

## WHITE PAPER

**VIBRANT WELLNESS**
**WHEAT ZOOMER - ENA OVERLAP**
**Table of Contents**

1. Wheat Sensitivity
2. Autoimmune Disease
3. Overlap Between Wheat Sensitivity and Autoimmune Disease
4. Our Study: High Frequency of ENA Antibodies in Wheat Sensitivity
5. Conclusion
6. References

**1. Wheat Sensitivity**

Wheat is a complex grain, composed of various molecules of carbohydrates, proteins, and fat. For some individuals, wheat ingestion is associated with a cascade of symptoms from gastrointestinal distress such as diarrhea, constipation, bloating, cramping, and altered bowel habits to extra-intestinal symptoms, including fatigue, joint pain, depression, and cognitive difficulties. Among a broad spectrum of wheat-related disorders, celiac disease is the best known: a chronic immune-mediated enteropathy in which dietary gluten leads to small bowel inflammation and villous atrophy in genetically susceptible individuals. For other types of wheat sensitivities, the Wheat Zoomer is an extremely useful tool in assessing the possible causes for different individuals. The Wheat Zoomer is a comprehensive wheat protein antibody panel covering IgG and IgA to 18 proteins listed in Table 1. All the key proteins of wheat are arrayed on the Vibrant Wheat Zoomer chip as overlapping 18-mer peptides covering the entire protein. The method of manufacturing the peptide microarray has been elaborated elsewhere<sup>1</sup>. There is also measurement of total IgA and IgG, which are often low in individuals with gluten sensitivity (though the fact that 10% of the general population may have a genetic IgA deficiency may confound the results).

**Table 1.** Protein probes of Wheat Zoomer

PANEL	PROTEINS
Celiac <sup>1</sup>	Transglutaminase 2, deamidated gliadin peptide (DGP)
Transglutaminase <sup>2,3</sup>	Transglutaminase 3, Transglutaminase 6
Wheat Germ <sup>4</sup>	Wheat Germ Agglutinin
Gliadin <sup>5</sup>	$\alpha$ Gliadin, $\alpha$ - $\beta$ Gliadin, $\gamma$ -Gliadin, $\Omega$ Gliadin, Gluteomorphin, Prodynorphin
Glutenin <sup>6</sup>	Low Molecular Weight Glutenin, High Molecular Weight Glutenin
Non-gluten Protein <sup>7</sup>	Serpin, Farnins, Amylase/Protease Inhibitors, Globulins, Purinin

**2. Autoimmune Disease**

A normally functioning immune system produces a response against harmful or foreign substances such as bacteria, viruses, parasites, etc. Autoimmune diseases arise when the body attacks its own constituents as it fails to recognize what is self and non-self. There are more than 80 known autoimmune diseases. The National Institute of Health estimates that more than 20 million people in

## 2. Autoimmune Disease

the US are affected by autoimmune disease.<sup>8</sup> Antinuclear Antibodies (ANA) and the specific ones against Extractable Nuclear Antigens (ENA) have been recognized as diagnostic features of systemic autoimmune disorders (e.g., systemic lupus erythematosus, mixed connective tissue disorder, etc).

Table 2. Vibrant America ANA+ENA panel

ANA IFA										
Speckled		Nucleolar		RIM		Cytoplasmic		Centromere		Homogeneous
ENA Panel										
Jo-1	Sm	RNP	SSA (Ro)	SSB (La)	Scl-70	Chromatin	Centromere	Histone	RNA POL III	

## 3. Overlap between Wheat Sensitivity and Autoimmune Disease

Over the last few years, wheat sensitivities have been associated with many disorders.<sup>9,10</sup> Remarkably, in patients with autoimmune thyroiditis (Hashimoto's thyroiditis, Graves' disease), Addison's disease, autoimmune insulin-dependent diabetes mellitus, Sjogren's syndrome, and autoimmune hepatitis, the frequency of celiac disease is much higher than that in the normal population. Carroccio et al reported in a retrospective study that the prevalence of ANA was 24% in celiac patients and 46% in non-celiac wheat sensitive patients. The same group also researched a prospective cohort and found ANA's prevalence to be 7.5% in celiac patients and 28% in non-celiac wheat sensitive patients.<sup>11</sup> In another comparative study by Volta et al., ANA was observed in 49% of the patients with celiac disease, reflecting a predominant autoimmune profile, as compared to 37% in the non-celiac wheat sensitive patients.<sup>12</sup>

## 4. Our Study: High Frequency of ENA Antibodies in Wheat Sensitivity

In our recently published study, we researched the frequency and level of ANA and 10 ENA-antibodies in seropositive wheat sensitive subjects and seronegative controls.<sup>13</sup> Our data present a strong tendency towards autoimmunity in wheat sensitive individuals, characterized by the presence of anti-ENA biomarkers. The combined evaluation of autoimmune antibodies is appropriate when existence of additional autoimmune diseases is suspected in wheat sensitive individuals.

### Methods

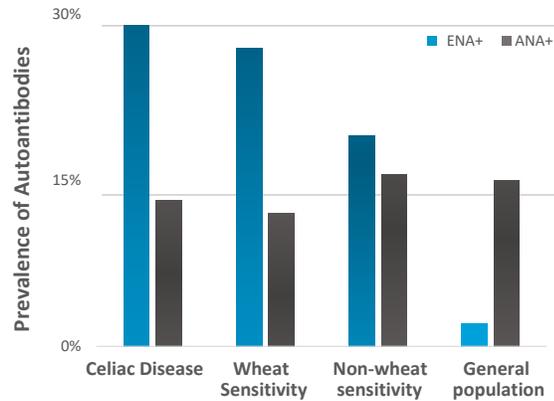
A total of 713 subjects, who showed symptoms related to wheat ingestion, were referred to Vibrant America Clinical Laboratory from December 2015 to November 2017. Serum samples were collected from all subjects and tested with the Wheat Zoomer and ANA+ENA panel. Retrospective analysis was completed using de-identified clinical data and test results.

### Results

(A) Prevalence of ANA and anti-ENA

In the retrospective analysis, 38 (5%) were seropositive in the celiac panel, 491 (83%) were seropositive in the Wheat Zoomer but not the celiac panel, and 84 (12%) were seronegative in both panels. **The prevalence of anti-ENA was reported to be less than 2% in the general population;<sup>14</sup> however, our study found it to be much higher in the celiac disease subjects (29%) and the wheat sensitive subjects (27%), compared with a smaller proportion of seronegative controls (19%).** ANA was detected in similar portions of the celiac disease subjects (13%), the Wheat Zoomer positive subjects (12%), and seronegative controls (15%), which is also very close to the reported occurrence of ANA positivity (15%) in the healthy population.<sup>15</sup>

According to our study and other studies, a subgroup (20%~30%) of the autoimmune patients would have their anti-ENA detected 1~2 years earlier than ANA being detected.<sup>16,17</sup> In the present study, the prevalence of anti-ENA was also observed to be much higher than that of ANA among the subjects. We hypothesize that the prevalence of ANA in this cohort would become more disparate after a longer period of time as a higher proportion of them already carried anti-ENA.



**Fig 1.** Prevalence of ANA and anti-ENA in celiac disease, Wheat zoomer seropositive subjects, seronegative controls, and general population.

## Results

### (B) Frequency of Anti-ENA Antibodies

As shown in Table 3, anti-histone was the most frequently found antibody among all anti-ENAs (73% in celiac disease, 60% in Wheat Zoomer positive, and 38% in non-wheat sensitivity). The antibodies against Jo-1, Sm and RNP were the least prevalent. All 10 anti-ENA antibodies were detected in the Wheat Zoomer positive subjects. The absence of some anti-ENA in the celiac disease subjects and the non-wheat sensitive subjects might be due to limited sample sizes.

**Table 3.** Frequency of anti-ENA in seropositive wheat sensitive subjects and seronegative controls

ENA	Celiac Disease (n=38)		Wheat Zoomer positive (n=591)		Non-Wheat Sensitivity (n=84)	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
<i>Jo-1</i>	0	0	3	2%	0	0
<i>Sm</i>	0	0	7	4%	0	0
<i>RNP</i>	0	0	1	1%	0	0
<i>SSA (Ro)</i>	0	0	14	9%	1	6%
<i>SSB (La)</i>	1	9%	18	11%	1	6%
<i>Scl-70</i>	0	0	14	9%	0	0
<i>Chromatin</i>	1	9%	24	15%	4	25%
<i>Centromere</i>	1	9%	17	11%	1	6%
<i>Histone</i>	8	73%	96	60%	6	38%
<i>RNA polymerase III</i>	1	9%	29	18%	1	6%

## Results

### (C) Prevalence of Wheat Protein Antibodies in ANA Positive or ENA Positive Subjects

Within the cohort of wheat sensitive subjects, we analyzed the distribution of wheat protein antibodies among the ANA positive and anti-ENA positive subjects. In both groups, gliadin antibodies were the highest (66% in ANA and 68% in ENA groups), followed by non-gluten proteins (61% in both groups). While alpha/gamma gliadin and its deamidated forms are the focus of most commercial tests, the Wheat Zoomer covers all known gliadins from all the different wheat species (alpha, gamma and omega gliadins) in both native and deamidated forms. In our study, the high frequency of gliadin antibodies in autoantibody positive subjects can be contributed to its high prevalence but also the utilization of a more sensitive and comprehensive testing tool. Moreover, non-gluten proteins including serpin, farinins, amylase/protease inhibitors, globulins, purinins have been shown to be immune reactive in celiac disease patients.<sup>18</sup> In our case, we confirmed the prominent presence of non-gluten proteins is in other types of wheat sensitive subjects as well.

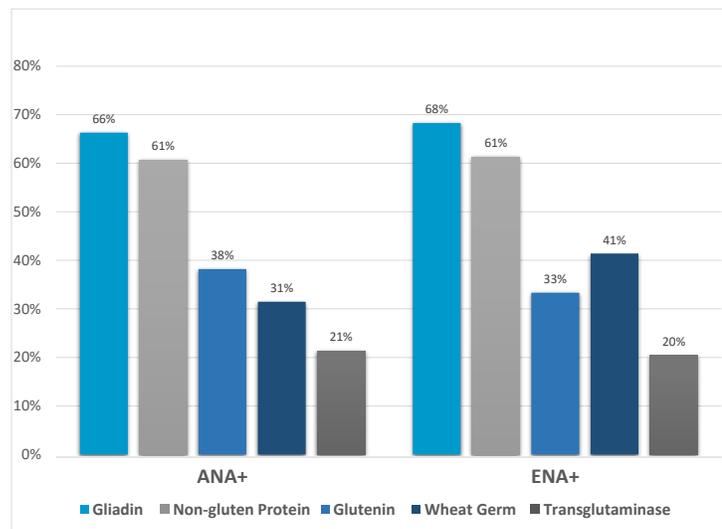


Fig 2. Prevalence of wheat protein antibodies in Wheat Zoomer positive subjects with ANA positivity or ENA positivity.

## 5. Conclusion

The presented data showed that high proportions of wheat-related disease subjects carry ENA-antibodies that are important specific biomarkers of autoimmunity. Therefore, evaluation of autoimmune antibodies is appropriate when existence of an additional autoimmune disease is suspected in patients with wheat-related disorders.

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