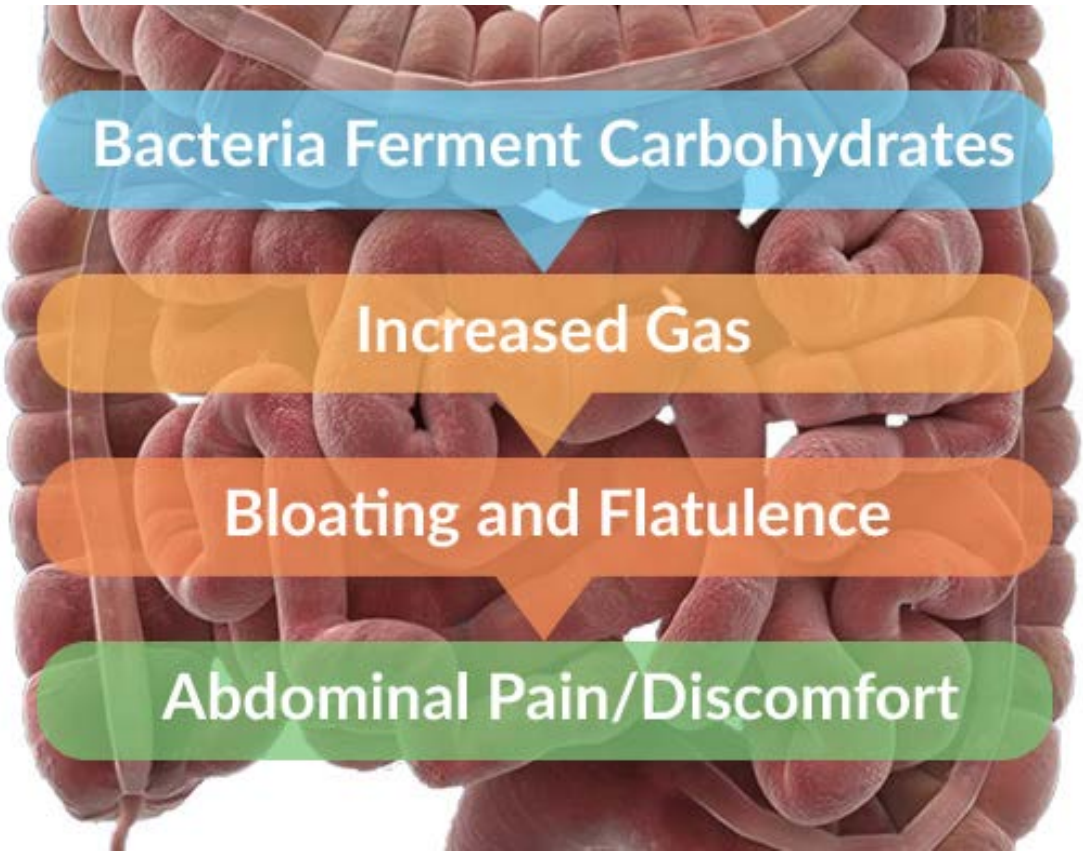

Figure 2. The Pathogenesis of SIBO. If coliform gram negative bacteria take up residence in the small intestine, the bacteria can ferment ingested carbohydrates, which increases gas production within the intestine, which results in bloating and flatulence, which then contributes to abdominal pain and/or discomfort.

Bacteria and the fermentation of carbohydrates produce toxic by-products that can further alter mucosa, gut immunity and damage the intestinal barrier. When the intestinal barrier is damaged, the body is at risk for autoimmunity. The mechanism of bacterial cytotoxin-induced autoimmunity is described below.

In the Microcosm

Older females are more likely to have IBS with SIBO than men.

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Due to the range of clinical symptoms expressed in patients with irritable bowels and/or SIBO, and the overlap in symptom presentations with other gastrointestinal disorders (Celiac disease, lactose intolerance, Crohn's disease and inflammatory bowel disease) identifying causes and accurate diagnosis can be challenging.

The diagnosis of SIBO has also been a challenge in that sensitive, specific and less invasive methods of detection has not been identified. Problems exist with each clinically-available assessments.[6] Popular breath tests for SIBO include lactulose hydrogen breath test (LHBT) and glucose hydrogen breath test (GHBT). LHBT results too many false positives, while GHBT has low sensitivity. The "gold standard" is the jejunal aspirate culture, which is a costly and invasive test.

Pimentel et al. have shown the sensitivity and specificity of assessing antibodies to bacterial cytotoxins[8] and to cytoskeletal protein,[7] in patients with D-IBS and in patients with D-IBS with SIBO. This combination of antigen assessments provides clinically useful information and can help differentiate D-IBS from IBD patients with chronic diarrhea.[8] Furthermore, Array 22 identifies, altered gut microbial populations, SIBO, intestinal cell damage, and even possible Celiac disease. See interpretation table below.

MECHANISMS OF BACTERIAL CYTOTOXIN- INDUCED AUTOIMMUNITY

As described above, altered gut microbiome can occur due to a variety of reasons. Once the gram-negative bacteria, thrive in their new environment, they may use their cytolethal distending toxins (CDTs) to infiltrate gut epithelial cells. CDTs have three subunits A, B and C designated as CdtA, CdtB, CdtC respectively. CdtA and CdtC bind to the epithelium cell wall, CdtB then enters the cell where it acts on the cell's nucleus to disrupt cell cycle and cause apoptosis (cell death).

The programmed death of epithelial cell results in a broken intestinal barrier allowing for the translocation of bacterial toxins such as CDTs and lipopolysaccharides (Array 2) from the gut into circulation. Systemic bacterial products contribute to systemic inflammation.

Cytoskeletal proteins from intestinal epithelial cells are also found in other body barriers. This production of antibodies against gut cytoskeletal proteins due to broken intestinal barrier may contribute to autoimmunity against the cytoskeletal proteins.

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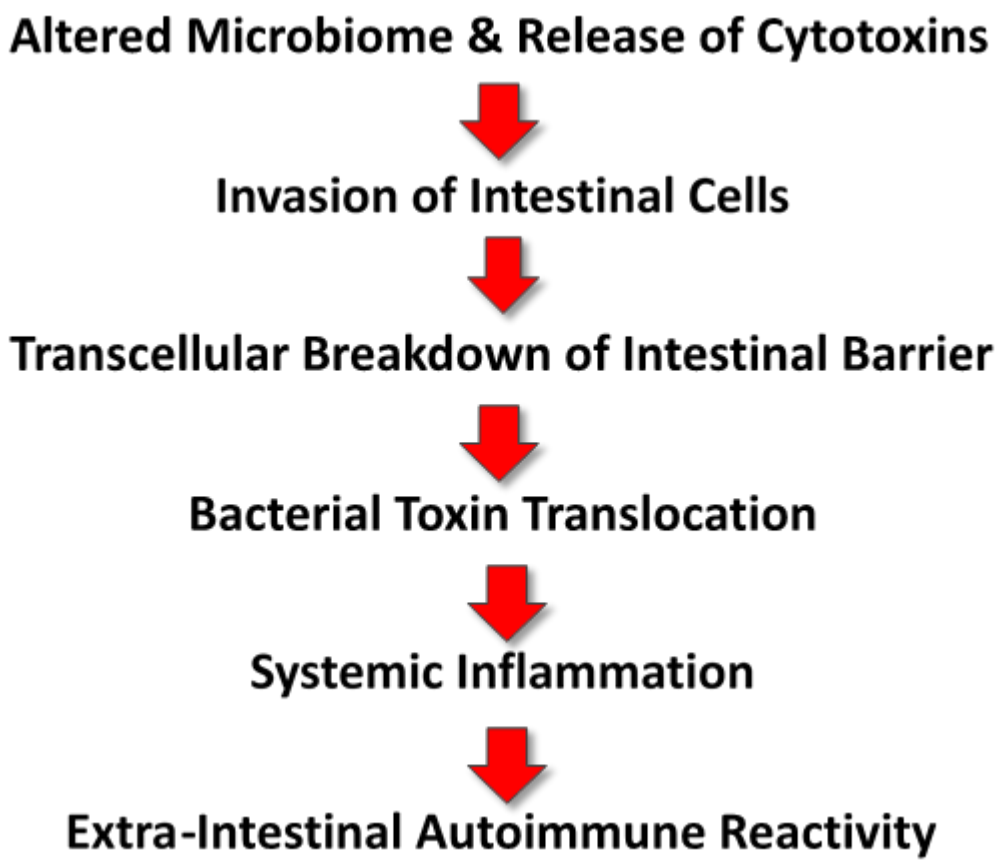

Figure 3. IBS/SIBO Mechanisms of Extra-Intestinal Autoimmunity. Something so small as bacteria can have far-reaching effects. When the intestinal microbiome is altered, cytotoxins from gram negative bacteria are released. These cytotoxins assist the bacteria in the invasion of epithelial cells where they attack the cell's DNA. This causes a breakdown in the intestinal barrier, releasing bacterial cytotoxins and other immunogens into the bloodstream. The systemic inflammation due to these immunogens may play a role in autoimmune reactivity in the body.

Mechanisms of extra-intestinal autoimmunity due to irritable bowels with or without SIBO may include a breakdown of the intestinal barrier followed by systemic inflammation or antibody cross-reactivity. See **Figure 3**. These three scenarios are known to play a role in autoimmunity. For greater details on the pathogenesis of autoimmune reactivity in relation to the antigens assessed on Array 22, please refer to the corresponding white paper. Furthermore, the survival of intestinal cells with DNA damage by CdtB may promote genomic instability, which can lead to gastrointestinal tumor initiation and/or progression.[13]

Some gram negative, diarrhea-causing bacteria, such as *Shigella*[14],and *Rickettsia*,[15] also employ molecular mimicry in order to bind to vinculin as an entry point into the host cell. *Shigella* injects invasion proteins into the epithelial cell which reorganizes the actomyosin network. Actomyosin calls vinculin to the cell interior wall where *Shigella* can access it. *Shigella* has engineered two vinculin binding sites that mimic those on talin. Vinculin is fooled, binds to *Shigella* and thus draws the bacterium into the cell.

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In the Microcosm

Bacteria can infiltrate and damage intestinal epithelial cells by:

- Molecular mimicry
- Excreting CDTs

CLINICAL ASPECTS OF ARRAY 22

The Cyrex System™ is a collection of arrays that assist the practitioner in identifying broken essential barriers, environmental triggers of autoimmunity and biomarkers of autoimmune reactivity, which can appear up to 10 years before the onset of clinical disease. Array 22 comprises both an environmental trigger (Bacterial Cytotoxins) and an indicator of a broken intestinal barrier (Cytoskeletal Proteins).

Cyrex's Array 22 – Irritable Bowel / SIBO Screen assesses IgG, IgA and IgM antibodies to Bacterial Cytotoxins and to Cytoskeletal Proteins. Cytotoxins are lethal xenobiotics released by commensal coliform bacteria. Bacterial cytotoxins can destroy epithelial cells of the intestinal barrier. Therefore, Cyrex also assesses antibodies to cytoskeletal proteins. Once this epithelial cell damage occurs, bacterial cytotoxins can freely enter circulation. Like systemic lipopolysaccharides (Array 2 – Intestinal Antigenic Permeability Screen), when bacterial cytotoxins enter the blood stream, they produce chronic inflammation and can open the blood brain barrier (Array 20 – Blood-Brain Barrier Permeability Screen). With Array 22, one can identify a trigger of irritable bowels and the specific intestinal barrier damage they cause.

In the Microcosm

Array 22, one can identify a trigger of irritable bowels and the specific intestinal barrier damage they cause

ARRAY 22 ANTIGEN ROLL CALL

The Array 22 – Irritable Bowel / SIBO Screen™ consists of cytoskeletal proteins and bacterial cytotoxins.

Please note: *A special white paper for these Array 22 antigens is available on the professional side of the Cyrex website. The white paper provides specific and detailed information about the antigens, the mechanisms of how bacterial cytotoxins destroy intestinal epithelial cells, and their role in autoimmunity.*

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Below are definitions of each antigen; please review the white paper for more in depth education on Array 22.

Bacterial Cytotoxins. Bacterial Cytotoxins are the cytolethal distending toxin, subunit B (CdtB) that is released by *Escherichia coli*, *Salmonella*, *Shigella* and *Campylobacter jejuni*. Utilizing subunits A and C, gram-negative bacteria can bind to human cells, allowing CdtB to infiltrate the cell. Inside the cell, CdtB contributes to cytoskeletal damage, which may induce apoptosis. CdtB is the first bacterial toxin known to act in the nucleus of a target cell. Bacteria such as *E. coli*, *Salmonella*, *Shigella* and *C. jejuni*, are members of the bacteria that participate in diseases that involve the disruption of a mucosal or epithelial layer.[16] By producing cytotoxins, these bacteria affect the delicate environment of the small intestine, and then gain entry into the cell, where by binding to the cellular DNA, they induce apoptosis. They find their way to the submucosa, regional lymphnodes, and into circulation where the immune system responds by producing antibodies against them. Indeed *in vitro* studies have shown that human epithelial cells are native targets of the CdtB expressed by these bacteria.[reviewed in 13] Cell infiltration by CdtB induces DNA damage which signals growth arrest at the G_2/M interphase of the cell cycle.[17] The epithelium is an early line of defense in the oral cavity and the gastrointestinal system against microbial assault. When damaged, bacteria collectively gain entry into the underlying connective tissue where microbial products can affect processes and pathways culminating in the destruction of the epithelial barrier and the underlying tissue. Antibodies against Bacterial Cytotoxins indicate gut dysbiosis with the potential for causing intestinal barrier damage.

Cytoskeletal Proteins. Cytoskeletal Proteins is the collective name given to intercellular adherent junctions that are involved in the integrity and functionality of the epithelial barrier. The major cytoskeletal proteins assessed in Array 22 are α-actinin, talin and vinculin. Alpha-actinin forms a lattice-like structure and stabilizes the muscle contractile, additionally α-actinin associates with signaling molecules. Talin is found in focal adhesions where it links integrins to the actomyosin network and either directly or indirectly interacts with α-actinin and vinculin. Vinculin is a cytoskeletal protein associated with cell-cell and cell-matrix junctions, where it is thought to function as one of several interacting proteins involved in anchoring F-actin to the membrane. Antibodies to Cytoskeletal Proteins may indicate intestinal barrier breakdown. A common mechanism of cytoskeletal breakdown is the infiltration of intestinal cells by bacterial cytolethal distending toxin-B (CdtB). Increased intestinal permeability to large macromolecules is a common consequence of mucosal inflammation, production of proinflammatory cytokines that disturb body homeostasis and enhanced exposure to environmental triggers including the external pathogens.[18] The broken barrier and entry of environmental antigens into the bloodstream may trigger autoimmune reactivity, which can lead to autoimmune disease.[19] If antibodies to Bacterial Cytotoxins are elevated in conjunction with Cytoskeletal Proteins, it may indicate irritable bowel syndrome with diarrhea (D-IBS) with small intestinal bacterial overgrowth (SIBO).[7 8]

Please note: Antibodies against α-actinin, talin and vinculin have been observed in extra-intestinal disorders including psoriasis, lupus nephritis and autoimmune hepatitis-1.[20 21 22 23]

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Influencing Factors

The development of bacterial cytotoxin and/or cytoskeletal protein antibodies depends not only on the amount and duration of the pathogen antigen exposure, but also on the genetic background,[24 25] diet[26 27 28 29] and lifestyle[30 31 32 33] of the person.[9]

Genetic

Although genetic and environmental factors both play a central role in autoimmunity, many times it is not clear which one is the main link to the heterogeneity of autoimmune prevalence. The importance of genes in autoimmunity was emphasized when it was noticed that the risk of autoimmunity is increased in twins and siblings of affected individuals.[34] Thereafter, gene analysis studies have confirmed the genetic relevance and suggested different methods for predicting the development of autoimmune conditions such as SLE, RA, DM1, and MS on an individual basis.[35 36 37] Immune and neurological system genetic variations that influence mucosal immunity and gut motility are thought to play a role in irritable bowel conditions. Although specific genes have been identified for Crohn's disease, researchers are still studying possible genes for D-IBS, with little success in duplicating positive studies via second cohort.[reviewed in 38] Familial grouping of irritable bowels is common; a family member of an individual with IBS is 2–3 times more likely to have IBS.[reviewed in 38] Thus, supporting a genetic predisposition theory. One risk locus, 7p22.1, which includes the genes KDELR2 (KDEL endoplasmic reticulum protein retention receptor 2) and GRID2IP (glutamate receptor, ionotropic, delta 2 (Grid2) interacting protein), shows promise in the first genome-wide association study on IBS conducted in 2014.[39]

Environmental Exposures

The development of bowel disorders may be influenced by the genes a person inherits together with the way the person's immune system responds to certain environmental triggers, such as stress, infectious agents and toxic chemicals.

It is important to consider:[9 12 40]

- Dietary risk factors
    - High sugar intake
    - High salt intake
    - Low protein intake
- Medical history
    - H. pylori infection
    - Proton pump inhibitor
    - Hypochlorhydria
    - Vagotomy
    - Gastrectomy
    - Gastric by-pass
    - Medications (opiates, tricyclic antidepressants, anticholinergics)
    - Connective tissue diseases
    - Resection of ileocecal valve
    - Duodenal and jejunal diverticulosis

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  - Mucosal and/or systemic immune deficiency
  - Celiac disease
  - Cirrhosis
  - Chronic pancreatitis
- Stress
  - Childhood exposure to victimization (verbal, physical, sexual abuse)
  - Loss of a parent at a young age
  - Learned illness behavior
  - Depression
  - Anxiety

Array 22 can be used to:

- Identify the overgrowth of large intestinal bacteria in the small intestine and the release of bacterial cytotoxin.
- Evaluate a breach of intestinal barrier by bacterial cytotoxins and their entry into circulation.
- Assist in setting guidelines for treatment of IBS/SIBO and reduced risk of igniting the autoimmune process.

Array 22 is recommended for patients who:

- Have irritable bowels.
- Exhibit symptoms of malabsorption, including weight loss, anemia or fatty stools.
- Have associated conditions such as fatigue, reflux, skin disorders, obesity or food intolerances.

CLINICAL INTERPRETATION FOR ANTIBODY ARRAY 22 – IRRITABLE BOWEL / SIBO SCREEN

Pimental et al. from the Gut Motility Program, Division of Gastroenterology, Cedars-Sinai Medical Center in Los Angeles, California, have conducted extensive research on bacterial cytotoxins and cytoskeletal proteins antibodies.[7] [8] The table below puts their work in a concise format..

Array 22 test results are not diagnostic for any clinical condition or disease. These reports may be used in conjunction with other pertinent clinical data for the purposes of diagnosis.

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Table 1. Interpretation of Array 22 – Irritable Bowel / SIBO Screen

Bacterial Cytotoxins	Cytoskeletal Proteins	Clinical Significance
+	-	Altered gut microbial populations, possible Celiac (follow up with Array 3 – Wheat/Gluten Proteome Reactivity & Autoimmunity)
-	+	Intestinal epithelial cell damage, increased intestinal barrier permeability, intestinal barrier autoimmunity
+	+	IBS and/or SIBO, altered gut microbial populations, intestinal epithelial cell damage, increased intestinal barrier permeability, intestinal barrier autoimmunity
-	-	If results are negative in a symptomatic patient, follow up with Arrays 2 and 10.

Array 22 contains an element of Environmental Triggers assessments available through the Cyrex System™ as well as an element within the Essential Body Barriers assessments. Depending on the result of Array 22, it may be logical to identify increased blood-brain barrier permeability (Array 20), or to assess gut pathogens (available on Array 12) or to assess Biomarkers of Autoimmune Reactivity (Arrays 5, 6, 7/7X). **Table 2** is a guide to potential follow up testing after Array 12.

Table 2. Suggested follow up testing. Based on Array 22 results and additional pertinent clinical data, these are suggested follow up testing for Array 22.

Positive Result	**Consider Follow Up Array**					
	2	**5**	**7/7X**	**12**	**20**	**3/4/10**
Bacterial Cytotoxins	X			X		X
Cytoskeletal Proteins	X	X	X		X	X

If Negative Result in a symptomatic patient, follow up with Arrays 2 and 10.

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SPECIMEN REQUIREMENT

2 mL serum
Ambient

RELATED TESTING

- **Antibody Array 2 - Intestinal Antigenic Permeability Screen**
- **Antibody Array 5 – Multiple Autoimmune Reactivity Screen**
- **Antibody Array 3 – Wheat/Gluten Proteome Reactivity and Autoimmunity Screen**
- **Antibody Array 4 – Gluten-Associated Cross-Reactive Foods and Food Sensitivity**
- **Antibody Array 10 – Multiple Food Immune Reactivity Screen**
- **Antibody Array 12 – Pathogen-Associated Immune Reactivity Screen**
- **Antibody Array 20 – Blood Brain Barrier Permeability**

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