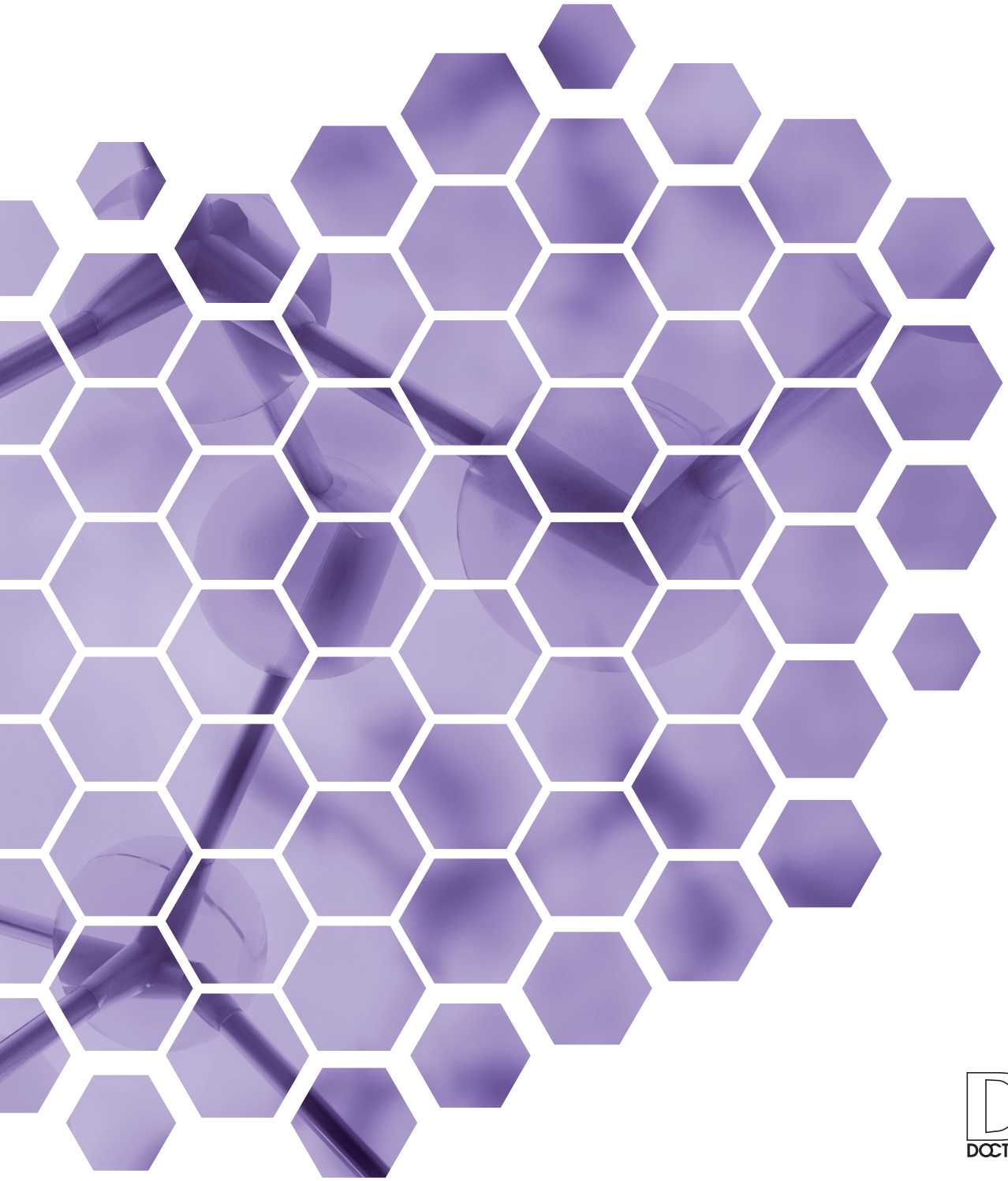
# Celiac Disease and Gluten Sensitivity

## RESOURCE GUIDE



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*By Jack A. Maggiore, PhD, MT(ASCP), Assistant Director, Chemistry Laboratory and R&D at Doctor's Data.*

### Key Points

- The diagnosis of celiac disease and non-celiac gluten sensitivity may now be made without the need for an invasive biopsy of the intestines
- Clinicians can now draw upon fully validated, sensitive, and specific FDA-cleared serum tests to identify the autoimmune response to gluten
- The Doctor's Data team has successfully validated procedures for using fingerstick blood for the key markers that identify celiac disease and gluten sensitivity, as well as aiding in monitoring the effectiveness of therapies

# Identifying Celiac Disease and Gluten Sensitivity with Minimally Invasive Testing

Celiac disease is an autoimmune disease that occurs in genetically predisposed individuals, in which the ingestion of gluten leads to an immune attack of the villi of the small intestines. Healthy villi are essential for the digestion and absorption of both macro- and micronutrients. Individuals with undiagnosed celiac disease are prone to malnutrition, vitamin deficiencies, and anemia. The prevalence in the United States is approximately 1 in 133, or close to 3 million people. This prevalence increases to 1 in 22 in individuals with a first-degree relative with the disease.[1]

Until recently, the criteria for diagnosis of celiac disease has required an intestinal biopsy to identify evidence of damaged villi secondary to the autoimmune attack. Now, due to enhancements of the clinical laboratory assays for the serologic markers of the immune response associated with celiac disease, a clinical diagnosis may be made without the need for highly invasive surgical procedures.[2] Sensitive and specific laboratory assays for the identification and quantitation of the immunoglobulins produced against tissue transglutaminase (tTG) and deamidated gliadin peptide (DGP) are now part of the celiac disease diagnostic algorithm. When these markers are found elevated in genetically predisposed people who are consuming a gluten-containing diet and present with symptoms of celiac disease, a diagnosis of celiac disease is strongly supported.[2] After further genetic testing to identify the histocompatibility leukocyte antigens (HLA) haplotypes associated with celiac disease, namely HLA-DQ2/DQ8, a diagnosis may be made.

### Symptoms of Celiac Disease[2]

Diarrhea
Gastrointestinal inflammation
Bloating or cramping
Foul or fatty stools
Weight loss
Fatigue
Muscle weakness

Celiac disease is unique in that the trigger and the genetic predisposition are known and that symptoms may fully resolve with lifelong avoidance of the trigger. Because of this, accurate and reliable testing becomes the cornerstone of diagnosis.

More complicated, however, is the categorization of individuals who continually test negative for the markers of celiac disease, but continue to display the serious gastrointestinal symptoms after consuming a gluten-containing meal. This is a very real and even more prevalent condition, referred to as non-celiac gluten sensitivity.[1]

Gluten sensitivity, as its name implies, comes with many of the same symptoms of celiac disease, which are resolved with the avoidance of gluten-containing foods. Previously, individuals were considered gluten sensitive after the successful implementation of a gluten elimination diet. Rarely, if ever, were such individuals tested for the immunologic markers against gluten, or its more specific protein components

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**LAB #: B000000-0000-0**
**PATIENT: Sample Patient**
**ID: P0000000000**
**SEX: Male**
**DOB:** **AGE: 21**

**CLIENT #: 12345**
**DOCTOR:**
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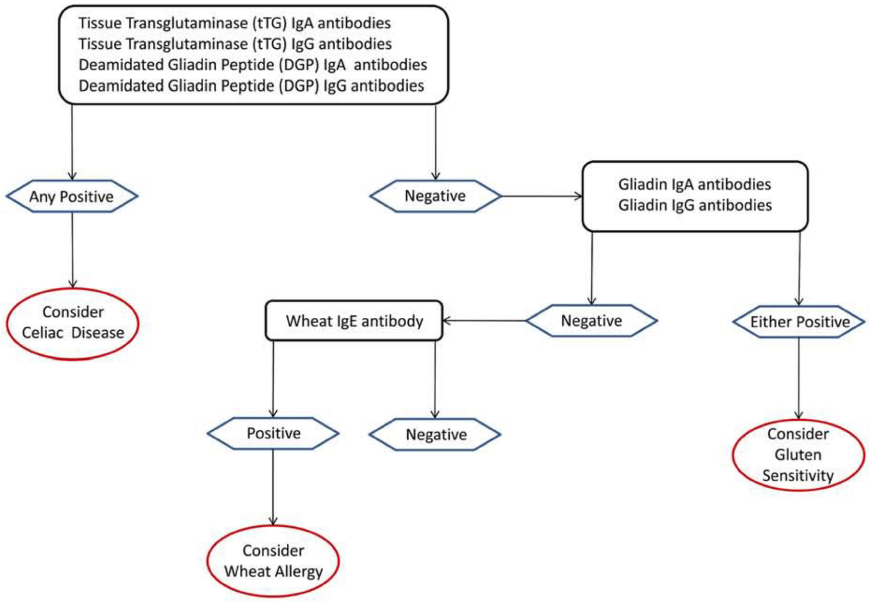
**3755 Illinois Ave.**
**St. Charles, IL 60174 U.S.A.**

## *Celiac & Gluten Sensitivity; serum*

| ANTIBODIES | RESULT/UNIT | REFERENCE INTERVAL | NEG / WEAK POS / POSITIVE |
|---|---|---|---|
| Tissue Transglutaminase (tTG) IgA | **1490 U** | < 20.0 | |
| Tissue Transglutaminase (tTG) IgG | **47.7 U** | < 20.0 | |
| Deamidated Gliadin Peptide (DGP) IgA | **78.8 U** | < 20.0 | |
| Deamidated Gliadin Peptide (DGP) IgG | **477 U** | < 20.0 | |
| Gliadin IgA | **13.0 U** | < 20.0 | |
| Gliadin IgG | **35.0 U** | < 20.0 | |
| Wheat IgE | **0.05 IU/mL** | < 0.08 | |
| | | | PERCENTILE: 2.5th 16th 50th 84th 97.5th |
| Immunoglobulin A (IgA) | **48 mg/dL** | 66- 433 | |

Celiac Disease/Gluten Sensitivity/Wheat Allergy Cascade




**SPECIMEN DATA**

Comments:

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Date Received: 06/24/2015 <dl: less than detection limit
Date Completed: 06/30/2015
Method: Chemiluminesent, Immunoassay



*This sample report includes a proposed diagnostic algorithm to differentiate between celiac disease, gluten sensitivity, and wheat allergy, largely based on guidelines from the American College of Gastroenterology.*[2]

of wheat—the gliadins. Now with the advent of FDA-cleared methods for the gliadin antibodies, a diagnosis of gluten sensitivity may be further supported, and the days of making a presumptive diagnosis following dietary avoidance of gluten will quickly become archaic.

While antibodies toward more specific antigenic determinants of the gliadin molecule have been identified, this degree of diagnostic specificity is unwarranted and extremely costly, especially as a front-line test to screen for non-celiac gluten sensitivity. The FDA-cleared methods in use at Doctor's Data for detecting gliadin antibodies identify and quantify IgA and IgG produced against the entire gliadin molecule, including all of its characterized antigenic epitopes, without compromise in analytical sensitivity.

### Recommendations for Celiac Disease Diagnosis[2]

1. Immunoglobulin A (IgA) anti-tissue transglutaminase (tTG) antibody is the preferred single test for detection of celiac disease in individuals over age 2.
2. When there exists a high probability of celiac disease wherein the possibility of IgA deficiency is considered, total IgA should be measured. Alternately, include both IgA and IgG-based testing in these high-probability patients.
3. In patients with low IgA or selective IgA deficiency, IgG-based DGPs and tTG testing should be performed.
4. If the suspicion of celiac is high, intestinal biopsy should be pursued even if serologies are negative.
5. All diagnostic serologic testing should be done with patients on a gluten-containing diet.
6. Antibodies directed against native gliadin are not recommended for primary detection of celiac.
7. Combining several tests in lieu of tTG IgA alone may marginally increase the sensitivity for celiac disease but reduces specificity and therefore are not recommended in low-risk populations.

## Minimally Invasive Dried Blood Spot Testing

At Doctor's Data, we continuously explore ways to make testing convenient and minimally invasive. We have employed our expertise in dried blood spot technologies to develop a menu of blood tests to assist clinicians in diagnosing celiac disease and gluten sensitivity.

Now, with just a few drops of blood from a fingerstick, Doctor's Data is able to detect and quantify the immunologic markers formed against gliadin and DGP using the BIO-FLASH Chemiluminescent Analyzer from Inova Diagnostics. Following extensive method validation protocols, the Doctor's Data team has confirmed that analyzing eluted blood spots is equivalent in sensitivity and specificity to testing serum for these immunologic markers of celiac disease and gluten sensitivity.

This means that clinicians can take advantage of highly standardized analytical methods combined with the convenience of a fingerstick sample that can be collected in the clinician's office or the patient's home. With the added stability of the dried blood sample, which remains viable for 21 days, and the economical transportation of the specimen using standard shipment, many of the barriers to testing have been eliminated.

## Advantages of Fingerstick Testing over Venipuncture

Collection of a dried blood spot sample has become relatively simple and is virtually pain-free. Today's micro lancets are safe and designed to overcome user error. These single-use, self-retracting, pressure-triggered products provide a controlled depth of lancing. Most lancets are calibrated to yield a free-

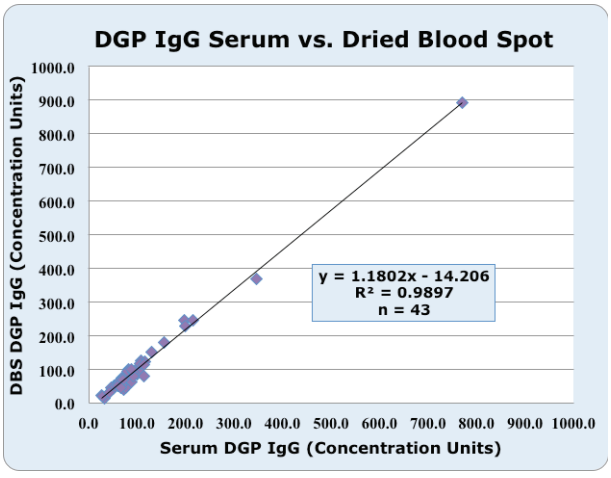
flowing capillary blood sample that minimizes the effects of tissue fluid contamination while also promoting quick healing. What's more, blood spot testing generally requires 100 to 200 µl compared to up to 7mL for a blood draw—venipuncture requires up to 70 times more blood.

Another advantage of dried blood spot testing is the ability of clinicians to expand service across a wide geographic area. If a physician cannot physically examine a patient or collect a blood specimen, the sampling kit allows a patient to collect a specimen at home. They simply send the kit to a central laboratory for testing and results are reported to the ordering physician for interpretation.

| Benefits of Dried Blood Spot Specimen Collection |
|---|
| **Healthcare Professional Advantages** |
| Saves costs and time |
| Expanded area of service and outreach testing |
| Enhanced sample stability and transportability |
| Reduced biohazardous waste |
| No phlebotomy needed |
| Improved patient compliance |
| **Patient Advantages** |
| Self-collection convenience |
| Time and cost savings |
| Smaller sample size |
| Reduced exposure to infection |
| Less pain and anxiety |
| Diminished bruising and faster healing |

## Performance Validation of Dried Blood Spot Testing for Celiac and Gluten Sensitivity at Doctor's Data

As shown in the graph, a strong degree of correlation (R2 = 0.989) was demonstrated between serum and eluted blood spot samples for the immunoglobulin G antibody to deamidated gliadin peptide (DGP IgG) across the analytical range (2.8 to 1000 concentration units), indicating that the same established cutoff for positivity of 20 concentration units in serum may likewise be used for dried blood spot samples. A similar high degree of correlation (R2 = 0.970, n=43) was realized in DGP IgA between paired serum and blood spot samples. Because tTG is found inside red blood cells, testing for the presence of antibody toward tTG is not feasible using blood spot extractants, as any circulating antibody in serum would become quickly bound to the tTG released from the cellular matrix during the blood elution process, thus causing false negative outcomes. While this somewhat limits the test menu for celiac serology in blood spots, the presence of antibodies to DGP meets the diagnostic criteria for celiac disease even in the absence of tTG testing. Doctor's Data offers DGP antibody testing in both IgA and IgG, which serves to enhance the detectability of an immune response, even in individuals who may be IgA deficient, which is prevalent among young children and many immune-compromised seniors.




*A comparison of serum and blood spot sample results for deamidated gliadin peptide (DGP) IgG.*

Doctor's Data uses the Inova BIO-FLASH® chemiluminescence system for celiac disease testing, whose QUANTA Flash® serum assays for DGP and tTG have been cleared by the FDA.

Serum testing for anti-gliadin antibodies (AGA) is also FDA-cleared and performed using an immunoassay. Similar to DGP, Doctor's Data also demonstrated 100% concordance between serum and dried blood spot results in paired samples for AGA IgA and IgG using the established serum cutoff value of 20 units for both IgA and IgG, (n = 75).

Assessment of analytical precision for each of the DGP and AGA methods in blood spots demonstrates a coefficient of variability of less than 7% at the clinical decision level, which strongly parallels the precision performance of the analysis of serum. This high level of concordance and correlation, in conjunction with the high degree of analytical reproducibility, leads to the confidence that Doctor's Data has in our ability to offer celiac disease and gluten sensitivity serologic testing in blood spots.

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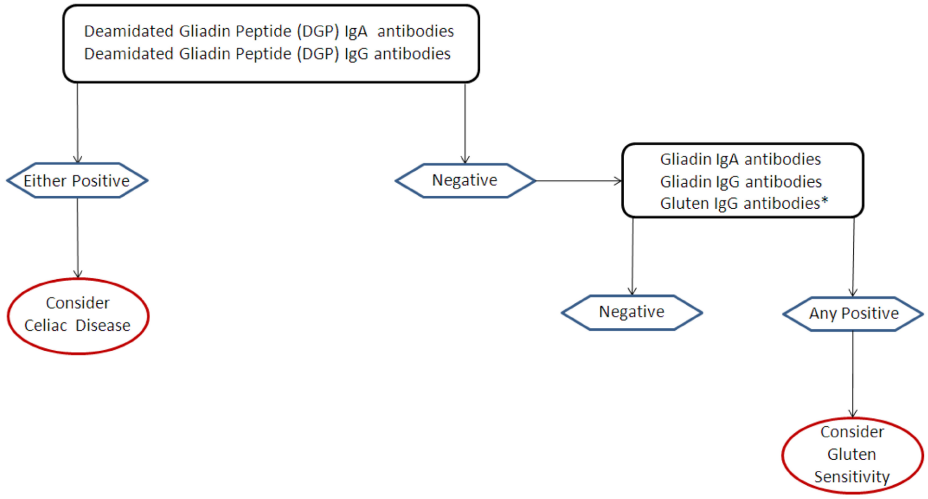
**LAB #: B000000-0000-0**
**PATIENT: Sample Patient**
**ID: P000000000**
**SEX: Male**
**DOB: 01/01/1962** **AGE: 52**

**CLIENT #: 12345**
**DOCTOR:**
**Doctor's Data, Inc.**
**3755 Illinois Ave.**
**St. Charles, IL 60174 U.S.A.**

## *Celiac & Gluten Sensitivity; blood spot*

| ANTIBODIES | | | | | |
|---|---|---|---|---|---|
| | RESULT/UNIT | | REFERENCE INTERVAL | NEG / WEAK POS / POSITIVE | |
| Deamidated Gliadin Peptide (DGP) IgA | **< 1.9** | **U** | < 20 | | |
| Deamidated Gliadin Peptide (DGP) IgG | **< 5.2** | **U** | < 20 | | |
| Gliadin IgA | **34** | **U** | < 20 | | |
| Gliadin IgG | **25** | **U** | < 20 | | |
| Gluten IgG* | **9.6** | **µg/mL** | < 3 | | |

Celiac Disease/Gluten Sensitivity Cascade

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|---|---|---|---|
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| Date Received: | 05/17/2014 | <dl: | less than detection limit |
| Date Completed: | 05/19/2014 | *Gluten IgG assay is for research use only. Not for use in diagnostic procedures. | |
| Method: | Immunoassay | | |



*Sample celiac and gluten sensitivity test results using dried blood spot technology.*

## Proven Partnership with Massachusetts General

Massachusetts General Hospital, one of the top centers of excellence in celiac disease and gluten sensitivity in the U.S. has teamed with Doctor's Data to investigate the utility of dried blood spot testing for screening newborns and infants of parents who have tested positive or have been genetically determined to be at an increased risk for these conditions. Several dried blood spot samples in which serum disposition was blinded to Doctor's Data were sent to our laboratory for analysis. The tables that follow summarize the preliminary findings of the sample comparisons, which exhibit 100% concordance for the serologic markers tested. One of the goals of this study is to demonstrate the utility of dried blood spot testing for celiac disease and gluten sensitivity in infants and children.

| ID | BIO-FLASH QUANTA Flash Assays | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Serum DGP IgA Mass General | | Blood Spot DGP IgA Doctor's Data | | Serum DGP IgG Mass General | | Blood Spot DGP IgG Doctor's Data | |
| | Units | Class | Units | Class | Units | Class | Units | Class |
| 08C | 5.2 | NEG | <5.2 | NEG | 2.8 | NEG | 3.0 | NEG |
| 18C | 9.2 | NEG | 10.0 | NEG | 2.8 | NEG | 5.0 | NEG |
| 63A | 8.3 | NEG | 7.1 | NEG | **31.4** | **POS** | **26.1** | **POS** |
| 64A | 5.2 | NEG | <5.2 | NEG | 2.8 | NEG | 4.1 | NEG |
| 11C | **24.7** | **POS** | **20.7** | **POS** | **28.9** | **POS** | **24.0** | **POS** |
| 65A | 5.2 | NEG | <5.2 | NEG | 3.3 | NEG | 4.2 | NEG |
| 66A | 5.2 | NEG | <5.2 | NEG | 2.8 | NEG | 3.0 | NEG |
| 67A | 5.2 | NEG | <5.2 | NEG | 2.8 | NEG | <2.8 | NEG |

*Deamidated gliadin peptide IgA and IgG—Preliminary data comparison between serum tested at Massachusetts General and blood spots tested at Doctor's Data demonstrating 100% concordance.*

| ID | Inova QUANTA Lite ELISA Assays | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Serum AGA IgA Mass General | | Blood Spot AGA IgA Doctor's Data | | Serum AGA IgG Mass General | | Blood Spot AGA IgG Doctor's Data | |
| | Units | Class | Units | Class | Units | Class | Units | Class |
| 08C | 8.0 | NEG | 9.9 | NEG | 3.8 | NEG | 5.0 | NEG |
| 18C | 36.9 | POS | 21.0 | POS | 16.4 | NEG | 16.1 | NEG |
| 63A | 6.7 | NEG | 10.1 | NEG | 45.7 | POS | 45.2 | POS |
| 64A | 5.0 | NEG | 5.0 | NEG | 6.1 | NEG | 6.0 | NEG |
| 11C | 56.0 | POS | 45.0 | POS | 56.5 | POS | 54.5 | POS |
| 65A | 3.7 | NEG | 6.1 | NEG | 7.7 | NEG | 8.0 | NEG |
| 66A | 10.5 | NEG | 8.1 | NEG | 3.6 | NEG | 7.2 | NEG |
| 67A | 5.7 | NEG | 6.0 | NEG | 7.2 | NEG | 6.4 | NEG |

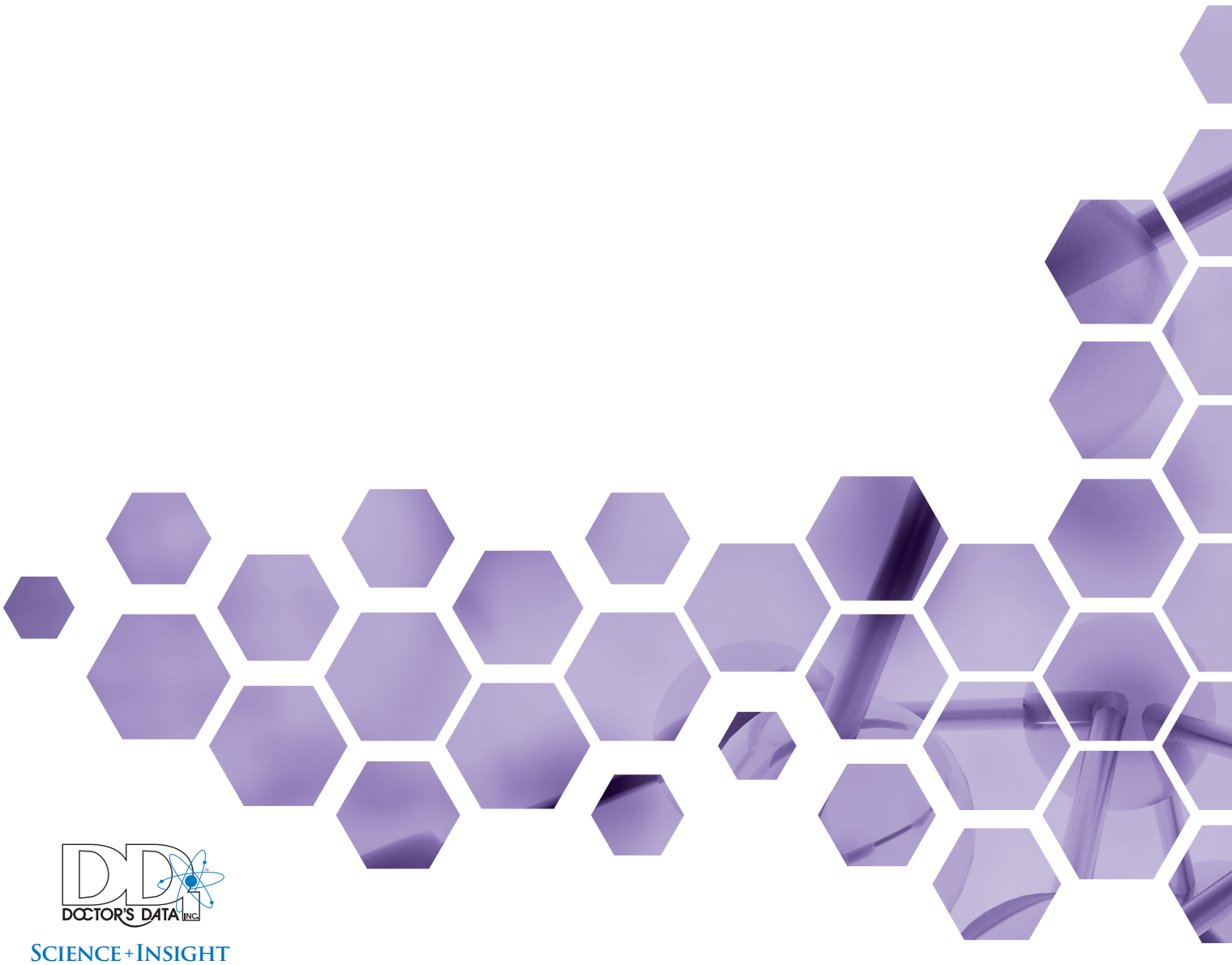
*Anti-gliadin antibody IgA and IgG—Preliminary data comparison between serum tested at Massachusetts General and blood spots tested at Doctor's Data demonstrating 100% concordance.*

If you have a patient with known or suspected intestinal permeability, a chronic inflammatory bowel disorder, or imbalanced microflora of the gut, consider Doctor's Data's expanded menu for assessing celiac disease and gluten sensitivity, which may be primary to the cause of the symptoms, or secondary to the intestinal injury or inflammation.

For more information about celiac disease and gluten sensitivity testing, visit *www.doctorsdata.com*

## References

1. Fasano A. Surprises from celiac disease. *Scientific Am*. 2009;301:54-61.
2. Rubio-Tapia A, Hill ID, Kelly CP, et al. ACG clinical guidelines: diagnosis and management of celiac disease. *Am J Gastroenterol.* 2013;108(5):656-76.



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