

# RESULTS: DRIED BLOOD SPOT TEST

Accession #: 100035536 • Patient: BRAD

**Patient:** BRAD

**Sex:** Male

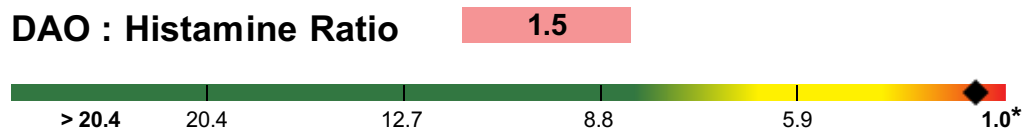
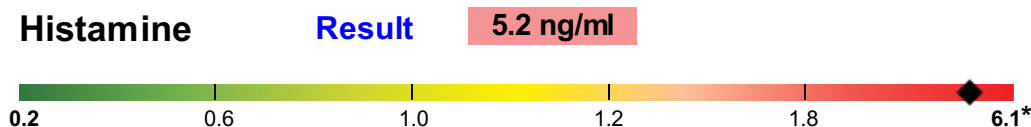
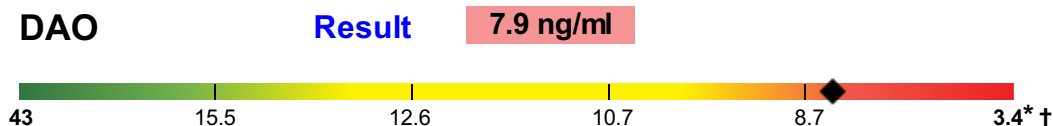
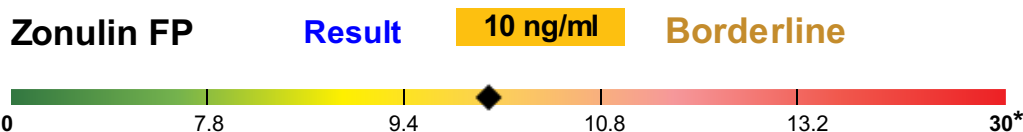
**Age:** 43 yr

**Date of Birth:** 1978-11-11

**Accession #:**

100035536

## ZONULIN FAMILY PEPTIDES (ZONULIN FP) + DIAMINE OXIDASE (DAO) / HISTAMINE



\*The reference range represents a quintile distribution, each quintile being 20 % of a population determined from archival data.

† DAO values are shown in ng/ml, where the conversion factor is 1U/ml = 1.25 ng/ml

## RESULTS: DRIED BLOOD SPOT TEST

### GENERAL COMMENTARY Zonulin Family Peptides (Zonulin FP)+ Diamine Oxidase (DAO) / Histamine

The comments provided here are for educational purposes only. The results in this report should not be interpreted as diagnostic, nor should they be viewed as treatment recommendations. Those decisions are the responsibility of the health care professional. Moreover, the reference range shown in this report is derived from a normal distribution of results, that encompass 95% of randomly selected individuals in a population (see below).

The FLUIDS iQ Zonulin FP test, which uses the most advanced, polyclonal antibody immunoassay developed to date, is our test for Permeability. By its design, this test measures pre- haptoglobin 2 (see below), known as Zonulin, as well as other very closely related peptides implicated in intestinal permeability.

#### Zonulin

Zonulin 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, and a small family of closely related peptides, bind to specific receptors only on the apical (luminal) surface of the intestinal epithelia and trigger a cascade of biochemical processes that induces tight junction (TJ) disassembly and a subsequent permeability increase of the intestinal epithelia.<sup>1</sup> This is often referred to as “leaky gut”.

Intestinal permeability changes due to Zonulin have been implicated in many diseases and dysfunction, notably in Celiac Disease and Type I Diabetes, but also in others including the brain, respiratory system and skin.

The gluten glycoprotein,  $\alpha$ -gliadin, can activate Zonulin signaling, irrespective of the genetic expression of autoimmunity. This generates a two-way response: Not only can fluid exit, but intestinal contents are able to gain entry in the opposite direction, into the bloodstream. This gliadin-Zonulin ‘leakage’ effect is longer and more pronounced (up to 5-fold greater) in the enterocytes, or intestinal cells, of people with Celiac Disease (CD).<sup>2</sup>

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 30 times as much Zonulin as non-CDs and 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.<sup>3</sup>

This strongly suggests that something besides gluten may be contributing to permeability changes in people with celiac disease. It may be that certain types of intestinal dysbiosis (improper balance of bacteria and yeasts in the intestines) 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 implicated in corticosteroid use, as well as the pathogenesis of insulin dependent diabetes (type 1) and juvenile nonalcoholic fatty liver disease. There is also evidence of its implication in multiple sclerosis, rheumatoid arthritis, skin diseases, as well as inflammatory bowel disease and obesity.<sup>4,5</sup>

Zonulin also 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 the  $\alpha$ -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 and resulting in activation of a cerebral inflammatory response.

## RESULTS: DRIED BLOOD SPOT TEST

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.<sup>6</sup>

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 in asthmatics may play a role in their susceptibility to environmental allergens. 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.<sup>3</sup>

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.<sup>7</sup>

### Why Test for Zonulin FP?

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.<sup>8,9</sup> For that reason Zonulin is gaining acceptance as a non-invasive marker of intestinal wall integrity and developing disorders.

The Zonulin FP range in this report is from 0 to 30 ng/ml. It should NOT be interpreted as meaning that this entire range is the optimal range for Zonulin FP. Rather, it represents the range for 95% of randomly selected individuals in a population and includes individuals with no disorders or disease, through to those with diagnosed inflammatory and/or autoimmune disorders.

Values between 0 and 7.8 are considered as Optimal (green). If there are gut issues, they are not sufficient to have an effect on gut permeability.

Values between 7.8 and 10.8 are Borderline (yellow-orange). The effects of gut inflammation, often caused by a combination of dysbiosis and enzyme imbalances, are beginning to have an effect on permeability.

Values from 10.8 to 30 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 FP levels much greater than 30 ng/ml and are noted as Above Range.

## RESULTS: DRIED BLOOD SPOT TEST

### Histamine and Diamine Oxidase

Histamine is a nitrogenous compound known to be involved in twenty three different physiological functions which include local immune responses, neurotransmission, as well as regulation of physiological function in the gastrointestinal tract.

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. The latter is often thought to be the underlying cause of gut inflammation and dysfunction, when in fact 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).<sup>11</sup> HIT is an acquired problem and is seen in approximately 3% of the population. Symptoms may occur in up to 20% 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.<sup>11</sup>

### 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.

The Histamine reference range is from 0.2 to 6.1 ng/ml:

- Below the reference range levels, < 0.2 ng/ml, indicate a low probability of HIT.
- Above the reference range levels, > 6.1 ng/ml, indicate a high probability of HIT.
- Levels between 0.2 to 6.1 ng/ml indicate an increasing probability of HIT as the value approaches the upper limit of the reference range.<sup>10</sup>

### High levels of histamine:

- 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)

## RESULTS: DRIED BLOOD SPOT TEST

- 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")
- Gastrointestinal bleeding
- Histamine-rich foods
- 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
- Medications:
  - Non-steroidal anti-inflammatory drugs (eg: Ibuprofen-Motrin, ASA-Aspirin)
  - Antidepressants (eg: Effexor, Zoloft, Prozac, Cymbalta)
  - Immune modulators (eg: Enbrel, Humira)
  - Anti-arrhythmics (eg: Propranolol, Norvasc, Cardizem)
  - H2 blockers (eg: Zantac, Pepcid, Tagamet)

### Low levels of histamine (Histapenia):

- 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 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).

## RESULTS: DRIED BLOOD SPOT TEST

An impaired histamine degradation, based on reduced DAO activity and the resulting histamine excess, may cause numerous symptoms mimicking an allergic reaction.<sup>11</sup>

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.

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.

However, if the histamine levels are normal, but the DAO levels are very low, it suggests a possible genetic deficiency of diamine oxidase.

The DAO reference range is from 43 to 3.4 ng/ml, with no significant daily variations or gender differences:

- Below the reference range levels, < 3.4 ng/ml, indicate a high probability of HIT.
- Above the reference range levels, > 43 ng/ml, indicate a low probability of HIT.
- Levels from 43 to 3.4 ng/ml indicate an increasing probability of HIT.<sup>11</sup>

### Low levels of DAO:

- 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

## RESULTS: DRIED BLOOD SPOT TEST

Determination of DAO activity, together with a detailed history, helps to differentiate food allergy and histamine intolerance. It should be performed in suspected patients who have headaches, urticaria, pruritus, diarrhea and hypotension, where food allergy has been excluded.<sup>12</sup>

Individuals who are unable to metabolize histamine will often improve with a variety of antihistamines. 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.<sup>13</sup>

### 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. It should be noted that even if DAO levels are normal, symptoms may still occur if the histamine levels are very high.

### References

1. Vanuytsel T et al. Tissue Barriers 2013; 1: 1-9;
2. Fasano A et al. Lancet 2000; 355: 1518-19;
3. Fasano A. Ann N Y Acad Sci 2012; 1258: 25-33;
4. Sapone A et al. Diabetes 2006; 55: 1443-1449;
5. Pacifico, L et al. World J of Gastroenterol 2014; 20: 17107-17114;
6. Skardelly M, et al. Transl Oncol 2009; 2: 117-120;
7. Rittirsch D, et al. Am J Physiol Lung Cell Mol Physiol 2013; 304: L863-L872;
8. Turner JR. Am J Pathol 2006; 169: 1901-1909;
9. Lee SH. Intest Res 2015; 13: 11-18;
10. Jean Dyer BS et al. J Allergy Clin Immunol 1982; 70 (2): 82-87.;
11. Maintz L, Novak N. Am J Clin Nutr 2007; 85:1185-1196;
12. Music E, et al. Food Allergy and Anaphylaxis Meeting 2011, Venice, Italy. 17-19 February, 2011;
13. Farhadi A, et al. J Gastroenterol and Hepatol 2003; 18: 479-497.;
14. Luk et al. J Clin Invest 1980; 66: 66-70.;
15. Honzawa et al. Inflamm Bowel Dis 2011; 17: Letter to Editor.;
16. Cai et al. Gastroenterology Research and Practice Vol 2019.