

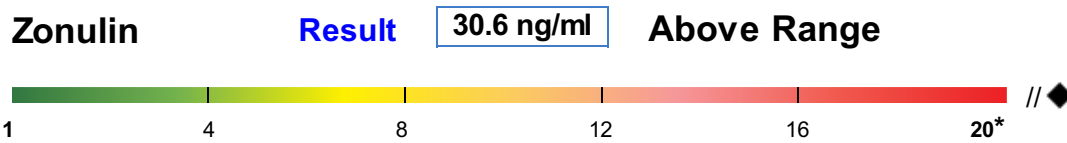
**RESULTS: DRIED BLOOD SPOT TEST**

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 Smith  
**Address:** 123 Main St., Anytown, Ontario W4W 4W4

**Accession #:** 123456789  
**Sample received:** 2015-06-02  
**Report issued:** 2015-06-19  
**Sample collection:** 2015-05-30

**ZONULIN**

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



Dr. Aron Gonshor PhD, DDS, FRCD(C), FAO • Laboratory Director

**GENERAL COMMENTS**

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).

**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 apical (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.<sup>1</sup> This is often referred to as “leaky gut”.

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 and Type I Diabetes, but also in others including the brain, respiratory system and skin.

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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 'leaky gut' 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. 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>

Levels of Pre-Haptoglobin 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.<sup>3</sup>

## RESULTS: DRIED BLOOD SPOT TEST

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### 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.<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 range in this report 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 and includes individuals 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 gut issues, they are not sufficient to have an effect on gut permeability.

Values between 6 and 10 are Borderline (yellow). 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 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.

### References

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