Intestinal iQ™

Supporting Gastrointestinal Health

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Chapter 1: Intestinal Biomarkers

1.1 Zonulin

Zonulin (Pre-Haptoglobin 2) is a protein found in intestinal cells, with production and release mimicking the effect of certain bacterial toxins on the tight junctions of the small intestine. Zonulin binds to a specific receptor only on the luminal surface of the intestinal epithelia and triggers a cascade of biochemical processes that induces tight junction (TJ) disassembly and a subsequent permeability increase of the intestinal epithelia[1].This has often been referred to as 'leaky gut syndrome', paracellular leakage, or antigenic hyperpermeability.

Zonulin is the only physiological modulator of intercellular TJs described, to date, that is involved in the control of macromolecule movement and the balancing of the tolerance/immune response. Intestinal permeability changes due to Zonulin have been implicated in many diseases and dysfunction, notably in Celiac Disease (CD) and Type I Diabetes, but also in others chronic inflammatory disorders, including multiple sclerosis, eczema, asthma, non-celiac gluten sensitivity and recurring upper respiratory infections (URI)[2].

Celiac Disease is an autoimmune enteropathy triggered by the ingestion of gluten-containing grains in genetically susceptible individuals. It is a complex genetic disorder in which many gene associations have been identified. The status of HLA surface markers has been identified as accounting for up to 40% of the genetic load. The presence of either HLA-DQ2 or DQ8 is necessary but not sufficient to develop CD, as 40% of the general population also has either DQ2 or DQ8.

The α-gliadin fraction of the gluten glycoprotein can activate Zonulin signaling, irrespective of the genetic expression of autoimmunity. In genetically predisposed individuals, the access of α-gliadin to the submucosa may permit the interaction of T cells with antigen-presenting cells, including macrophages, leading ultimately to the antigen-specific adaptive immune response causing the autoimmune insult of the intestinal mucosa seen in patients with CD[3].

Zonulin signalling generates a two-way response: Not only can fluid exit, but intestinal contents are able to gain entry in the opposite direction; that is, into the apical area of the cell and eventually into the bloodstream. This gliadin-Zonulin 'leakage' effect is longer and more pronounced (up to 5-fold greater) in the intestinal cells of those with CD[3].

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When intestinal tissue is taken from CDs in remission and from non-CD controls with digestive complaints, results show that CDs may produce up to 20-30 times as much Zonulin as non-CDs and may have a three-fold greater intestinal permeability, even though the non-CDs are eating diets containing gluten, while the CDs have been gluten free for over two years[4]. This strongly suggests that something besides gluten may be contributing to the increased permeability in those with celiac disease. It may be that certain types of intestinal dysbiosis, such as improper intestinal balance of the microbiome, virome and mycobiome, may prime genetically susceptible individuals to develop CD in

response to gluten. In addition, many people who suffer from CD also suffer from other autoimmune disorders.
Increased levels of Zonulin are seen with the use of corticosteroids, NSAIDs[5], Diabetic medications[6], juvenile non-alcoholic fatty liver disease, as well as in the pathogenesis of insulin dependent Diabetes-type 1. In the latter, researchers have detected elevated Zonulin levels in 70% of patients who proceeded to develop type 1 diabetes 3.5 +/- 0.9 years later[8].

A very recent study has shown that elevated Zonulin concentrations in serum, as an expression of an impaired intestinal barrier, are a factor of relevance not only for the emergence of diabetes type 2, but also remain detectable even after a long history of the disorder, as well as correlating with the quality of metabolic control and BMI. A significant correlation was also detected between Zonulin and renal-function parameters; for example, a significant positive correlation between Zonulin concentration and Glomerular Filtration Rate (GFR) and a negative correlation with Urinary Albumin/Creatinine Ratio (UACR). The authors conclude that these correlations indicate that Zonulin may possess further effects in other organs that extend beyond the already known influence on the intestinal barrier[7].

There is also evidence of its implication in inflammatory bowel disease and obesity[8,9], rheumatoid-arthritis[5,9], asthma[10,11], as well as in multiple sclerosis (MS). Patients with MS show increased permeability of both the blood-brain-barrier (BBB) and the intestine. In addition, intestinal permeability and intestinal Zonulin are increased during the pre-clinical phase of neurological symptoms, suggesting a role for Zonulin in disease development[11].

Therefore, Zonulin plays an important role in permeability changes in the brain, working as a gatekeeper, not only in the intestine, but also at the blood brain barrier (BBB). This is clearly in evidence when there is an intake of foods containing α-gliadin or similar proteins. The resulting high Zonulin levels leads to disassembly of the TJs in the vascular epithelium, permitting many molecules, including toxins, to slip through the BBB, resulting in activation of a cerebral inflammatory response. Whether this due to microglial activation, as seen in traumatic brain injury[11], or other neurological processes, is an area that is becoming of great interest for clinicians. The resulting symptoms may include anxiety, depression, brain fog, slow mental processing, and emotional disturbances. Over time, this chronic inflammation may progress to neurodegenerative conditions such as dementia, Alzheimer's, and Parkinson's disease[12,13].

Zonulin is also involved in the regulation of airway and lung permeability through its action on the TJs of the respiratory epithelial and/or endothelial barriers. This can be seen in asthma, a complex clinical syndrome characterized by airflow obstruction, airway hyper responsiveness and inflammation. Increased intestinal permeability, as well as permeability in similar tight junctions of the respiratory epithelium in asthmatics, may play a role in their susceptibility to environmental allergens. This relationship is now an active area of research[14]. Recent evidence has shown that serum Zonulin levels are high in a subset of subjects affected by asthma, with 40% of asthmatic patients exhibiting increased intestinal permeability. This suggests that, besides inhalation, an alternative route for the presentation of specific antigens or irritants may occur through the gastrointestinal mucosal immune system, following their intercellular passage through the TJs[4].

The effect of Zonulin is also evident in lung infections, including Acute Lung Injury (ALI). The role of Zonulin in ALI links the regulation of permeability with the inflammatory response through direct activation of the complement system, specifically the generation of complement C3a and C5a. This suggests that Zonulin facilitates development of ALI both by enhancing albumin leak and complement activation[15]. This makes testing for complement a possibly desirable screening tool, with Zonulin testing as confirmation.

Levels of Pre-Haptoglobulin are controlled by the absence or presence of the relevant Haptoglobin (HP) gene on chromosome 16. There are 3 variants. The HP 1-1 genotype with zero copies of the Zonulin gene, the HP 2-2 with two copies and the HP 1-2 with one copy. The HP 1-1 variant is highly decreased in several immune-mediated diseases, such as Celiac, Crohn's and Schizophrenia, whereas the HP 1-2 and 2-2 are significantly increased. In addition, with the HP1-1 variant the Zonulin levels remain in the very low range, even when some form of inflammatory or autoimmune disease is highlighted by other biomarkers.[4] The percentage of individuals with this variant is estimated to be in the 10-20% range. These individuals are thought to produce a Zonulin-like molecule that acts in a similar fashion to Zonulin (personal communication).

Why test for Zonulin?

Zonulin plays a pivotal role in the control of the tight junctions of the small intestine. As mentioned above, increased Zonulin levels are seen in many conditions and diseases associated with increased intestinal inflammation, with changes in permeability preceding clinical manifestations by up to a year[16,17]. For that reason Zonulin is gaining acceptance as a non-invasive marker of intestinal wall integrity and developing disorders.

1.2 Histamine and Diamine Oxidase

Histamine is a biogenic amine, known to be involved in 23 different physiological functions, which include local immune responses, neurotransmission, as well as regulation of physiological function in the gastrointestinal tract.
Histamine is synthesized from the amino acid histidine by the pyridoxal phosphate-dependent (Vitamin B-6) L-histidine decarboxylase[18]. This reaction can be modified by ascorbic acid. Histamine is either stored, or rapidly metabolized and inactivated, by either of two degradative enzymes; histamine-N methyltransferase or diamine oxidase (DAO), the latter being the principal enzyme observed in the digestive tract.
The testing of histamine, together with DAO levels, provides important information that is not revealed with standard food sensitivity tests. Food sensitivity reactions are thought to be the underlying cause of gut inflammation and dysfunction. The intestinal epithelial lining can become inflamed as a result of immune activity initiated by certain food antigens, with this inflammatory effect on the cell lining leading to a subsequent disruption in normal DAO production. However, often the reason may actually be an imbalance of accumulated histamine and the capacity for its degradation by enzymes such as DAO. This imbalance may result in a condition known as Histamine Intolerance (HIT). Very

recent work has highlighted the daily fluctuations of histamine and DAO in symptomatic individuals[19]. The results showed that decreased DAO activities correlate with elevated histamine levels in a significant subgroup (24%) of those suspected of Histamine Intolerance, and that these findings help discriminate these individuals from those with food intolerance that exhibit similar clinical symptoms.

Histamine intolerance is not really a diagnosis as much as it is a description of a series of symptoms. The more accurate description of this condition is Mast Cell Activation Disorder (MACD), which is characterized by the accumulation of mast cells that are genetically altered (mastocytosis) and/or by the abnormal release of mast cell mediators. The latter is referred to as Mast Cell Activation Syndrome. Because mast cells are found in all human tissues, and mast cell mediator receptors are found on almost every cell in the body, MCAD has the potential to affect all body organ systems[20]. The term HIT will be utilized in the present document. HIT is an acquired problem and is seen in approximately 3% of the population. Symptoms may occur in up to 20% of the population when histamine-rich food is consumed together with DAO inhibitors, such as alcohol. Women represent about 80% of those affected, with most of them aged 40 or over. It is important to note that the risk of developing this intolerance is increased in those individuals who suffer from inflammatory intestinal diseases or cross-sensitivities.

Why test for Histamine?

Histamine is involved in many inflammatory and allergic processes, including both immediate and delayed hypersensitivity reactions. Histamine excess can be triggered by its release in the body as a result of a variety of environmental triggers, from the ingestion of foods with high histamine content, a deficiency in DAO, or both. Antigen inhalation can similarly induce mast cells and eosinophils to infiltrate in the esophageal epithelium via a histamine-mediated mechanism[21].

High levels of histamine - signs, symptoms and conditions:

- Runny nose, sneezing, congestion
- Itching, hives, skin flushing
- Dizziness or vertigo
- Headache, migraine
- Nausea, vomiting
- Intestinal cramps, gas
- Diarrhea
- Abnormal menstrual cycle
- Shortness of breath
- Abnormal heart rate
- High blood pressure
- Severe allergic reactions (anaphylaxis)
- Abnormalities may also arise in the following:
  - Memory
  - Body temperature
  - Circadian rhythm
  - Locomotion
  - Learning

What causes high histamine levels?

- Allergies (IgE reactions)
- Gluten Intolerance
- Small Intestinal Bacterial Overgrowth (SIBO)
- Intestinal Permeability (“leaky gut syndrome”)
- Gastrointestinal bleeding
- Histamine-rich foods
- Medications:
  - Non-steroidal anti-inflammatory drugs (eg: Ibuprofen-Motrin, ASA-Aspirin)
  - Antidepressants (eg: Effexor, Zoloft, Prozac, Cymbalta)

• DAO deficiency or DAO-blocking foods: alcohol, energy drinks, and tea
• Genetic mutations (common in people of Asian descent)
• Inflammatory bowel diseases: Crohn's, ulcerative colitis

- Immune modulators (eg: Enbrel, Humira)
- Antiarrhythmics (eg: Propanolol, Norvasc, Cardizem)

Low levels of histamine (Histapenia): Signs, symptoms and conditions:

• Fatigue
• Sleep-wake disorders
• Depression and anxiety in older adults; paranoia in younger people
• Convulsions

What causes low Histamine levels?

Excess copper can create low levels by decreasing histamine in the brain. In turn, the lowered levels of histamine allow more copper to accumulate. High copper in the brain may lead to a state of restlessness, insomnia, violence, depression, irritability, paranoia, and high blood pressure.

Why test for DAO?

The ingestion of histamine rich food, alcohol or drugs, as well as almost any inhalant allergens, that release histamine or block DAO, may provoke an imbalance of accumulated histamine and the capacity for its degradation, already referred to above as Histamine Intolerance (HIT). An impaired histamine degradation, based on reduced DAO activity and the resulting histamine excess, may cause numerous symptoms, mimicking an allergic reaction[22].

DAO activity does not depend on the DAO alone, but also on cofactors such as vitamin C, vitamin B6, copper or manganese ions. Copper is a central component of DAO. A deficiency in copper can result in insufficient DAO being produced. Vitamin B6 is a cofactor of DAO. If vitamin B6 is missing, DAO is unable to degrade histamine. Therefore, in assessing HIT via the DAO activity test one should also consider determining the levels of these cofactors.

The symptoms of HIT can be caused by low DAO activity because the above-mentioned cofactors are not sufficiently available. By quantifying the levels of these co-factors it can also be determined which one needs to be supplemented.

If the DAO levels are in the normal range but the histamine levels are high, it may indicate that the issue is not insufficient DAO, but rather an overproduction of histamine, due to factors such as gut dysbiosis. In such a case, improving intestinal function together with a low-histamine diet would probably be sufficient, with no additional intervention required.

However, if the histamine levels are normal, but the DAO levels are very low, it suggests a possible genetic deficiency of diamine oxidase. In such cases what may be most helpful is to increase the level of diamine oxidase, most easily accomplished by DAO supplementation.

Low levels of DAO: Signs, symptoms and conditions:

- Skin rash and pruritis (itching), urticaria (hives), eczema, psoriasis
- Nasal congestion, asthma
- Headache, migraine
- Chronic fatigue
- Anxiety, depression
- Inflammation, irritable bowel syndrome (IBS)
- Estrogen dominance, dysmenorrhea, Premenstrual Syndrome (PMS)
- Muscular pain, fibromyalgia
- Rheumatoid arthritis
- Hypertension, hypotension, arrhythmia
- Multiple sclerosis and other neurological conditions

Determination of DAO activity, together with a detailed history, helps to differentiate food allergy and histamine intolerance[19]. It should be performed in suspected patients who have symptoms such as headache, urticaria, pruritus, diarrhea and hypotension, where food allergy has been excluded[23]. Individuals who are unable to metabolize histamine will often improve with a variety of antihistamines. DAO acts on histamine both inside and outside the body. In the body DAO is found principally in the kidneys and the thymus gland, but it is not reliably found in other organs. The digestive tract is essentially an area outside the body, with input of material at one end and output at the other. Only the digested products enter the body through the epithelium and pass into circulation. DAO is found in the ileum and jejunum of the small intestine. Because DAO formation occurs in the gastrointestinal system, lower than normal levels are suggestive of poor digestive dysfunction, as well as problems in the intestinal barrier[24].

The Importance of the DAO: Histamine Ratio

The DAO: Histamine Ratio is helpful in highlighting the imbalances in DAO and histamine levels.

High Ratio: There is enough DAO enzyme available for histamine degradation, or that there is a relatively low level of free histamine in the system.

Low Ratio: There is insufficient DAO enzyme available to degrade the relative amount of free histamine. The lower the ratio gets to 1, the more evident that becomes. It should be noted that even if DAO levels are normal, symptoms may still occur if the histamine levels are very high.

DAO: A Biomarker of Intestinal Barrier Integrity

The degradative enzyme, Diamine Oxidase (DAO), is the principal enzyme observed in the digestive tract which scavenges extracellular Histamine. Recent research has begun to shed light on another important aspect of DAO activity, which is unique among intestinal mucosal enzymes: Circulating blood levels of DAO represent a reliable marker of mucosal maturation & integrity[25]. Indeed, serum DAO activity has been shown to correlate with intestinal permeability of the small intestine[26].

Intestinal Barrier Changes

Intestinal barrier damage is due to a number of potential causes, amongst them mechanical, microbial and/or enzymatic action. These vectors of damage can be due to:

- Genetic predisposition
- Dysbiosis
- Infections
- Loss of luminal mucous barrier, with a concomitant decrease in levels of SIgA and antimicrobial peptides (AMPs).
- High luminal histamine levels from endogenous or exogenous sources, or both. Increased histamine can occur due to gut microbial action, a diet rich in histamine or histidine, an increased mast cell response as part of an immune reaction and acute or chronic stress.

DAO is synthesized by mucosal cells of intestinal villi crypts. The damage caused by one or more of the factors above can lead to a loss of barrier integrity, which in turn can lead to further inflammatory reactions. All of this will often lead to increased intestinal permeability, which will then enter into a vicious cycle of more inflammation and barrier damage. During the period of initial damage, the mucosal cells release increased amounts of DAO which increases its serum concentration. Therefore, a change in blood DAO concentration is an indication of damage to the intestinal cavity. High DAO in blood is tightly linked to abnormal intestinal barrier function in an acute stage[27].

With more chronic damage, as often seen in cases of inflammatory bowel diseases (IBD), such as Chron's disease or Ulcerative Colitis, one begins to encounter apoptosis of the crypt cells within the villi. This decrease or loss of DAO levels in the gut lumen & blood is a sign of chronic or late stage mucosal damage to the barrier, and is a sign of breakdown in cell architecture. More specifically, it is a decrease in DAO production capacity due to the destroyed or dysfunctional specialized mucosal cells[26].

This makes DAO a sensitive & accurate marker for monitoring Crohn's Disease activity and other inflammatory bowel conditions.

The DAO:Histamine Ratio and Intestinal Permeability

As stated previously, the DAO:Histamine Ratio is helpful in highlighting the imbalances in DAO and histamine levels.

High Ratio: Normally a high ratio is an indication that there is enough DAO enzyme available for histamine degradation, or that there is a relatively low level of free histamine in the system. A very high ratio, due to high DAO in the face of low Histamine, could be a sign of early breakdown in barrier integrity.

Low Ratio: When low levels of the DAO enzyme are available to degrade normal or high amounts of free Histamine, it is often a sign of abnormally low production of DAO, due to a breakdown in cell architecture, or even cell destruction, as seen in cases of IBD.

1.3 Total IgG Food Sensitivity

Reactions to foods are common. They fall into 3 general categories: Food allergy, auto-immune reactions and food sensitivities. Most reactions, are caused by food sensitivity, rather than by food allergy. People often confuse the two, given that food sensitivity can cause some of the same signs and symptoms as food allergy[28].

The body's immune system is categorized by five distinct types of antibodies, often signified by the following letters: E, M, G, A, D. The immunoglobulin G group, most often shown as IgG, represents about 80% of all antibodies found in blood and is responsible for most food sensitivity reactions.

Food allergy, also known as a Type I Hypersensitivity, is associated with IgE antibodies and affects a small percentage of the population. IgE food allergy is potentially a very serious health condition and can be triggered by any food, even in small amounts. It is caused by a rapid mast cell reaction, causing the release of histamine and other protein cytokines, which in turn produce an immediate hypersensitivity reaction, with symptoms appearing in minutes or hours. These symptoms, which appear very quickly and usually last no more than several hours, usually involve skin rashes, swelling of the lips and throat, vomiting or diarrhea, as well as possible difficulty breathing, a precipitous drop in blood pressure and occasionally a severe, life threatening reaction, called anaphylaxis.

Food sensitivity, or Type III hypersensitivity, is a delayed reaction that may take weeks or months to fully develop after food intake. When a reactive food is consumed, antibodies form complexes with food protein antigens, but the resulting immune complexes are normally eliminated by the macrophages of the immune system. If a large number of these complexes is produced and not entirely eliminated, the remaining complexes can enter various tissues of the body, resulting in an inflammatory response and possible symptoms[29].

These symptoms, related to an IgG food sensitivity reaction, may take months to appear and become clinically apparent. This IgG-mediated inflammatory response is not short acting, as is the IgE allergic reaction. Rather, the reactions associated with an IgG immune response may

last for many weeks or months, **even after the offending food has been eliminated from the diet**[30].

The FLUIDS iQ® Total IgG Food Sensitivity Test measures the presence of IgG antibodies to specific food proteins that are produced by the immune system when certain foods are eaten. The patient's serum is introduced to proteins prepared from a group of different foods. If a specific union occurs between the protein antigen and the patient's serum IgG antibody, a complex is formed. A food elimination diet can be established and improvement of symptoms can be monitored.

Food sensitivities can develop at any time during one's life and can change significantly throughout one's lifetime. As stated above, unlike food allergies, symptoms may be delayed for days or weeks after exposure, making a diagnosis more difficult.

Common Symptoms of Food Sensitivity:

- Abdominal and stomach pain, bloating, cramps
- Constipation and diarrhea
- Gastritis
- Headache, migraines, fatigue
- Itchy skin
- Bronchitis, sinusitis, rhinitis
- Weight control problems
- Water retention

It should be noted that not all reactions to food are due to an immune system reaction. Indeed, reactions may be caused by a condition known as **lactose intolerance**, which can result in abdominal pain, cramps and bloating after the intake of various dairy products. Lactose intolerance is due to a lack or deficiency in lactase, the enzyme that breaks down lactose sugar in milk, and is not an immune reaction.

As mentioned in the previous section, certain individuals are sensitive to pro-inflammatory proteins such as histamine. There is an increasing understanding that overproduction is a root cause. Overproduction typically is due to two main causes. The first is gut dysbiosis, since we know that certain bacteria produce histamine and other types degrade it. If there is an overrepresentation of the histamine producing bacteria, this could lead to excess histamine production. The second cause is mast cell activation syndrome (see above), which involves an overactivation of mast cells. Although poorly understood, genetics almost certainly plays a role Whatever the precipitating cause, these individuals will exhibit many symptoms associated with 'histamine intolerance'. This may be due to the ingestion of foods that are high in histamine, a deficiency in diamine oxidase, the enzyme that breaks down histamine, or a combination of both.

Although most health professionals agree that foods can produce IgG antibody-antigen immune complexes, there are varying opinions on whether these complexes cause inflammation and

subsequent symptoms.

However, there is a growing body of evidence that IgG food sensitivity reactions are involved in a number of conditions and diseases that show improvements when the reactive foods are eliminated from the diet. These conditions include obesity[31], migraines[32] and irritable bowel syndrome[30], as well as fibromyalgia, arthritis, GERD and metabolic syndrome. There is also growing evidence of its role in conditions such as ADD/ADHD and autism.

The common feature of all food-induced inflammatory reactions is that they trigger the release of mediators, such as cytokines, prostaglandins, and others. Their release comes from a myriad of white blood cells, including neutrophils, monocytes, eosinophils and lymphocytes. This holds true whether the reactions are immediate or delayed, whether they are controlled by the innate or adaptive immune systems and whether their mediation is cell or humoral. In short, all food induced inflammatory reactions involve mediator release, which is the single most important event that may lead to all the effects suffered during the reaction, including the generation of symptoms.

1.4 Candida albicans

What is Candida?

The microbiome contains trillions of microbes, including bacteria, fungi, viruses and parasites. When this delicate ecosystem becomes imbalanced it can be associated with weight gain, cardiovascular diseases and various forms of autoimmunity. *Candida albicans* is a member of the fungal family, but is typically referred to as a yeast and is found in about 80% of fungal isolates. It naturally coexists alongside other microbes in many areas of the body, including the skin and the mucous membranes of the mouth, throat, gastrointestinal tract and vagina. In low concentrations, *Candida* helps maintain a healthy balance of microorganisms in these different areas. It is normally found in small amounts in the body, and is the only fungal species belonging to the 'normal' microflora. It also supports the immune system, aids in digestion, and is able to promote vitamin and mineral absorption from food. However, if the body develops *Candida* overgrowth, or candidiasis, it can create an imbalance.

***Candida Overgrowth**:*

A) Common Signs and Symptoms

- Skin changes (acne and eczema) and nail fungal infections
- Bowel issues (constipation and diarrhea)
- Mood changes (brain fog, anxiety or depression)
- Inability to lose weight

The skin and mucous membrane infections may be exacerbated by factors such as pregnancy, diabetes mellitus, immune deficiency and therapies with cytostatic drugs or antibiotics. Infections of

organs like the lungs may cause death in immune suppressed patients with a cellular immune deficiency[33]

B) Causes

There are many different diet, lifestyle and medical factors that can cause *Candida* to grow out of control.

- **Use of antibiotics**. Antibiotics destroy bacteria that help control *Candida*. As these bacteria are depleted, *Candida* is free to multiply.
- **A diet high in processed foods and sugar**. *Candida,* and other yeast species, multiply in a high sugar environment,
- **A weakened immune system**. *Candida* is an opportunist, so an overgrowth often occurs when recovering from illness, while the immune system is still weak. This is further accentuated by the use of antibiotics in combatting the illness.
- **Stress**. Too much stress can create changes in the gut environment. If the intestine is already imbalanced, due to the use of antibiotics or diet, an increase in stress can make this imbalance worse and enhance the overgrowth of *Candida* and other yeasts.
- **Hormonal imbalances.** Hormonal therapy, for HRT or birth control, can lead to increased *Candida* overgrowth. Specifically, women who have increased levels of estrogen are at a higher risk of yeast infection due to *Candida* overgrowth. An imbalance of the natural estrogen and progesterone ratio is a significant sign of this overgrowth.

C) Confirmation of Candida overgrowth

Visual signs, such as skin rashes, are important as evidence of fungal infections. So are high stress, the recent use of antibiotics, as well as a high carbohydrate diet.

Blood Tests: Blood tests, either in serum or blood spot, can give a quick indication of *Candida* overgrowth. The test checks for levels of antibodies; Specifically, the immunoglobulins IgM, IgG and IgA. A blood test is often the preliminary test, given that it reflects an activated immune response to either an acute or chronic infection. Due to colonization of mucous membranes with *Candida albicans*, and its passage into the host's blood stream, the humoral immune system is stimulated, which results in the production of antibodies against *Candida albicans*[34].

a. IgM antibodies: These are the first of the immunoglobulin isotypes formed after a primary exposure to the *Candida* antigen, reflecting a present infection. Typically, these antibodies develop as the predominant immunoglobulin early in the course of an infection and then decrease in number over a short period of time, measured in days. Infections of the bloodstream have serious consequences unless controlled quickly and the rapid production of IgM, together with its efficient activation of the complement system, are important initiators of that control. This complement activation assists the phagocytic system in the elimination of

antigens from the intravascular space[35]. Upon reinfection, IgM antibody levels may often not be as elevated as in the earlier infections.

b. IgG antibodies: These immunoglobulins are the most predominant isotype formed from secondary exposure to the Candida antigen, and reflect a past or more prolonged ongoing infection[36]. They are produced in increasing numbers as the IgM antibody levels decrease after the primary exposure. IgG antibodies are smaller in size than IgM and diffuse easily out of the blood into the tissues. They activate complement and assist the phagocytic system in eliminating the antigen from the extravascular spaces[37].The IgG antibodies represent the largest class of human immunoglobulins and are evenly distributed throughout both intra and extravascular fluids. Specific IgG antibodies may remain for many years after an infection has been eliminated.

c. IgA antibodies: These antibodies represent only 15-20% of human serum immunoglobulins. However, they are by far the most predominant antibody class found in seromucous secretions[35], playing an important role in the local mucosal immune responses that occur in the digestive, respiratory and vaginal tracts, as well as in saliva and tears[37]. High levels of serum IgA antibodies are thought to be associated with mucosal, epithelial, tracheobronchial, and genitourinary candida infections. IgA is less potent than IgG as an opsonin; that is, it is less able to bind to foreign microorganisms, so as to make them more susceptible to phagocytosis. Also, unlike IgG, IgA is a weak activator of complement. This distinction is not surprising, since IgG functions mainly in the body tissues, where accessory cells and molecules are available, whereas IgA functions mainly on epithelial and mucosal surface environments, where complement and phagocytes are not normally present. IgA therefore functions chiefly as a neutralizing antibody[38].

Stool Tests: A subsequent stool test can help to find out what types of yeast - including *Candida* - exhibits overgrowth in the gastrointestinal tract. This is most often part of a comprehensive stool analysis, and it tests for the *Candida* antigen.

Candida is an opportunist and its overgrowth indicates an active state of dysbiosis, which is important in weakening the gut cell barrier lining and leading to increased gut permeability. This is a serious condition in which bacteria from the gut are able to pass into the bloodstream. In terms of treatment, restoration of yeast balance can help in the normalization of the intestinal lining and its permeability.

D) Decreasing and Eliminating Overgrowth

After determining that there is a *Candida* overgrowth, there are a number of lifestyle changes, as well as supplements or medications, that can help to bring the situation under control.

1. ***Decreasing the intake of Certain Foods***

Refined foods, that have a high sugar content, allow *Candida* to thrive. Therefore, an early step should be the elimination, or significant decrease in the intake the foods that have high levels of the refined carbohydrates and sugars, but also other foods that may enhance *Candida* overgrowth. These include starchy fruits and vegetables, grains, beans and any foods or drinks that contain yeast, such as alcohol, vinegar, sauerkraut and kombucha. This type of elimination procedure should be done with the assistance of a trained health care professional. The latter individual can create a personalized program, as well as a means of monitoring progress.

2. **Sleep, Exercise, and Stress Reduction**

Factors such as adequate and effective sleep, exercise and stress reduction all impact the gut microbiome. If the gut microbiome is already in an imbalanced state, the addition of problematic sleep, inadequate exercise and undue stress patterns can worsen the imbalance. The aim should be to have at least seven hours of sleep per night, allowing for three or four cycles to occur during that period of sleep. Light exercise, such as walking, Pilates and yoga are excellent ways to get movement during the day, especially if elimination or decrease in sugar and carbohydrates is being attempted at the same time. The restorative exercises like yoga, Pilates, and light walking may be preferable to higher intensity exercise during the period of *Candida* overgrowth treatment.

3. **Supplements**

Supplements, especially herbal varieties like oregano oil and garlic extract, have been shown to help restore yeast balance. The addition of a probiotic yeast, such as *Saccharomyces boulardii* (*S. boulardii*), can also create balance by increasing competition, so that *Candida* does not grow out of proportion[38].

4. **Medications**

Diet, lifestyle changes and supplements can significantly decrease levels of *Candida*. However, there are situations where Candida overgrowth requires even stronger measures. In those circumstances, when the growth is significant, there may be a requirement for treatment with prescription-strength antifungals. This type of treatment should be approached after careful consideration with, and the supervision of, a trained health care professional.

Chapter 2: Digestive Enzymes and Absorption

2.1 Stomach: Hypochlorhydria

(Parietal Cell Insufficiency and Low Hydrochloric Acid)

Effects of Hypochlorhydria

• Small Intestinal Bacterial Overgrowth (SIBO) and increased pH alkalinity
• Chronic *Candida* Infections (thrive in alkaline pH)
• Mineral/Vitamin Deficiencies (d/o > pH): Vitamins A, D, B_9 (folic acid) & B_{12}, Ca, Mg, Zn, Fe, Cr, Mo, Mn, Cu
• Conditions linked to acne, rosacea, alopecia, eczema, lupus and vitiligo

Origins of Hypochlorhydria

• Aging
• Stress
• Fasting: Need ~600-800 cal/day to concentrate enough H+ ions
• Viral or bacterial infection (fever)
• Debilitating chronic condition
• Protein Pump Inhibitors, H-2 blockers, and antacid abuse
• H Pylori infection
• Sympathetic Adrenergic Hyperactivity

2.2 Pancreatic & Biliary Enzyme Secretion Insufficiency

Causes and Results of Insufficiency

• Hormone Imbalance
• Age-related and/or damaged microvilli
• Abnormal Stress Response
• Entero-Endocrine imbalance
• Inhibitors in food
• Suppression by alcohol abuse
• Pancreatitis, cystic fibrosis, diabetes, gallstones, and inflammation
• pH imbalance
• Free radical oxidation
• Nutrient imbalance or insufficiency
• Eating only cooked foods
• Exposure to radiation or toxins
• Hereditary weakness
• Drugs and infection

Chapter 3: Intestinal iQ™ Treatment Protocols

Staging of Treatment

1. Food Plans: Diet Protocols
2a. Enzyme Balancing
2b. Intestinal Protection
3. Inflammation Regulation
4. Immune Regulation
5. Intestinal Permeability Regulation
6. Dysbiosis: Probiotics, Prebiotics, Anti Spasmotics
7. Repair – GI Replenishment
8. Stress Regulation:
   - Cortisol Evaluation
   - General Hormone Evaluation & Balancing
9. Summary of Treatment Therapeutics

1. Food Plan: Diet Protocols

Diet protocols are most often based on some form of elimination diet. This type of management is used to highlight the foods that can produce symptoms and may constitute an important underlying cause of a myriad of chronic health problems.

From a restorative perspective, elimination diets have been considered as having 4 stages.

Step 1 – Preparatory

By combining a clinical assessment with the results of a Total IgG Food Sensitivity test profile, the clinician is armed with the evidence that can help in determining which foods should be temporarily removed from the diet. The average timeline for this phase is from 4-12 weeks. Some foods may have to be avoided for longer periods and in some cases indefinitely. Fundamental to the success of this phase is the guidance given to the patient prior to the start of the diet. This guidance includes, but is not restricted to, the following:

1. Providing education and resources to the patient, to help them navigate food labels, meal plans, prepare foods and choose alternatives in order to ensure nutrient needs are met
2. Encouraging the removal of offending foods from the home and the shopping cart.
3. Creating a journal to record consumed foods, their dates of elimination and consumption, and a method of tracking any changes in symptoms.

Paramount to success in this phase is the proper education on reading labels, since many patients are consuming problematic foods and are unaware that they are doing so.

Step 2 – Elimination

In order to gain the greatest benefit from the diet it is critical that the foods that showed a significant reaction on the test be avoided, both by eliminating that food in its whole form, or as an ingredient in other prepared foods. For many clinicians who use the FLUIDS iQ® Food Sensitivity test, **this includes foods that elicit reactions that begin in the upper levels of 'low', through the level of 'moderate' reactivity and levels above.**

Step 3 – Reintroduction or Inoculation

Foods that have been eliminated should be reintroduced one food at a time with a minimum of 4 days between additions, so as to allow for clear monitoring of any untoward reactions. The foods showing the most sensitivity on the IgG profile should be added last. If reactions occur, the patient should be told to remove the offending food again and then monitored to evaluate if the symptoms subside. If no symptoms occur, the food in question can be added back to the diet. This same procedure should be performed for each of the eliminated foods. It should be noted that introduction of this problematic food should be attempted again at the end of the food reintroduction schedule. Should a reaction occur again this food may need to be eliminated long term.

Step 4 - Maintenance

As shown in previous sections, elimination diets are to be viewed as a part of an overall assessment of gastrointestinal health. That means that the results of the elimination diet should be combined with the knowledge gained from the patient's clinical presentation, the results of IgG Food Sensitivity testing, as well as tests for intestinal permeability and inflammation. It is this ensemble of actions that will lead to an overall strategy, based on observation and objective measurement.

See Appendix: Column 1
- Elimination diet from Functional Medicine.[41]
- Core Food Plan[42]

2. Enzyme Balancing & Intestinal Protection

Nutrient intake balance requires post ingestive mechanisms. The first stage at which post ingestive balancing occurs is within the gastrointestinal tract, by the differential release of digestive enzymes.

2A. Enzyme Balancing

Gastric Function

Natural health products to consider, with dosages/day

Gastric Mucosal Healing:
• Pepsin (HCl replacement and/or stimulation): 90-180 mg
• Betaine HCl: 650 mg
• Gentiana root: 10 mg
• Prolyl Endoprotease (in Aspergillus Niger): Dosages: Variable
- Optimal function at pH 4-5. Stable at pH 2. Resistant to Pepsin digestion
- Degrades Gluten 60 x faster than a Prolyl Oligopeptidase

Pancreas

Generic Digestive Enzyme Formulas

Digestive Enzyme Combinations:

a. Amylases - hydrolize starches into oligosaccharides & maltose
b. Lipases - hydrolize tryiglycerides into fatty Acids & glycerols
c. Proteases - hydrolize proteins into amino acids
    i. Food based, animal based, or grown on Aspergillus
    ii. Trypsin: Hydrolizes protein to oligopeptides
    iii. Lactose/Dairy Enzymes
    iv. Gluten Enzymes

Therapeutic options with dosages

• **Pancreatin** (Pancreatic Enzymatic Extract)
    - Contains: Amylases, Proteases, Lipases, Trypsin
• **Bromelain** (Proteolytic Enzyme)

- Post eating: Enhances enzymatic digestive support

Dosages
• Amylase - Never < 24,000 U/G
• Neutral Protease - Never < 6,000 U/G
• Lactase - Never < 4,000 U/G
• Cellulase - Never < 340 U/G

Other Natural Products to Consider
- Nicotinic Acid 300 (Pancreatic output): 1500 mg

Biliary Function

• Fat emulsification
• Cholesterol removal
• Xenobiotics & drug elimination
• Emulsifies fat-soluble vitamins
• Incomplete digestion
• Steatorrhea (fatty stool)
• Diarrhea
• Gallstones

Natural Health Products to Consider

• Choline 3xdaily: 250- 350 mg
• Inositol 3xdaily: 25 to 100 mg
• L-Methionine 3xdaily: 250-350 mg
• Beetroot
• Bile salt extract with fatty meals: 100 mg
• Artichoke leaves (Cynarascolymus): 300 mg
• Berberis (biliary insufficiency): 500 - 1500 mg
• Taurine (biliary stasis): 1200 mg
• Cynara sp. (Artichokes): 300 mg
• Bile salts (ox bile): 500 - 1000 mg
• Chelidonium Majus: 1:4 botanical tincture up to 30%, 2-10 ml
• Curcumin (Biliary tract inflammation): 300 - 1800 mg
• Taraxacum Officinale (Hepatic Efflux): 1:4, 20%, 5 - 10 ml
• Phosphatidylcholine (lecithin): up to 500 mg daily
• Black radish (Raphanus sativus niger)

See Appendix: Column 2a

2B. Intestinal Protection

• **Mucin Layer Enhancement and Integrity**: Protects luminal side of intestinal cells
• **Modifying Mucosal Immunity:**
- Secretory Immunoglobulin A (sIgA)

- Blocks undesirable antigens

Natural Health Products to Consider:

• **Mucin Layer Enhancement and Integrity**
- Zinc Carnosine: 75-150 mg
- Nicotinic Acid
- Ascorbate flush (Optimize gastric microcirculation): 4000 mg
- Astaxanthin (Carotenoid: phospholipid bilayer antioxidant and gastric protection): 500 mcg
- Phosphatides (phospholipid bilayer and gastric integrity): 900 mg
- Arabinogalactans (mucosal and secretory immunity): 1500 mg
- N-acetylglucosamine - metabolic fuel for fibroblasts and epithelial glycosaminoglycan synthesis: 2-4 gm/day
- L-Glutamine: Enterocytes & microvilli repair: 5 gm, 2x/day
- Quercetin: Additional anti-inflammatory and anti-oxidant effects: 300 mg, 2x/day

• **Modifying Mucosal Immunity**
- Vitamin A (Trans Retinol): Increases sIgA production and improves probiotic adhesion
- Ghee (clarified butter): Increases sIgA production
- Strain specific Lactobacillus or a *Bifidobacterium* probiotics (also for gut flora normalization)

See Appendix: Column 2b

3. Inflammation Regulation

Emollients: To reduce local intestinal Inflammation
- Licorice root extract: 500 mg, 2x/day
- Slippery elm: 100 mg, 2x/day
- Marshmallow: 200 mg, 2x/day
- Curcumin (Inflammatory Modulation): 300 mg, up to 4x/day
- Aloe: 100 mg
- Ginger: 100 mg, 2x/day
- Bromelain: 500 mg, 2x/day
- Boswellia:200 mg, 2x/day
- Quercetin: 200 mg, 3x/ day

See Appendix: Column 3

4. Immune Regulation

Natural Health Products to consider
- Vitamin D-3 Titrate: up to 10,000 IU/day
- Ganoderma and other medicinal mushroom: 300 mg, up to 3x/day
- Bovine Colostrum: 5 gm, 2x/day
- Lactoferrin: 250 mg, up to 4x/day

See Appendix: Column 4

5. Intestinal Permeability Regulation

Increased Intestinal Permeability

- Intestinal inflammation
- Food sensitivities
- Celiac disease & other autoimmune disorders
- Chemotherapy patients
- Toxins:
  - Endogenous: From resident microflora
  - Exogenous: NSAIDs, undigested food, alcohol, drugs and foreign microbes

Re-establishing Intestinal Tight Junctions

Generic Treatment Options:

• **Bone Broth:** Source of:
- Proline: Supports collagen formation. Repairs TJs
- Glycine: Key amino acid (AA) in collagen formation
- L-Glutamine: Essential, free AA. Fuel for small intestine

• **Raw Cultured Dairy Products**
- Contain Probiotics which increase the production of Short Chain Fatty Acids
- Fermented milk (Kefir, Amasai), yogurt
- Butter, raw cheese

• **Fermented Vegetables**
- Sauerkraut, Kimchi, Kvass
- Organic acids to Stabilize pH
- Kombucha

• **Flavonoids**
  - **Quercetin**: enhances Tight Junction (TJ) barrier function through assembly & expression of TJ proteins
  - **Genistein**: Blocks tyrosine phosphorylation of TJ proteins, induced by oxidative stress & acetaldehyde, enteric bacteria & inflammatory cytokines
  - **Myricetin**: Polyphenol with antioxidant properties
  - **Epigallocatechin Gallate** (EGCG): Polyphenol, abundant in tea

• **Sprouted Seeds**:
- Source of Fiber (prebiotic) for commensal growth
- Chia seeds, Flaxseeds, hempseeds

• **Natural Health Products:**
- L-Glutamine
- N-Acetyl Glucosamine
- Zinc Carnosine
- Curcumin
- D-Limonene

See Appendix: Column 5

6. Dysbiosis: probiotics, Prebiotics, antispasmotics

Types of Prebiotics

- 2-Fucosyllactose (2-FL) – selective microbial growth
- Isomalto-Oligosaccharide
- Fructo-oligosaccaride (FOS)

Prebiotics Benefits

• Symbiotic relationship with probiotics.
• Feed beneficial bacteria, improving their viability.
• Selectively stimulates the growth of beneficial bacteria such as *lactobacilli* and *bifido* bacteria.
    - Inulin, oligofructose, and FOS have been demonstrated to increase fecal *bifodo* bacteria at low intakes of 5-8 gm/day[43].
    - Galacto-oligosaccharides (GOS) and lactulose have been shown to increase growth of *lactobacilli* and *bifido* bacteria[44].
• Improved gut barrier function and balanced gut microbiota.
• Improved immunity.
• Decrease in possible pathogenic subpopulations (ie, *clostridia*).
• Increased short chain fatty acid (SCFA) production.
• Improved absorption of minerals, such as calcium and magnesium.
• Prevention of the growth of gut lesions, such adenomas and carcinomas, possibly reducing the risk factors involved in colorectal disease.
• Improvement of liver function in cirrhotic patients.
• Reduce inflammation and symptoms associated with inflammatory bowel disease.
• Enhance satiety, weight loss and prevent obesity.
• Eating foods that contain prebiotics such as bananas, onions, garlic, leeks, asparagus, artichokes, and whole grains can increase the number of good bacteria in the gut.

Precision Probiotics
Strain: Specific and Proper Dosing is Essential

Please ensure the probiotic is human strain and has been studied to have a specific effect. Nonspecific use of probiotics is not recommended. Many different strains have been shown to be

helpful. Here is some general information on the 2 more common clinically beneficial types.

***Lactobacillus* Spp.**
• High levels associated with higher bacterial gene richness in the gut
• Altered levels in Irritable Bowel Syndrome (IBS)
• Lower levels correlate with symptom severity in IBS
• Increased levels seen in the obese compared to lean controls

***Bifidobacterium* Spp.**
• High levels associated with higher bacterial gene richness in the gut
• Modulates local and systemic immune responses
• Lower levels in Inflammatory Bowel Disease (IBD)
• Lower levels in IBS; low levels correlate with symptom severity in IBS
• Lower levels seen in type 2 diabetes, pediatric allergy, and autism
• Higher levels in the obese compared to lean/overweight;
• Levels decrease after weight loss and gastric-bypass surgery

See Appendix: Column 6

7. Repair – GI Replenishment

Cell and Tissue regeneration

See Appendix: Column 7

8. Stress Regulation: Hormone evaluation

Cortisol Regulation

See Appendix: Column 8

9. Summary of Treatment Therapeutics

• Avoid foods that enhance intestinal permeability
    - Include: gluten, dairy/lactose, spicy foods/ capsicum, FODMAPs
    - Processed foods with artificial flavours and colours
• Stop or greatly diminish the use of NSAID
• Determine HPA axis stressors and treat accordingly
• Decrease or stop hard physical exercise or activity
• Increase intake of fresh fruits and vegetables to enhance the diversity and amount of phytonutrients

• **Consider the following supplemental nutrients:**
    - Omega-3 fatty acids, ALA, EPA, DHA (diet and supplementation)

- Glutamine (4 to 8 grams daily)
- Vitamin D (1,000 IU/day minimum; test and dose to desired serum levels)
- Probiotics (mixed strain combination, 20-40 billion CFU; consider high doses for long-standing intestinal barrier issues or when associated with IBD)
- Prebiotics (precursor for important short-chain fatty acids - may be contraindicated if FODMAPS are to be avoided)
- Zinc (25 mg daily with other minerals)
- Iron (only when iron deficiency is confirmed)
- Flavonoids (for quercetin and related compounds, dose not as important as consistent daily consumption from foods and supplementation)
- Colostrum/Lactoferrin/IgG
- Berberine (consider adding 1 g/day when subject is obese, insulin-resistant or has type 2 diabetes)

(Thanks to Dr Tom Guilliams)

Chapter 4: The Intestinal iQ™ Biomarkers:

4.1 Zonulin

The Zonulin range is from 1 to 20 ng/ml (*).

**This range should NOT be interpreted as meaning the optimal range for Zonulin. Rather, it represents*
the range for 95% of randomly selected individuals in a population, including those with no disorders or
disease, through to those with diagnosed inflammatory and/or autoimmune disorders.

Values between 1 and 6 are considered as Optimal (green). If there are intestinal issues, they are not sufficient to have an effect on permeability.

Values between 6 and 10 are considered as Borderline (yellow). The effects of intestinal inflammation, often caused by a combination of dysbiosis and enzyme imbalances, are beginning to have an effect on permeability.

Values from 10 to 20 are considered as Elevated (red). Within this red portion of the range one may find individuals showing signs and symptoms of enzyme deficiencies, dysbiosis, acute or chronic inflammatory disease and those with established autoimmune disorders. A small percentage of individuals with acute disorders may show Zonulin levels much greater than 20 ng/ml and are noted as Above Range.

Those individuals with a Zonulin value less than 1 ng/ml are either very optimal or are part of a population with a HP 1-1 genetic variant that makes them unable to produce Zonulin. These individuals are thought to be producing proteins that are serving a similar function to that of Zonulin. Research is presently ongoing to find these entities and elucidate their action.

Treatment Staging:
- Based on multiple factors, including Zonulin levels
- Staging can be found in Chapter 3. Shown below in brackets.

Below 1 ng/ml:
- If also minimal IgG Food Sensitivity Reaction – Optimal Range
- If also significant IgG Food Sensitivity Reaction
Genetic Variant HP1-1: No Zonulin production

Between 1-4 ng/ml:
- Core food & Elimination diet plans **(Stage1)**
- Start Enzyme Balancing & Intestinal Protection **(2a & b)**

Between 4-8 ng/ml:
- Core food & Elimination diet plans **(1)**
- Enzyme balancing & Intestinal Protection **(2a & b)**
- Inflammatory Regulation **(3)**
- Immune regulation **(4)**
- Hormonal System: Cortisol level evaluation **(8)**

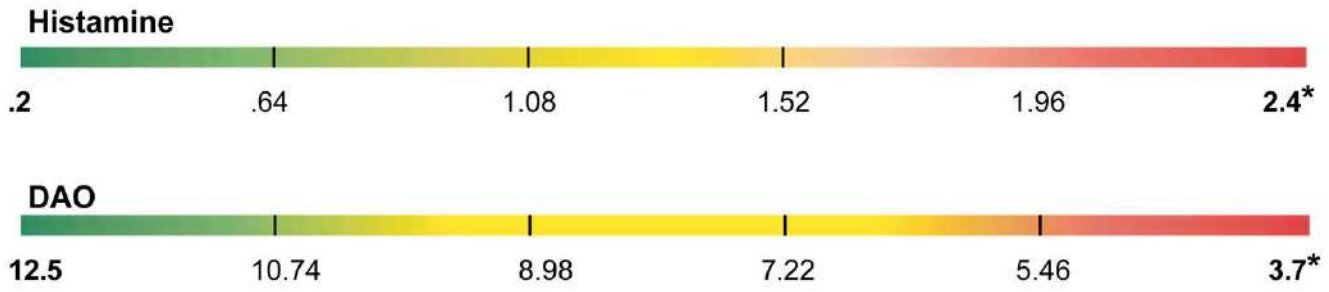
Between 8-12 ng/ml:
- Core food & Elimination diet plans **(1)**
- Enzyme balancing &Intestinal Protection **(2a & b)**
- Inflammatory Regulation **(3)**
- Immune Regulation **(4)**
- Gut flora normalization – Probiotics & Prebiotics **(6)**
- Repair - GI replenishment **(7)**
- Hormonal System: Cortisol level evaluation **(8)**

Between 12-20 ng/ml:
- Core food & Elimination diet plans **(1)**
- Enzyme balancing & Intestinal Protection **(2a & b)**
- Inflammatory inflammation **(3)**
- Immune Regulation **(4)**
- Intestinal Permeability Regulation **(5)**
- Gut flora normalization - Probiotics **(6)**
- Repair - GI replenishment **(7)**
- Hormonal System: General Evaluation **(8)**

4.2 Histamine / Diamine Oxidase (DAO)

Histamine reference range: 0.2 to 2.4 ng/ml*.
• Below reference range, < 0.2 ng/ml. Low probability of Histamine Intolerance (HIT).
• Above reference range, > 2.4 ng/ml. High probability of HIT.
• Levels moving from between 0.2 to 2.4 ng/ml indicate an increasing probability of HIT as the value approaches the upper limit of the reference range[10].

DAO reference range: 12.5 to 3.75 ng/ml* (10-3 U/ml), with no significant daily variations or gender differences.
• Below reference range, < 3.75 ng/ml. High probability of HIT.
• Above reference range, > 12.5 ng/ml. Low probability of HIT.
• Levels moving from 12.5 to 3.75 ng/ml indicate an increasing probability of HIT, as the value approaches the lower limit of the reference range[12]
.



*These ranges should **NOT** be interpreted as meaning the optimal range for Histamine or Diamine Oxidase (DAO). Rather, it represents the range for 95% of randomly selected individuals in a population, including those with no disorders or disease, through to those with diagnosed inflammatory and/or autoimmune disorders.

Histamine: > 1.08 (Increasing imbalance)
- Vit: B5 (acetylation), B6, B12/folate (methylation)
- Zn, Mg

DAO: < 8.98 (Increasing imbalance)
- Vit: B6, B12, C
- Iron
- DAO administration with each histamine containing meal

Combinations
Histamine > 1.08, **DAO** > 8.98 (normal range)
- Vit: B1, B6, E
- Zn, Bioflavanoids

Histamine > 1.08, **DAO** < 8.98, **Zonulin** < 6 (optimal range)
- Vit: B6, B12, C, Zn
- Zn, Mg, Iron

Histamine > 1.08, **DAO** > 8.98 (normal range), **Zonulin** > 6
- Vit: B1, B2, B6, B12, E
- Zn, Mg, Iron, K
- Thioles, Bioflavanoids

Histamine > 1.08, **DAO** < 8.98, **Zonulin** > 6
- Vit: B2, B6, B12, C
- Zn, Mg, Iron, Cu, Ca (calcium)

Sample report

FLUIDS iQ®

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RESULTS: DRIED BLOOD SPOT TEST

Accession #: 123456789 • Patient: Jane Doe

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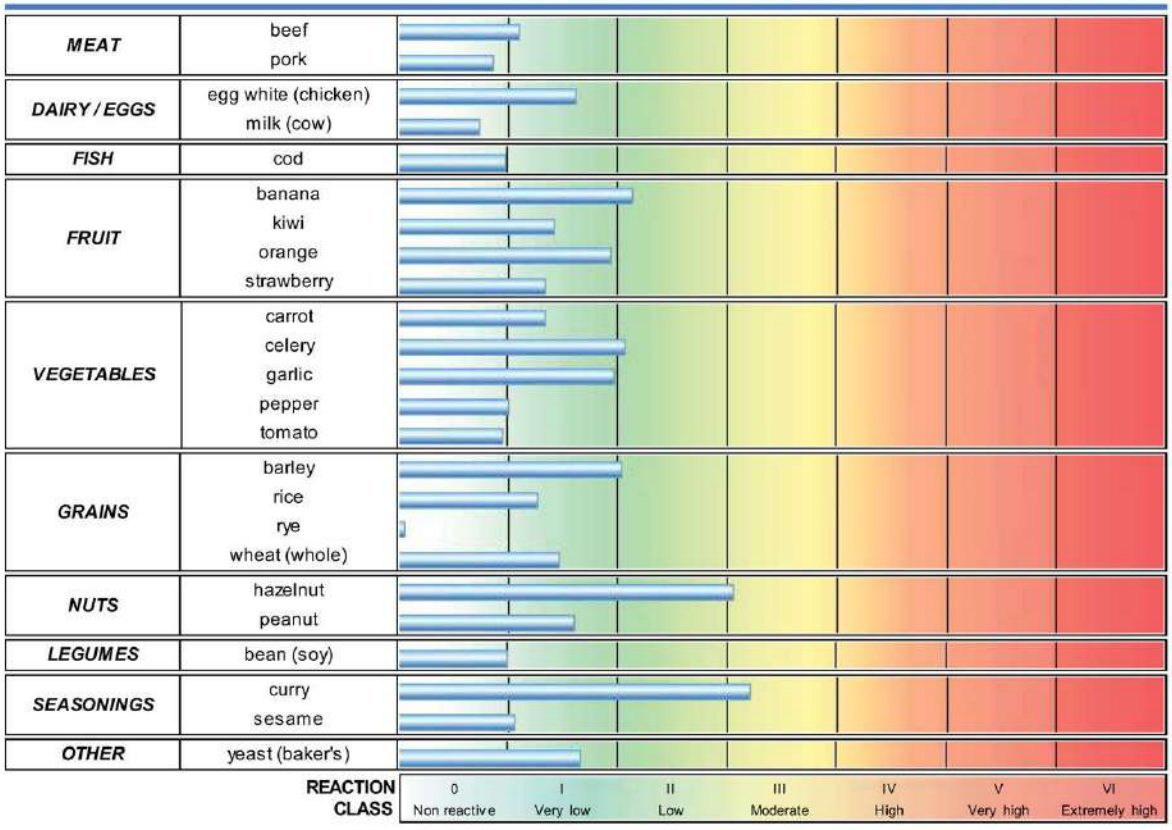
Patient: Jane Doe
Sex: Female **Age:** X yr **Date of Birth:** xxxx-xx-xx

Health Care Professional: Dr John Doe
Address: 123 Main St., Anytown, Quebec W4W 4W4

Accession #: 123456789
Sample received: 2016-08-05
Report issued: 2016-08-10
Sample collection: 2016-08-02

TOTAL IgG 24 FOODS PANEL



FLUIDS iQ®

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RESULTS: DRIED BLOOD SPOT TEST

Accession #: 100041052 • Patient: Jane Smith

Convert to pdf, Save or PRINT >>

Patient: Jane Smith
Sex: Female **Age:** 36 yr **Date of Birth:** 1984-06-14
Health Care Professional: John Smith

Accession #: **100041052**
Sample received: 2020-07-02
Report issued: 2020-07-08
Sample collection: 2020-06-26

CANDIDA SUITE (IgM, IgG, IgA)

Analyte	Result (U/ml)	Reaction	Reference Range* (U/ml) Non Reactive	Indeterminant	Reactive
Candida IgM	0.29	Non Reactive	< 9	9 - 11	> 11
Candida IgG	0.06	Non Reactive	< 9	9 - 11	> 11
Candida IgA	0.03	Non Reactive	< 9	9 - 11	> 11

*Reference range derived from a normal distribution of results, encompassing 95% of a randomly selected population

The comments provided here are for educational and research purposes only. These analytical results, on their own, should not be interpreted as being diagnostic or treatment recommendations. They must be correlated to clinical observations and diagnostic tests. Decisions are the responsibility of the health care professional.

Definitions

Non Reactive: Values are considered Non Reactive when no significant level of the Candida albicans antibody has been detected.
Indeterminant: Values are considered Indeterminant, or borderline, due to cross reactivity with other Candida species that may have pathological potential. The level of the antibody does not permit a clear description or classification of the reactivity within the range of the assay. A follow-up test within 2 to 4 weeks may be helpful in that determination[8].
Reactive: Values within the Reactive Range show that Candida albicans is detected, and may indicate a past, active or prolonged infection, depending on the level of the antibody. Significantly elevated levels of antibody have been observed in patients with active infection.

4.4: Candida Suite -IgM, IgG, IgA

Increased IgM antibody levels appear early in the course of an infection overgrowth and will usually reappear with renewed exposure, but to a lesser extent[45].

Increased IgG antibody levels may suggest chronic overgrowth of *Candida*. IgG antibodies increase well after the initial exposure and may persist in the body for many months, or even years after an

infection has been eradicated [46]. IgG antibodies represent approximately 75% of antibodies in human serum and are the most common type of antibody found in blood circulation.

Increased IgG and IgM antibody levels suggest a recent or recurrent *Candida* overgrowth[47].

Increased IgA antibody levels may suggest that there is mucosal overgrowth. This overgrowth is most often situated in the digestive tract, but can also occur in the oral, nasal, urinary and respiratory tracts[48].

Low IgM antibody levels (Deficiency) are seen in individuals with the rare immune disorder, Selective IgM Deficiency (SIgMD)[28]. These individuals have very little or no IgM antibodies, but exhibit normal levels of IgG and IgA antibodies. They commonly present with chronic and recurrent infections.

Low IgG antibody levels (Deficiency) may be primary or secondary. Researchers don't know what causes primary IgG deficiency, but genetics may play a role. Secondary IgG deficiency may be caused by aging, malnutrition, medicines such as chemotherapy, and chronic viral infections[29].

Low IgA antibody levels (Deficiency) may be associated with a heightened risk of chronic infections[30]. Research has also linked IgA deficiency to allergies, asthma and autoimmune diseases[31]. It is also observed in cases of Selective IgA Deficiency (SIgAD), defined as a primary immunodeficiency that is characterized by very low or undetectable levels of Immunoglobulin A (IgA) in the blood and secretions, but with no other immunoglobulin deficiencies. SIgAD is the most common form of primary immunodeficiency in the western world[32].

Chapter 5: Gut-Brain-Axis and the Microbiome

Emerging evidence suggests a significant interplay between the gastrointestinal milieu; ie. microbial metabolites, intestinal immunity, as well as the enteric nervous system and the brain. Trafficking of information exists in a bi-directional, communicative signaling process primarily through direct perturbation of receptors, paracrine secretions and the vagal nerve. It is important for clinicians to consider the mood, emotional and psychological status of patient's undergoing gastrointestinal assessment and treatment.

Gastrointestinal Microflora

Altered colonic microflora: Direct impact on all gastrointestinal mechanisms

– Impacts gut-associated lymphoid tissue (GALT) function and systemic and local cytokine production.
– Impairs food digestion & synthesis of essential nutrients such as biotin.
– Small Intestinal Bacterial Overgrowth (SIBO) may lead to significant **dysbiosis** and systemic health challenges. For severe SIBO, antibiotics such as Rifaximin may be helpful.

Therapeutic Options and Dosages

For microbiome alteration, eradication, or countering bacterial overgrowth

Herbal Antimicrobials:

• Allicin 1%: 750 mg
• Oregano (10:1): 150 mg
• Hydrastis and Berberines: 300 mg
• Rosemarinic Acid 6%: 200 mg
• Thymus Vulgaris: 720 mg
• Salvia Officinalis: 225 mg

Prebiotics must be added with caution and typically later in a microbiome protocol, as these functional foods and fibres may cause GI distress if significant overgrowth is still present.

Prebiotic and Probiotic Foods to be considered:
• Root Vegetables:
    - Celery, Turnip, Carrots, Beets,
• Fermented foods:
    - Kombucha, Kefir
• Prebiotics and Functional Fibres:
    - Rice Fibre, Larch, Psyllium, Xylo-oligosaccharides (XOS)

Notes:

- Baking soda or other alkalinizing agents between meals may have an overall beneficial effect on the growth of symbiotic microorganisms.

- Emerging evidence is suggesting that the state of the microbiome is a reflection of a patient's overall lifestyle and not simply dietary choices. Please consider a patient's pace of life, sleep habits, emotional health and exercise routines when attempting to optimize the status of the human microflora.

References:

1. Vanuytsel T et al. The role of Haptoglobin and its related protein, Zonulin, in inflammatory bowel disease. *Tissue Barriers* 2013; 1: 1-9.

2. Sturgeon C, Fasano A. Zonulin, A Regulator of Epithelial and Endothelial Barrier Functions, And Its Involvement in Chronic Inflammatory Diseases. *Tissue Barriers* 2016; 4: e1251384.

3. Fasano A et al. Zonulin, a newly discovered modulator of intestinal permeability, and its expression in celiac disease. *Lancet 2000*; 355: 1518-19.

4. Fasano A. Zonulin, regulation of tight junctions, and autoimmune diseases. *Ann N Y Acad Sci* 2012; 1258: 25–33.

5. Jenkins RT et al. Increased intestinal permeability in patients with rheumatoid arthritis: a side-effect of oral nonsteroidal anti-inflammatory drug therapy? *Br J Rheumatol*. 1987; 26:103-107.

6. Fukui H. The Gut Impacts Diabetic Management Tomorrow: The Recent Messages from Intestine and Microbiota. *J ClinNutri & Diabet* 2016; 2: 1-13.

7. A. Hasslacher C et al.Serum zonulin as parameter of intestinal permeability in longstanding type 2 diabetes: correlations with metabolism parameter and renal function. *Journal of Diabetes, Metabolic Disorders & Control* 2018; 5: 58-62.

8. Sapone A et al. Zonulin upregulation is associated with increased gut permeability in subjects with type 1 diabetes and their relatives. *Diabetes* 2006; 55: 1443-1449.

9. Pacifico, L et al. Increased circulating zonulin in children with biopsy-proven nonalcoholic fatty liver disease. *World J of Gastroenterol* 2014; 20: 17107-17114.

10. Nouri M, et al. Intestinal barrier dysfunction develops at the onset of experimental autoimmune encephalomyelitis & can be induced by adoptive transfer of auto-reactive T cells. *PLoS One* 2014; 9: 1-11.

11. Fasano A. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. *Physiol Rev* 2011; 91:151-75.

12. Kyeong Jeong H et al. Brain Inflammation and Microglia: Facts and Misconceptions. *Exp Neurobiol* 2013; 22: 59-67.

13. Skardelly M, et al. Expression of zonulin, c-kit, and glial fibrillary acidic protein in human gliomas. *Transl Oncol* 2009; 2: 117–120.

14. Swindle EJ et al. Breakdown in epithelial barrier function in patients with asthma: Identification of novel therapeutic approaches. *J Allergy Clin Immunol* 2009; 124: 23-34.

15. Rittirsch D, et al. Zonulin as prehaptoglobin2 regulates lung permeability and activates the complement system. *Am J Physiol Lung Cell Mol Physiol* 2013; 304: L863–L872.

16. Turner JR. Molecular basis of epithelial barrier regulation: from basic mechanisms to clinical application. *Am J Pathol* 2006; 169: 1901-1909.

17. Lee SH. Intestinal Permeability Regulation by Tight Junction: Implication on Inflammatory Bowel Diseases. *Intest Res* 2015; 13: 11-18.

18. Dyer J et al. Measurement of plasma histamine: description of an improved method and normal values. *J Allergy Clin Immunol* 1982; 70: 82-87.

19. B. Pinzer TC et al. Circadian profiling reveals higher histamine plasma levels & lower DAO serum activities in 24% of patients with suspected histamine intolerance compared to food allergy & controls. *Allergy* 2018; 73: 949-957

20. Molderings GJ et al. Mast cell activation disease: a concise practical guide for diagnostic workup and therapeutic options. *J Hematol & Oncol* 2011; 4: 1-10.

21. Yu S et al. Antigen inhalation induces mast cells and eosinophils infiltration in the guinea pig esophageal epithelium involving histamine-mediated pathway. *Life Sci* 2008; 82: 324-330.

22. Maintz L, Novak N. Histamine and histamine intolerance. *Am J Clin Nutr* 2007; 85:1185-1196.

23. Music E, et al. Serum diamine oxidase (DAO) activity as a diagnostic test for histamine intolerance. Food Allergy and Anaphylaxis Meeting 2011, Venice, Italy. 17-19 February, 2011.

24. Farhadi A, et al. Intestinal barrier: An interface between health and disease. *J Gastroenterol and Hepatol* 2003; 18: 479–497.

25. Luk et al. Diamine Oxidase (Histaminase). A circulating marker for rat intestinal mucosal maturation and integrity. J Clin Invest 1980; 66: 66-70.

26. Honzawa et al. Clinical Significance of Serum Diamine Oxidase Activity in Inflammatory Bowel Disease: Importance of Evaluation of Small Intestinal Permeability. *Inflamm Bowel Dis* 2011; 17: Letter to Editor.

27. Cai et al. Diagnostic and Clinical Significance of Serum Levels of D-Lactate and Diamine Oxidase in Patients with Crohn's Disease. *Gastroenterology Research and Practice* Vol 2019.

28. Sicherer SH, Sampson HA. Food Allergy.*J Allergy Clin Immunol* 2010; 125: S116-125.

29. Janeway CA, Travers P, Walport M, Shlomchik M. Immunobiology: The Immune System in Health and Disease. 5th ed. New York: Garland Science, 2001.

30. Mullin G et al. "Testing for Food Reactions: The Good, The Bad, and the Ugly." *Nutr Clin Pract* 2010; 25: 192-98.

31. Wilders-Truschnig M, Mangge M, Lieners C et al. IgG antibodies against food antigens are correlated with inflammation and intima media thickness in obese juveniles. *Exper Clin Endocrinol Diabetes* 2008; 116: 241-245.

32. Arzani et al. Gut-brain Axis and migraine headache: a comprehensive review. *The Journal of Headache and Pain* 2020; 21: 1-12.

33. Gunther LS et al. Prevalence of *Candida albicans* and non-*albicans* isolates from vaginal secretions: comparative evaluation of colonization, vaginal candidiasis and recurrent vaginal candidiasis in diabetic and non-diabetic women. *Sao Paulo Med J* 2014; 132: 116–120.

34. Duggan S et al. Host response to Candida albicans bloodstream infection and sepsis. *Virulence* 2015; 6: 316-326

35. Janeway CA. *Immunobiology: The Immune System in Health and Disease*. 5th edition - Garland Science, 2001.

36. Macpherson AJ, Slack E. The functional interactions of commensal bacteria with intestinal secretory IgA. *Current Opinion in Gastroenterology* 2007; 23: 673–678.

37. Burns CA et al. Immunoglobulin A Antibody Levels in Human Tears, Saliva, and Serum. *Infection and Immunity* 1982; 36: 1019-1022.

38. Murzyn A et al. The effect of Saccharomyces boulardii on Candida albicans-infected human intestinal cell lines Caco-2 and Intestin 407. *FEMS Microbiol Lett* 2019; 310: 17-23.

39. Alpay K, Ertas M et al. Diet restriction in migraine, based on IgG against foods: A clinical double-blind, randomised, cross-over trial. *Cephalalgia* 2010; 30: 829–837.

40. Isolauri E, Rautava S, Kalliomaki M. Food allergy in irritable bowel syndrome: new facts and old fallacies. *Gut* 2004;53: 1391-1393.

41. https://experiencelife.com/article/the-institute-for-functional-medicines-elimination-diet-comprehensive-guide-and-food plan/

42. https://richmondfunctionalmedicine.com/nutrition/core-food-plan/

43. Costabile A., Kolida S., Klinder A., Gietl E., Bauerlein M., Frohburg C., Landschutze V., Gibson G.R. A double-blind, placebo-controlled, cross-over study to establish the bifidogenic effect of a very-long-chain inulin extracted from globe artichoke (*Cynara scolymus*) in healthy subjects.*Br. J. Nutr* 2010;104:1007–1017.

44. Watson D., O'Connell Motherway M., Schoterman M.H.C., van Neerven R.J., Nauta A., van Sinderen D. Selective carbohydrate utilization by lactobacilli and bifidobacteria.*J. Appl. Microbiol* 2012; 114: 1132-1146.

45. Bassetti M et al. What has changed in the treatment of invasive candidiasis? A look at the past 10 years and ahead. *J Antimicrob Chemother* 2018; 73, Suppl 1: i14–i25.

46. Stanley PJ et al. Serum IgG subclasses in chronic and recurrent respiratory infections. *Clin. exp. Immunol* 1984; 58: 703-708.

47. Gonzalez-Quintela A et al. Serum levels of immunoglobulins (IgG, IgA, IgM) in a general adult population and their relationship with alcohol consumption, smoking and common metabolic abnormalities. *Clinical and Experimental Immunology* 2007; 151: 42–50.

48. Fagarasan S, Honjo T. Inteatinal IgA Synthesis: Regulation of Front-Line Body Defences. *Nature Reviews Immunology* 2003; 3; 63–72.

Appendix:

FLUIDS iQ® offers the information in the following appendix as an aid to the practitioner. FLUIDS iQ® does not necessarily recommend the use of supplements, nor does it in any way recommend one supplement or company over another. All the decisions on supplementation and diet plans are made by the health professional. The lists of supplements companies are in alphabetic order.

Company	Product	1 Diet Plans	2a Enzyme Balancing			2b Intestinal Protection		3 Inflammation Regulation	4 Immune Regulation	5 Permeability Regulation	6 Dysbiosis Intestine		7 GI Repair Replenish	8 Stess Regulation Cortisol	
			Stomach	Pancreas	Biliary	Mucin	sIgA				Small	Large		Low	High
AOR	Chanca Piedra														
	PRO Adapt														
	PRO Adrenal Glandular														
	PRO Destress														
	PRO GI Calm														
	PRO GI Repair														
	Probiotic 3														
	PRO Dysbio X														
Arthur Andrews Medical	Devigest														
	Neprinol														
	Syntol														
Aromtech	Omega 7 500mg														
Biotics Research Canada	ADHS														
	ADB-5 Plus														
	A.D.P. (Oregano Oil)														
	Aqueous Zinc														
	Berberine HCl														
	Betaine Plus HP														
	Beta-Plus														
	Beta-TCP														
	Bio -C Plus 1000														
	Bio-HPF (H-Pylori)														
	Bio-Protect														
	BioDoph-7 Plus														
	BioDophilus Caps														
	Bio--D-Mulsion 1000														
	BioDophilus -FOS														
	Bio-Immunozyme Forte														
	BioMega 1000														
	Biome Balance														
	Bromelain Plus CLA														
	Curcum-RX														
	Cytozyme AD														
	Cytozyme-LV														
	Cytozyme Pan														
	Dismuzyme Plus Granules														
	Gastrazyme														
	GI Resolve														
	GlucoBalance														
	Hydro-zyme														
	IAG														

	Immuno-gG														
	Intenzyme Forte														
	KappArest														
	Livotrit Plus														
	Liquid Iodine Forte														
	Optimal EFA's														
	Phosphatidylcholine														
	Sacchoromyces Boulardii														
	ZN-Zyme Forte (Zinc)														
Biotics_Vital Life	Brain Dep														
	Candid A 1														
	DH-Adren'l														
	Endometab														
	Female														
	Immune-X														
	Infla-Mate														
	Intra-Cell														
	Joint Regen														
	Kid-Nee1														
	Liv-er														
	Lymph														
	Male+														
	Mesenchyme														
	Systemic DX														
	Thyro														
Da Vinci Labs	Serenity Now														
Doctors Choice	Adrenal Gland														
	Full Spectrum Enzymes														
	L-Taurine														
Douglas Laboratories	Ayur-Curcumin														
Ecotrend/Enzyme Science	Critical Digestion														
	Intolerance Complex														
	Neprinol														
Ecotrend/Natren	Bifidonate														
	Bulgaricum														
	Flora “50-14”;”20-14”;”5-14”														
	Superdophilus														
	Trenev Trio														
Ecotrend/Quality of Life	Kinoko Pro														
Ecotrend/Pekana	apo-STOM														
	apo-HEPAT														
	apo-INFLAMYAR														
	Neu-regen														
	Psy-Stabil														
Enzymedica	Acid Soothe														
	Lipid Optimize - LypoGold														
Experience Life		Elimination													

FitX	Reishi														
	Vitamin D														
Genestra	Bromelain Papain Plus														
	Pancreatin 840														
Innate Response	Adrenal Response														
	C Complete Powder														
	Digestive Enzymes														
	Magnesium 300 Powder														
Metagenics	GI Sustain														
	Licorice Plus														
	Lipo-Gen														
	Meta-Lipoate														
	OmegaGenics EPA/DHA 720														
	OmegaGenics EPA 1200														
	OmegaGenics Mega 10														
	SpectraZyme Pan 9x														
	UltraGI Replenish														
	UltraFlora Acute Care														
	UltraFlora Balance														
	UltraFlora IB														
	UltraFlora Intensive Care														
	UltraFlora Spectrum														
	UltraInflamX Plus 360														
Natural Factor	Ginger Root														
Ortho Molecular	Adapten-all														
	Adrenevive														
	Probio 225														
	Reacted Magnesium Powder														
Pure Encapsulations	Pancreatic Enzyme Formula														
Richmond		Core Food plans													
Wise Woman	Licorice Solid Extract														