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(54) **METHODS AND APPARATUS FOR DETECTION OF GLUTEN SENSITIVITY, AND ITS DIFFERENTIATION FROM CELIAC DISEASE**

VERFAHREN UND VORRICHTUNG ZUM NACHWEIS EINER GLUTENEMPFINDLICHKEIT UND ZU IHRER DIFFERENZIERUNG VON ZÖLIAKIE

MÉTHODES ET APPAREIL POUR LA DÉTECTION DE LA SENSIBILITÉ AU GLUTEN, ET SA DIFFÉRENCIATION DE LA MALADIE C LIAQUE

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Description

FIELD OF THE INVENTION

[0001] The present invention relates to methods and kits for aid in diagnosis of gut-related diseases and pathologies, including at least gluten immune reactivity and sensitivity, silent celiac disease, and Crohn's disease.

BACKGROUND OF THE INVENTION

[0002] Wheat allergy, celiac disease and gluten sensitivity are three distinct conditions that are triggered by the ingestion of wheat gliadin (1, 2). In these conditions, the reaction to gluten is mediated by both cellular and humoral immune responses, resulting in the presentation of different symptomatologies. For example, in wheat allergy a specific sequence of gliadin peptides cross-links two IgE molecules on the surface of mast cells and basophils that trigger the release of mediators such as histamines and leukotrienes (3).

[0003] Celiac disease (CD) is an autoimmune condition with known genetic makeup and environmental triggers, such as gliadin peptides. CD affects between 1-2% of the general population. Throughout this application, unless the context dictates the contrary, all ranges set forth herein should be interpreted as being inclusive of their endpoints, and open-ended ranges should be interpreted to include commercially practical values. Similarly, all lists of values should be considered as inclusive of intermediate values unless the context indicates the contrary.

[0004] Markers for confirming a diagnosis of this disorder are IgA against native, deamidated gliadin peptides and IgA anti-tissue transglutaminase (tTg) autoantibody. In comparison with CD, gluten sensitivity (GS) affects up to 30% of the population (4). According to two articles published in 2010 and 2011 by Sapone et al (5, 6), symptoms in GS may resemble some of the gastrointestinal symptoms that are associated with CD or wheat allergy, but it is emphasized that objective diagnostic tests for gluten sensitivity are currently missing (5, 6). While studying the innate and immune responses in CD compared to those in GS, the researchers found that TLR1, TLR2 and TLR4, which are associated with innate immunity, were elevated in mucosal GS but not in CD, while biomarkers of adaptive immunity such as IFN-g, IL-21 and IL-17A were expressed in mucosal tissue in CD but not GS. They believed that measurements of toll-like receptors and IFN-γ, IL-21 and IL-17A would enable them to differentiate between CD and GS (5, 6) with a method that is highly invasive and would require a biopsy. Immediate type hypersensitivity to gluten is IgE mediated, while delayed type hypersensitivity to gluten is an antibody- (IgG, IgA) and T-cell-mediated reaction, which is called celiac disease or gluten sensitivity with enteropathy (7). In the absence of IgG and IgA against tTg, elevated IgG and IgA against various wheat antigens and peptides indicate the loss of mucosal immune tolerance against wheat peptides and the development of gluten sensitivity (7). Due to antigenic similarities between wheat antigens and human tissue, both CD and GS can result in many autoimmune conditions, including Type 1 diabetes, arthritis, thyroiditis, and even neuroautoimmune conditions such gluten ataxia and multiple sclerosis (8-10).

[0005] It should be appreciated that the term "patients" refer to humans under the care of a health care professional. More broadly, however, the novel testing protocols and analyses disclosed herein could be applied to non-patient humans, and any other animal that could suffer from celiac disease, gluten sensitivity, and gut-related autoimmunities.

[0006] While GS patients, similar to CD patients, are unable to tolerate gluten and can develop the same or similar sets of gastrointestinal symptoms, in GS this immune reaction does not lead to small intestine damage (5, 6). This lack of induction of intestinal damage in GS and the association of CD with genetic markers HLA DQ2/DQ8 plus small intestinal damage make the diagnosis of CD much easier than GS. The less severe clinical picture in GS, the absence of tTg autoantibodies, and the dismissal of the significance of elevated IgG and IgA autoantibodies against various wheat proteins and peptides by many clinicians makes GS an extremely dangerous disorder. This is because the persistence of IgG and/or IgA antibodies in the blood for long periods of time, along with inducers of inflammatory cascades can result in full-blown autoimmunity. If this were to be the case, due to the severity of the resulting tissue damage even implementation of a gluten-free diet might not be able to help reverse the course of the autoimmune reaction induced by IgG and IgA antibodies against different wheat antigens and peptides.

[0007] A comparison between celiac disease and gluten immune reactivity/sensitivity is shown in Figure 1. According to this model, if two children, one with a negative genetic makeup (HLA DQ2/DQ8$^-$), and the other with positive (HLA DQ2/DQ8$^+$), are exposed to environmental factors, such as Rota virus, bacterial endotoxins, and some medications or their synergistic effects, the result can be a breakdown of mucosal immune tolerance in both children. The induction of mucosal immune tolerance against gliadin results in the production of IgA and/or IgG against native wheat proteins and peptides, which is the next step in the initiation of gluten sensitivity in both individuals that are HLA DQ2/DQ8$^-$ and HLA DQ2/DQ8$^+$.

[0008] However, in the individual with the positive genetic makeup, the IgG and IgA antibodies against gliadin along with biomarkers of inflammation can activate tTg, induce damage to the villi, and result in villous atrophy. Deamidation of a specific gliadin peptide leads to the formation of a complex between it and the tTg; the presentation of this complex by antigen-presenting cells to T

cells and B cells results in IgA or IgG production against tTg, deamidated gliadin and the gliadin-tTg complex. The formation of these antibodies and their detection in blood is the hallmark of CD, which is an inherited condition detected in 1-2% of the population. If CD is left untreated, the outcome could be autoimmunities and cancer.

[0009] In comparison, in an individual negative for HLA DQ2/DQ8, this breakdown in immunological tolerance and the concomitant production of IgA and or IgG against native wheat proteins and peptides may activate an inflammatory cascade. In the absence of tTg activation, however, villous atrophy does not occur. Furthermore, gliadin peptides do not go through de-amidation, and consequently IgG and IgA antibodies are produced only against native wheat and gliadin peptides.

[0010] With continuous exposure to wheat antigens and continuous mucosal immune tolerance, the wheat antigens and reacting antibodies form an unholy alliance of immune complexes, resulting in severe gluten immune reactivity and sensitivity. This immune reactivity and sensitivity is a non-inherited condition detected in up to 30% of the population. If this disorder is left unchecked, prolonged exposure to IgG and IgA antibodies against wheat antigens and peptides and their cross-reaction with different tissue antigens can result in various autoimmune disorders. Therefore, even in the absence of CD, GS might still provide a productive environment for other gluten-related autoantibodies that attack different organs.

[0011] Furthermore, a gluten-free diet usually is only recommended for those who meet the criteria for a diagnosis of CD, not of gluten immune reactivity and sensitivity. Unfortunately, that leaves many gluten-sensitive people suffering unnecessarily with very serious symptoms that put them at risk for complications, conditions that might be resolved with a gluten-free diet, if they only knew.

[0012] The journal article by Fälth-Magnusson K and Magnusson KE, "Elevated levels of serum wheat germ agglutinin in celiac children lend support to the gluten-lectin theory of celiac disease", published in 1995 in Paediatric Allergy and Immunology (Vol. 6, pg. 98-102), reports a study that compared the serum antibody levels of IgA, IgG and IgM to wheat germ agglutinin and to gliadin in children under investigation for celiac disease (CD), as compared to reference children.

[0013] The journal article by Leszczyńska J, Łącka A and Bryszewska M, "The use of transglutaminase in the reduction of immunoreactivity of wheat flour", published in 2006 in *Food and Agricultural Immunology* (Vol. 17, pg. 105-114), reports an investigation of the immune response to wheat flour modified by treatment with transglutaminase, under different conditions of temperature, incubation period and ratio of enzyme to wheat flour. Particular wheat protein fractions were examined for immune reaction by the use of an indirect non-competitive ELISA, testing commercially available monoclonal antihuman IgG/IgE conjugates with alkaline phosphate and human sera with elevated IgG as well as rabbit sera against QQQPP peptide. The investigation reported a decrease in gliadins immunoreactivity for transglutaminase treated wheat flour.

[0014] Thus, a new paradigm is needed for aid in diagnosing and distinguishing among various gut-related diseases, including gluten immune reactivity and sensitivity, silent celiac disease, celiac disease, and gut-related autoimmunity.

SUMMARY OF THE INVENTION

[0015] In one aspect, the present invention claims a method of making a determination that assists in differentiating celiac disease from (a) gluten immune reactivity and sensitivity and from (b) a diagnosis related to gluten immune reactivity/sensitivity and autoimmunity, using test results derived from analyzing a blood sample obtained from a human, as set out in claim 1. Further embodiments of the present invention are set out in the dependent claims.

[0016] The present disclosure provides apparatus, systems and methods in which antibodies are used as biomarkers to assist in diagnosing gluten immune reactivity and sensitivity, silent celiac disease, Crohn's disease and other gut- related pathologies.

[0017] In certain aspects of the present disclosure, whole blood, blood sera, saliva or other samples from a human or other animal are tested for antibodies to (a) a wheat antigen; (b) a gliadin antigen; and (c) one or more of a wheat germ agglutinin, a gluteomorphin, a glutenin, a de-amidated glutenin, a prodynorphin, and a dynorphin.

[0018] The test results can advantageously be used to assist in differentiating gluten immune reactivity or sensitivity from celiac disease, especially where the wheat antigen is de-amidated, and the gliadin antigen is selected from the group consisting of an α-gliadin-33-mer, an α-gliadin- 17-mer, a γ-gliadin-15-mer, an ω-gliadin-1 7-mer, and a glutenin-21-mer.

[0019] In certain aspects of the present disclosure, whole blood, blood sera, saliva or other samples from a human or other animal are tested for antibodies to one or more of a γ-gliadin protein or a peptide thereof such as γ-gliadin-15-mer, an ω-gliadin protein or a peptide thereof such as ω-gliadin-17-mer, wheat germ agglutinin, a gluteomorphin, a glutenin protein or a peptide thereof such as glutenin-21-mer, a de-amidated glutenin protein or a peptide thereof, a prodynorphin, and a dynorphin.

[0020] In certain aspects of the present disclosure, tests conducted and/or test results analyzed for antibodies that assist in distinguishing gluten immune reactivity and/or sensitivity, silent or atypical celiac disease relative to patently symptomatic (classical) celiac disease, Crohn's disease and chronic immune activation. Test plates and kits are disclosed that test for antibodies to at least three, five, seven or all of α-gliadin, γ-gliadin, ω-gliadin, glutenin, wheat germ agglutinin, gluteomorphin, prodynorphins, transglutaminase, and gliadin-bound

transglutaminase (gliadin-transglutaminase complex).

[0021] In certain aspects of the present disclosure, assays and assay kits of particular interest allow for testing IgA and/or IgG antibodies to one or more wheat antigens, a α-gliadin protein or one or more peptides thereof such as α-gliadin-33-mer and/or α-gliadin- 17-mer, a γ-gliadin protein or one or more peptides thereof such as γ-gliadin-15-mer, an ω-gliadin protein or one or more peptides thereof such as ω-gliadin-17-mer, wheat germ agglutinin, an opioid peptide such as one or more of gluteomorphin, prodynorphin and/or dynorphin, a glutenin protein or one or more peptides thereof such as glutenin-21-mer, a deamidated glutenin protein or one or more peptides thereof, a gliadin-transglutaminase complex, or combinations thereof.

[0022] In certain aspects of the present disclosure, the detection of antibodies can be performed with an immunoassay, including, but not limited to, ELISA assay, RIA assay, latex agglutination, beads assay, proteomic assays, and other immunoassays known to one of ordinary skill in the art.

[0023] Various objects, features, aspects and advantages of the inventive subject matter of the present invention will become more apparent from the following detailed description of disclosed embodiments, along with the accompanying drawing figures in which like numerals represent like components.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024]

Figure 1 is a schematic showing differentiation between celiac disease (right hand listing) and gluten immune reactivity/sensitivity (left hand listing), as contemplated herein.

Figure 2 is a diagram showing the layout of a sample microtiter plate having 12 different rows with 12 different antigens and peptides.

Figure 3 is a diagram showing the layout of a sample microtiter plate by which IgG or IgA is measured against 12 different antigens or peptides from wheat and associated tissue antigens (antigens or peptides are transparent).

Figure 4 is a diagram showing the layout of a sample microtiter plate by which IgG or IgA is measured with weekly negative and positive controls for quality control purposes (antigens or peptides are transparent).

Figure 5 is a diagram of an antibody testing protocol for celiac disease using tTg and various antigens according to the prior art (17).

Figure 6 is a diagram of a testing protocol for celiac disease using deamidated gliadin peptide according to the prior art (17).

Figure 7 is a diagram of a proposed testing protocol for celiac disease, gluten immune reactivity/sensitivity and autoimmunity using a repertoire of wheat antigens and peptides.

DETAILED DESCRIPTION OF THE DRAWINGS

[0025] According to certain aspects of the present disclosure, antibodies are used as biomarkers to assist in diagnosing and distinguishing gluten immune reactivity and sensitivity, silent celiac disease, Crohn's disease and other gut-related pathologies as opposed to the classical celiac disease against an array of wheat antigens and peptides.

[0026] In certain aspects of the present disclosure, a bodily fluid is tested for immunoglobulin G (IgG) and/or immunoglobulin A (IgA) antibodies to one or more of a whole-wheat antigen; an α-gliadin protein or one or more peptides thereof; a γ-gliadin protein or one or more peptides thereof; an ω-gliadin protein or one or more peptides thereof; a glutenin protein or one or more peptides thereof; one or more opioid peptides; a gliadin-transglutaminase complex (gliadin bound to transglutaminase); transglutaminase; wheat germ agglutinin; and combinations thereof.

[0027] In the present disclosure, the whole-wheat antigen may be deamidated and may be prepared by combining water-soluble and alcohol-soluble whole-wheat proteins. The α-gliadin protein or one or more peptides thereof includes α-gliadin-33-mer and/or α-gliadin-17-mer, with other α-gliadin peptides contemplated by the present disclosure. The γ-gliadin protein or one or more peptides thereof includes γ-gliadin-15-mer, with other γ-gliadin peptides contemplated by the present disclosure. The ω-gliadin protein or one or more peptides thereof includes ω-gliadin-17-mer, with other ω-gliadin peptides contemplated. The glutenin protein or one or more peptides thereof includes glutenin-21-mer, with other glutenin peptides contemplated by the present disclosure. The one or more opioid peptides includes exorphin peptides including gluteomorphin, prodynorphin and/or dynorphin, with other exorphin peptides contemplated by the present disclosure.

[0028] In the present disclosure, whole blood, blood serum/sera, saliva or other bodily fluid samples from a human or other animal are tested for antibodies to (a) a wheat antigen; (b) a gliadin antigen; and (c) one or more of a wheat germ agglutinin, a gluteomorphin, a glutenin, a de-aminated glutenin, a prodynorphin, and a dynorphin. It is disclosed that test results are considered particularly interesting where the wheat antigen is deamidated, and the gliadin antigen is selected from the group consisting of an α-gliadin-33-mer, an α-gliadin-17-mer, a γ-gliadin-15-mer, an ω-gliadin-17-mer, and a glutenin-21-mer. It is disclosed that test plates and kits can advantageously test for antigens to at least three, five, seven or all of α-

gliadin, γ-gliadin, ω-gliadin, glutenin, wheat germ agglutinin, gluteomorphin, prodynorphins, transglutaminase, and gliadin-bound transglutaminase.

[0029] Assays and assay kits are disclosed that allow for testing IgA and/or IgG antibodies to one or more wheat antigens, a α- gliadin protein or one or more peptides thereof such as α-gliadin-33-mer and/or α-gliadin- 17-mer, a γ-gliadin protein or one or more peptides thereof such as γ-gliadin-15-mer, an ω- gliadin protein or one or more peptides thereof such as ω-gliadin- 17-mer, wheat germ agglutinin, an opioid peptide such as one or more of gluteomorphin, prodynorphin and/or dynorphin, a glutenin protein or one or more peptides thereof such as glutenin-21-mer, a de-amidated glutenin protein or one or more peptides thereof, a gliadin-transglutaminase complex, or combinations thereof.

[0030] In the present disclosure, the detection of antibodies can be performed with an immunoassay, including, but not limited to, ELISA assay, RIA assay, latex agglutination, beads assay, proteomic assays, and other immunoassays known to one of ordinary skill in the art.

[0031] Following are exemplary descriptions of assays, and their use and analysis with respect to some test patients. Although other materials and methods similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred method and materials are now described in the exemplary description of assays to further illustrate the present invention.

EXAMPLE 1

ELISA Assay

A. Materials And Methods - Plate and Sample Preparation:

[0032] Wheat antigens and peptides. A whole-wheat antigen was prepared by combining water-soluble and alcohol-soluble proteins. Different gliadin peptides including α-gliadin 33-mer, 17-mer, γ-gliadin 15-mer, ω-gliadin 17-mer, gluteomorphin, prodynorphin, transglutaminases, and gliadin bound to transglutaminase, glutamic acid decarboxylase (GAD-65) HPLC grade were synthesized by Bio-Synthesis Inc. (Lewisville, TX). Wheat germ agglutinin (WGA) was purchased from Sigma/Aldrich (Saint Louis, MO).

[0033] The antigens and peptides were dissolved in methanol at a concentration of 1.0 mg/mL, then diluted 1:100 in 0.1M carbonate-bicarbonate buffer, pH 9.5, and 50 μl were added to each well of a polystyrene flat-bottom ELISA plate.

[0034] The ELISA plates were incubated overnight at 4°C and then washed three times with 200 μl Tris-buffered saline (TBS) containing 0.05% Tween 20 (pH 7.4). The non-specific binding of immunoglobulins was prevented by adding 200 mL of 2% bovine serum albumin (BSA) in TBS, and incubated overnight at 4°C. Plates were washed and after conducting quality control were kept at 4°C until used.

[0035] The enzyme conjugates included: Affinity Purified Antibody Phosphatase-labeled Goat anti-Human IgG (Jackson ImmunoResearch, Cat#109-055-008), and Affinity Purified Antibody Phosphatase-labeled Goat anti-Human IgA (Jackson ImmunoResearch, Cat#109-055-011).

[0036] Other additional reagents and materials included in the method as further described herein, includes: Phosphate-Buffered Saline Powder (Sigma, Cat#P3813-10PAK), Bovine Serum Albumin (Biocell, Cat#3203-00), Sodium Azide (Sigma, Cat#S-2002), Tween 20 (Sigma, Cat#P1379-1000ML), Glycerol (Sigma, Cat#G5516-500ML), Sodium Hydroxide (Sigma, Cat#S-5881), Magnesium Chloride (Sigma, Cat#8266), Diethanolamine (Sigma, Cat#D-8885), 1.0 N Hydrochloric Acid Solution (Sigma, Cat#H3162), 5mg Substrate Tablets: p-NPP (para-nitophenyl phosphate) (Sigma, Cat#S-0942), and Distilled water (D. H_2O).

[0037] The microwell plates were prepared and coated with the desired number of wheat-associated antigens and/or peptides. In the following case examples, 12 different wheat-assocaited antigens and peptides were coated on the microwell plates. Calibrator and positive controls and diluted patient samples were added to the wells and autoantibodies recognizing different wheat antigens bind during the first incubation. After washing the wells to remove all unbound proteins, purified alkaline phosphatase labeled rabbit anti-human IgG/IgA unbound conjugate were removed by a further wash step.

[0038] Bound conjugate was visualized with paranitrophenyl phosphate (PNPP) substrate, which gives a yellow reaction product, the intensity of which is proportional to the concentration of autoantibody in the sample. Sodium hydroxide was added to each well to stop the reaction. The intensity of color was read at 405 nm.

[0039] Plain red tops or red tiger tops (SST tubes) were used for specimen collection, although in certain aspects, other specimen collection apparatus are contemplated for this assay.

[0040] Blood samples were collected using aseptic venipuncture techniques and serum was obtained using standard procedures. In certain aspects, it is preferred that a minimum of about 50 μl of serum for the assay, which therefore corresponds to about 0.5 ml or more of blood.

B. Test Assay Procedure

[0041] The analytical procedure for IgG and/or IgA antibody array to assist in diagnosing and detection of gluten immune reactivity and sensitivity, silent celiac disease, Crohn's disease and other gut-related pathologies is now discussed. In some aspects, all reagents were allowed to reach room temperature before the test assay was commenced. The test assay procedure includes preparing the desired number of coated wells or plates with

the desired number and type of wheat-associated antigens and/or peptides. Once the microtiter wells are prepared, about 100 μl of 1:100 diluted control calibrator are added to Rows A and B of the microtiter plate as shown in Figure 3 using a multi-channel pipettor. About 100 μl of 1:100 diluted patient's test sample, here blood serum, was added to duplicate wells of rows C and D for Clinical Specimen 1, rows E and F for Clinical Specimen 2, and rows G and H for Clinical Specimen 3.

[0042] On a separate plate, the periodic negative and positive controls similar to clinical specimens in duplicates were conducted, as shown in Figure 4.

[0043] The plates were then incubated for 60 minutes at room temperature. After incubation, the wells were emptied and washed four times with PBS using an ELISA Washer. About 100 μl of optimally diluted alkaline phosphatase-labeled goat anti-human IgA was added to the IgA plate or about 100 μl of enzyme-labeled IgG was added to the IgG plate at optimal dilution.

[0044] The respective plates was then incubated for 30-60 minutes at room temperature. About ten minutes before the conjugate-incubation ends, a substrate solution was prepared by mixing 5 mg of a p-nitrophenyl phosphate tablet with 5 ml of substrate buffer, which was mixed well until the tablet completely dissolved. Washing four times with PBS using the ELISA washer was repeated. Then, about 100 μl of substrate solution was added to each well. The plate was then incubated for 30 minutes at room temperature with the avoidance of any exposure to direct sunlight. The reaction was stopped by adding about 50 μl of 3 N NaOH. The color intensity of the wells were read using a microtiter plate reader at 405 nm against a blank well, with the absorbance values of the calibrators, controls and unknown samples being recorded.

C. Calculation of Results

[0045] After the plate was read the plate at 405 nm to obtain the optical density values (OD405), the mean ODs of the negative controls, the mean ODs of the positive controls and the mean ODs of each clinical specimen were divided by the mean ODs of calibrators on Rows A and B to obtain each Index Value (IV).

[0046] The Index Value (IV) for each antibody was calculated against the 12 different antigens by dividing the mean OD of each duplicate sample by the mean OD of the calibrator control value (for example, divide the mean OD of wells C1 and D1 by the mean OD of wells A1 and B1, the mean OD of wells C2 and D2 by the mean OD of wells A2 and B2, the mean OD of wells C3 and D3 by the mean OD of wells A3 and B3, etc.).

[0047] The results were then compared to the established reference ranges.

$$\text{Index} = \frac{\text{Mean OD of patients}}{\text{Mean OD of calibrators}}$$

Index Calculation For Wheat Antigens	
Cal 1 (OD) Cal 2 (OD)	0.41 0.44
Sample 3 A (OD) Sample 3 B (OD)	3.77 3.79
Index	8.93

D. Interpretation of Results

[0048] Examples of IgG and IgA antibody patterns of 3 patients with celiac disease, 3 patients with gluten immune reactivity and sensitivity, and 3 patients with Crohn's disease with overlapping gluten sensitivity are shown in Tables 2-7. Crohn's disease, also known as regional enteritis, is a type of inflammatory bowel disease having a strong genetic component. It is often described as an autoimmune disease, but others consider it be a disease of immune deficiency.

[0049] Data interpretation and laboratory differentiation between celiac disease and gluten immune reactivity/sensitivity/autoimmunity are shown in Table 8. In particular, it is now contemplated that celiac disease can be differentiated from silent celiac disease, gluten immune reactivity/sensitivity and/or gluten-related autoimmunity as follows:

a. A serological pattern of silent celiac disease is indicated where the test results include positive results of IgA and/or IgG against any or combination of wheat proteins, α-gliadin 33-mer, deamidated α-gliadin 33-mer, α-gliadin 25-mer, α-gliadin 18-mer, α-gliadin 17-mer, γ-gliadin 15-mer, ω-gliadin 17-mer, glutenin, deamidated glutenin, gluteomorphin, prodynorphin and wheat germ agglutinin, and negative results against tranglutaminase-2, and at least one of IgA and IgG tests positive against at least one of transglutaminase-3 and transglutaminase-6.

b. A serological pattern of gluten immune reactivity and sensitivity is indicated where the test results include positive results for IgG and/or IgA against wheat antigens, in particular native α-gliadin, γ-gliadin, ω-gliadin, glutenin, deamidated glutenin, gluteomorphin, and wheat germ agglutinin, but not deamidated α-gliadin and not transglutaminase-2.

c. A diagnosis related to gluten immune reactivity/sensitivity and autoimmunity is indicated where the test results include a positive for IgG and/or IgA against wheat antigens, in particular native α-gliadin, γ-gliadin, ω-gliadin, glutenin, deamidated glutenin, gluteomorphin, and wheat germ agglutinin, and positive results for any IgG, IgA or IgM to glutamic acid decarboxylase, cerebellar tissue antigens or pep-

tides or other tissue antigens, and negative results for IgA to deamidated α-gliadin, deamidated glutenin or against transglutaminase-2, but positive IgA or IgG against transglutaminase-3 and/or transglutaminase-6.

[0050] Reportable ranges and reference ranges are show in Table 1, which may be updated from time to time. The reader will note that compared to established reference ranges at 2 standards above the mean, while IgA antibody against tTg and gliadin-tTg complex is highly positive (confirming the diagnosis of CD), the pattern and the strength of IgG and IgA antibody varies from antigen to antigen. For example, while both IgG and IgA against wheat antigens in all three patients with celiac disease was 4-6 fold higher than reference ranges, when the IgG and IgA was measured against α-gliadin 33-mer the IgG antibody level was significantly elevated in one and the IgA level in two out of three patients (Tables 2, 3).

[0051] When compared to patients with celiac disease, in patients with gluten immune reactivity and sensitivity none of the three patients showed a significant IgA reactivity against tTg and gliadin-tTg complex, while the IgG and IgA antibodies against wheat antigens and in combination with one or more wheat peptides (α-gliadin, γ-gliadin, ω-gliadin, glutenin, gluteomorphin, prodynorphin and wheat germ agglutinin) was significantly elevated. The IgA immune reaction against wheat antigens and peptides in combination with tTg and gliadin-tTg complex clearly distinguished between celiac disease and gluten immune reactivity/sensitivity, in which IgG and/or IgA antibodies are reactive against various wheat antigens and peptides but not against tTg and the gliadin-tTg complex (Tables 4, 5).

[0052] Tables 6 and 7 present the results of three patients with Crohn's disease. The pattern of IgG and IgA antibodies against tTg and gliadin-tTg complex clearly shows that these patients, in addition to Crohn's disease, are also suffering from gluten immune reactivity/sensitivity, and possibly also celiac disease (Tables 6, 7).

CASE STUDY EXAMPLES

[0053] Four different case reports, the first on a patient with celiac disease, the second with gluten sensitivity, the third with gluten sensitivity and autoimmunity, and the fourth with gluten sensitivity overlapping with Crohn's disease are shown below.

A. CASE REPORT #1: Diagnosis of Celiac Disease in the Elderly by the Use of IgA against Gliadin and Tissue Transglutaminase with Improvement on a Gluten-Free Diet

[0054] A 76 year-old man with longstanding dyspepsia, indigestion, tiredness and rapid weight loss was referred for gastrointestinal evaluation. Blood tests showed macrocytic anemia with low concentrations of folate and vitamin B-12. The patient's hemoglobin concentration was 79 g/L, albumin 32 g/L, and transglutaminase 212 μg/mL (normal range = 0-10 μg/mL. An urgent colonoscopy and duodenal biopsy was performed, which yielded macroscopically normal results. At this level his IgG and IgA concentrations against gliadin and transglutaminase were checked using FDA-approved kits. Both IgG and IgA against α-gliadin were very high; against transglutaminase, IgA but not IgG was 3.8-fold higher than the reference range. In view of the IgA positivity against gliadin and transglutaminase and diagnosis of celiac disease he was transfused with 2 units of packed cells and started on both a gluten-free diet and 20 mg of prednisone daily. Six months later he had gained about 12 pounds and showed few GI symptoms. Because of this improvement the patient became committed to the gluten-free diet. One year after the first performance of IgG and IgA antibody testing against gliadin and transglutaminase the repeat tests for these antibodies were negative, which is a further indication that disease management plus a gluten-free diet was instrumental in the treatment of this elderly patient with a silent celiac disease.

[0055] Discussion: According to Catassi et al. (1, 11), celiac disease (CD) is one of the most common lifelong disorders in western countries. However, most cases of CD remain undiagnosed mostly due to the poor awareness of the primary care physician regarding this important affliction (Catassi C, et al., Am J Gastroenterol, 102:1454-1460, 2007). Celiac disease is perceived as presenting GI symptoms accompanied by malabsorption. But many patients with celiac disease do not present GI symptoms. These individuals may have silent or atypical celiac disease, and the condition may present with iron deficiency, anemia, increased liver enzymes, osteoporosis or neurological symptoms (12). As used herein, the term "atypical celiac disease" refers to celiac disease in patients who have only subtle symptoms, and the term "silent celiac disease" refers to celiac disease in patients who are asymptomatic.

[0056] The increasing recognition of celiac disease is attributed to the use of new serological assays with higher sensitivity and specificity. Until recently celiac disease was incorrectly perceived as being uncommon and detected mainly during infancy or childhood. However, it is now recognized that most cases of CD occur in adults 40-60 years old. Patients in this age group may present their symptoms, lab test results and other examination signs in atypical fashion. In fact, according to a very recent publication, less than one in seven patients is correctly diagnosed with CD (13).

[0057] Consequently, as this case shows, if an adult patient presents with symptoms and signs suggesting malabsorption, testing for IgA antibody against gliadin and transglutaminase should be considered. If the test results are positive, celiac diseases should then be made a part of the differential diagnosis, based on which a gluten-free diet should be recommended. If the gluten-free diet should produce an improvement in symptoms, the

patient should commit to the diet regardless of age.

B. CASE REPORT #2: Gluten Immune Reactivity and Sensitivity induced by a Combination of Anesthetics, Antibiotics and Pain Medication

[0058] A 46 year-old woman was given her yearly checkup by her internist. Based on her medical examination and a normal CBC, chemistry including liver enzymes, and an autoimmune profile, she was classified as a healthy person. Gluten antibodies were not measured at that time. A few months later she went to her dentist for a root canal, bone graft and preparation for dental implantation. During five different visits over ten days she was treated with anesthetic material (mepivacaine), antibiotics and painkillers. Four months later the dental implant procedure was completed after local anesthesia with lidocaine with subsequent prescription of antibiotic (amoxicillin) and painkillers. Four hours later she developed a severe allergic reaction with localized edema, in particular the lips and periorbital area swelling. The patient became agitated and exhibited with a generalized itching, particularly her face, hands and feet. Tightness of the chest with wheezing and difficulty in breathing was an indication of allergic reaction to one or more of the medicines used. She was immediately treated with 0.01 mL per kg of body weight of adrenaline, intramuscularly supplemented by antihistamine treatment. However, while the allergic reaction was controlled, the patient developed severe vomiting and diarrhea with severe abdominal pain, which lasted for 8 days. Two weeks later, while the diarrhea had ameliorated, the patient continued to complain about bloating and abdominal discomfort with irritable bowel-like syndrome. She was referred to a GI specialist who detected nothing of note upon a thorough examination. The possibility of gluten sensitivity was then considered, and the patient was tested for HLA typing and IgG and IgA anti-gliadin and anti-transglutaminase antibodies. Immunological tests showed these results: IgG anti-gliadin 6.8 U/mL (normal range <20 U/mL); IgA anti-gliadin 4.1 U/mL (normal range <20 U/mL); IgG anti-tTg 2.1 U/mL (normal range <6 U/mL); IgA anti-tTg 1.2 U/mL (normal range <4 U/mL); and negative for HLA DQ2 and DQ8. Based on these findings gluten sensitivity and celiac disease were excluded, and it was concluded that the patient was suffering from psychogenic or idiosyncratic reaction associated with reaction to the anesthetic and its synergistic effect with the antibiotics. Ninety days later upon her follow-up visit the patient was still complaining about bloating and abdominal pain, particularly 1-3 hours after each meal. Repeat testing was ordered, and both a basic test and a comprehensive test was ordered for anti-gliadin and -tTg IgG and IgA along with ASCA and p-ANCA IgG, which are the suggested tests for suspected Crohn's disease and ulcerative colitis. Interestingly, almost 100 days after the first GI discomfort, while ASCA and p-ANCA were completely within the normal range, both the IgG and IgA against gliadin were 4 to 9-fold above the reference range (gliadin IgG = 79 U/mL; IgA = 54 U/mL). However, IgG and IgA antibodies against tTg were within the normal range. In addition, the IgG and IgA antibody testing was performed against an array of wheat, gliadin, glutenin, wheat germ agglutinin, gliadin-tTg complex and tTg antigens. IgG was detected against 8 out of 12 tested antigens and IgA against 6 out of 12 tested antigens at 2-7 fold higher than established reference ranges. Both IgG and IgA against tTg and gliadin -tTg complex were negative (see Tables 4 and 5, Sample #1). These results along with the positivity of the basic IgG and IgA test against gliadin but not against transglutaminase showed that the patient, due to allergic reaction to environmental factors, had lost tolerance to wheat antigens and had developed gluten sensitivity but not celiac disease. Despite the absence of elevation in IgG and IgA levels against tTg, due to the continuous GI discomfort and the elevated IgG and IgA against gliadin, a gluten-free diet was recommended, and a dietitian advised the patient to take probiotics and go on a restricted diet free of glutens and also of lectins, since the WGA level was also elevated for both IgG and IgA. Six months after the introduction of the diet and the probiotics, the patient's GI discomfort had subsided and she was back to normal health.

[0059] Discussion: The term gluten sensitivity refers to a state of heightened immunological responsiveness to gluten as indicated by the elevation of IgG, IgA or both against gliadin but not against transglutaminase (14). Gluten sensitivity begins with the loss of mucosal immune tolerance to wheat antigens and peptides due to environmental factors affecting the mucosal immune homeostasis.

[0060] In this case gluten sensitivity was confirmed based on GI symptoms and immunological testing, in particular IgG and IgA against gliadin and its associated proteins and peptides almost 100 days after the triggering factors had affected her state of immunological tolerance to wheat and associated antigens. It seems that in this patient the synergistic effects of anesthetics, antibiotics and painkillers resulted in dysregulation of her mucosal immune system, followed by a breakdown in immunological tolerance to wheat and other dietary proteins and peptides. This, possibly in combination with the effect of environmental factors on the activity of the digestive enzymes, resulted in the induction of the opening of tight junctions and the entry of undigested wheat proteins and peptides into the submucosa, lymph nodes, and the circulation. These antigens were subsequently presented by antigen-presenting cells to T cells and B cells. During this process gliadin-specific B cells are assisted by gliadin-specific T cells, leading to B-cell clonal expansion and the release of IgG and IgA antibodies to gliadin and associated proteins and peptides, which in this case was detected about 100 days after the original traumatic experience.

[0061] It is concluded herein that screening for gluten

sensitivity in patients with GI discomfort associated with the use of anesthetics and antibiotics may be easily and cost-effectively undertaken by measuring circulating IgG and IgA against gliadin and associated proteins and peptides. Failure to do so may not only deprive the patient of an accurate diagnosis and the proper treatment by implementation of a gluten-free diet, but may also result in unnecessary medical interventions with their associated side effects.

C. CASE REPORT #3: Gluten Immune Reactivity, Sensitivity and Autoimmunity

[0062] Here, a case report is described in which the original presentation led to an erroneous diagnosis of irritable bowel syndrome, resulting in incorrect medical intervention. The correct diagnosis of gluten immune reactivity and sensitivity was made after years of mistreatment.

[0063] A 49 year-old woman with abdominal pain, constipation, acid reflux and headache was examined by an internist. Investigation revealed normal CBC with hemoglobin of 10.8 g/dl and normal chemistry profile including liver enzyme. Over several visits detailed biochemical and immunological profiles including ANA, rheumatoid factor, T3, T4, and TSH levels were performed, all testing within the normal range. After repeated complaints about GI discomfort, the patient was referred for GI evaluation. Both endoscopy and H. pylori test results were normal. The patient was diagnosed with irritable bowel syndrome and put on β-blockers and nexium, which moderately improved her symptomatologies. Four years later, however, in addition to the old GI symptoms and headache, she presented symptoms of malaise, blurred vision and facial rash. She was intermittently sleepy and irritable, and experienced breathing problems. Further lab tests revealed her hemoglobin was 9.7 g/dl with MCV of 72 fL, a raised erythrocyte sedimentation rate (46 mm/1st hour), ANA of 1:80 (normal range <40), mild elevation in IgA smooth muscle antibody, double-stranded DNA and extractable nuclear antibodies were negative. Based on the available evidence, a diagnosis of systemic lupus erythematosus (SLE) was made by a rheumatologist, and treatment with steroids was commenced. There was some improvement in her overall state but her hemoglobin level continued to be low, while her ESR fluctuated. Two years later she developed difficulty in passing urine accompanied by tingling and sensory disturbance in her trunk and legs, which led to her being referred to a neurologist. Close questioning revealed a band-like sensation in the trunk and reduced visual acuity (8/46 in the right eye, 8/23 in the left eye) with minimal eye pain, but normal eye movement. Lab investigation came up with low hemoglobin, abnormal MCV, and low serum ferritin at 14 μg/L (normal range 10-150 μg/L), which confirmed iron deficiency. MRI scan of the brain showed extensive white matter abnormalities not typical of multiple sclerosis, but no abnormalities were detected in CSF examination. While blood and CSF examination showed no evidence of bacterial and viral infection including syphilis, mycobacteria, borrelia, EBV, CMV, HTLV, and Herpes Type-6, visual evoked potentials showed delay in both optic nerves. In view of these abnormalities, and since tests for gluten sensitivity had not been performed during the earlier investigations, the possibility of gluten sensitivity was considered. A comprehensive IgG and IgA panel was ordered against a repertoire of wheat proteins and peptides, as well as against tTg and various tissue antigens. This comprehensive gluten sensitivity and immune reactivity screen revealed IgG against wheat antigens, α-gliadin 33- and 17-mer, γ- and ω-gliadin, glutenin, gluteomorphin, prodynorphin, gliadin-tTg complex, wheat germ agglutinin, and glutamic acid decarboxylase 65 (GAD-65). IgA antibodies were detected against wheat antigens and wheat germ agglutinin (see Tables 4 and 5, Sample #3). Interestingly, both IgA and IgG tested against tTg were within the normal range.

[0064] Furthermore, antibodies against ganglioside, cerebellar, synapsin, myelin basic protein, collagen, thyroglobulin and thyroid peroxidase were tested, and all were 2-4 fold above the reference range. Upper GI endoscopy and biopsy revealed normal histology and intraepithelial lymphocytes. Overall the patient was diagnosed as having gluten sensitivity with its associated autoimmunities, including gluten ataxia, headache, white matter abnormalities, and neuromyelitis optica. A five-day course of intravenous methylprednisolone was implemented, and gradually the sensory, motor and visual symptoms improved. In addition, based on the very high levels of IgG and some IgA antibodies against a repertoire of wheat antigens and peptides, a gluten-free diet was introduced, and 12 weeks later marked improvement was observed in the patient's clinical symptomatology. She continued the 100% gluten-free diet under the observation of a dietitian, and the steroid treatment was stopped. Six months after introduction of the diet antibody tests against wheat antigens, peptides, and human tissue were repeated; more than 60% reduction in antibody levels was observed, and the patient became almost asymptomatic.

[0065] Discussion: From this data I have concluded that a patient may suffer from gluten immune reactivity and sensitivity without having abnormal tissue histology or flat erosive gastritis and antibody against tTg based on which a diagnosis of celiac disease is normally made. If patients with gluten sensitivity and immune reactivity are not detected in time based on the proper lab tests, in particular IgG and IgA antibodies against a repertoire of wheat proteins and peptides, patients' symptomatologies may mislead many clinicians into treating their patients for lupus, MS-like syndrome, neuromyeltitis optica, and many other autoimmune disorders. Therefore, measurement of IgG and IgA antibodies against a repertoire of wheat antigens and peptides is recommended for patients with signs and symptoms of autoimmunities so that intervention with a gluten-free diet will be instru-

mental in reversing the autoimmune conditions associated with gluten immune reactivity and sensitivity. Otherwise, untreated and/or mistreated, the patient will develop multiple autoimmune disorders.

D. CASE REPORT #4: Gluten Immune Reactivity and Sensitivity Overlapping with Crohn's Disease

[0066] Crohn's disease is an inflammatory disorder that often emerges during the second or third decade of life, affecting the terminal ileum in more than two-thirds of patients (15). A combination of genetic and environmental factors, including a shift in gut flora and dysfunctional responses against them, is believed to lead to dysregulated immunity, altered intestinal barrier function, and possibly autoimmunity (16).

[0067] Here, a 32 year-old man presented with gastrointestinal discomfort and diarrhea 2-3 times per month. Laboratory results including chemistry panel, CBC, iron, ferritin, transferrin, vitamin B-12, thyroid function, and urine analysis were within the median level of the normal range. Upon the second visit and continuation of GI symptoms he was referred to a GI specialist who ordered additional lab examinations. These tests were microbiological evaluation of the stool and blood tests for antibodies against *H. pylori,* Saccharomyces and gliadin. Stool testing with respect to the detection of Salmonella, Shigella, Yersinia, Campylobacter, enteropathogenic and enterohemorrhagic *E. coli* or *Clostridium difficile* came out negative. Regarding antibody examinations in the blood, IgG against *H. pylori* and IgA against Saccharomyces and gliadin were negative, but IgG against gliadin was moderately elevated at 59 U/mL (normal values = <20 U/mL). The IgG antibody elevations were considered non-specific or protective, and the patient was put on painkillers and sent home with no diagnosis of any specific disorder. Three years later after seeing the frequency of the watery diarrhea increase to 3-5 times daily and losing 12 pounds of his body weight in the last two months, the patient went to another GI specialist for a second opinion. Gastric and duodenal biopsies and endoscopy were performed. While the endoscopy of the upper GI tract revealed gastritis of the antrum, histologically, gastric and duodenal biopsy turned out to be negative. D-xylose absorption test was performed; the resulting value of 1.89 g/5h in urine was suggestive of malabsorption. Immunoserologically ANA titers were below 1:40, p-ANCA and c-ANCA were negative, but the IgA anti-Saccharomyces antigen (ASCA) was positive at 85 U/mL (normal = <10 U/mL). Based on the increased frequency of watery diarrhea, abnormal D-xylose absorption, and positive IgA anti-ASCA, the diagnosis of Crohn's disease was made. A therapeutical trial using cholestyramine was initiated but the frequency of the diarrhea remained unchanged. In addition the patient was treated with 230 mg of methylprednisolone, and 2 x 1000 mg of mesalazine. Two years after this treatment the patient developed entero-enteric fistulae in the terminal ileum with sigmoid affection. After admission to the hospital, ileocolectomy was performed and 22 cm of the ileum was resected. Upon his release remission maintenance with 3 x 500 mg of mesalazine was implemented.

[0068] For eight years following this treatment the patient continued to suffer from increasing frequency of watery diarrhea and lost an additional 14 pounds. During this period several additional treatment attempts were made using aspirin, loperamide, and budesonide, unfortunately without significant clinical improvement. Furthermore, the patient was losing more weight on a monthly basis. A complete review of the medical history revealed the fact that almost thirteen years earlier, gliadin IgG antibody had been found to be elevated, which was considered normal at the time. Since all classical treatments for Crohn's disease had failed to improve the clinical picture over all the years, a comprehensive test for the assessment of gluten immune reactivity and sensitivity was ordered. This included IgG and IgA against wheat, native and deamidated α-gliadin peptides, γ-gliadin, ω-gliadin, glutenin, gluteomorphin, prodynorphin, gliadin-tTg complex, transglutaminase, wheat germ agglutinin, and GAD-65.

[0069] Results depicted in Tables 6 and 7, Sample #3 show that the patient had a significant elevation of IgG antibodies against 11 out of 12 tested antigens, and IgA antibodies against wheat, α-gliadin 33-mer, ω-gliadin, prodynorphin, wheat germ agglutinin and GAD-65 were detected at 2-5 fold above the normal range. Based on these results, in addition to Crohn's disease a diagnosis of gluten sensitivity was also made. A diet consisting of rice, potato, and other gluten-free/yeast-free foods was commenced immediately, which led after six weeks to a complete cessation of diarrhea. Upon continuation of the gluten-free diet, not only did stool consistency become normal but the patient also started gaining weight. On follow-up one year later the patient was back to a normal state and had regained more than 80% of his lost weight.

[0070] Discussion: This case reports on the association of Crohn's disease with gluten sensitivity but not with celiac disease. Based on the impressive clinical response to the gluten-free diet plus the detection of IgG and IgA antibodies against various wheat antigens, and upon re-evaluation of the IgG antibody level detected 14 years earlier, the diagnosis of Crohn's disease with secondary malabsorption and gluten sensitivity was finally established. Since IgG antibodies against gliadin but not transglutaminase were detected, it can be argued that in this patient the disease initiated with gluten sensitivity and not Crohn's disease. The initial diagnosis of Crohn's disease was made despite the fact that a demonstration of duration exposure to gluten and risk of autoimmune disorders was published in 1999 (Ventura A et al., Gastroenterol, 117: 303-310, 1999); unfortunately, this was ignored.

[0071] It is contemplated herein that continuous exposure to environmental factors such as wheat antigen induced inflammation for a prolonged period of time, re-

sulting in inflammatory bowel disease or Crohn's disease.

Conclusions Regarding Case Reports

[0072] The foregoing case studies show the importance of proper laboratory testing for confirming diagnoses of celiac disease, gluten immune reactivity/sensitivity, and autoimmunity. They show that years of erroneous testing and misdiagnoses can lead to years of suffering. It is vital to get the most accurate information and the most accurate diagnosis, to distinguish between one condition and another. Figures 5 and 6 show a summary of the current state of testing as well as a proposed future direction for more accuracy in the diagnosis of celiac disease as proposed by Volta et al. (17). Figure 7 summarizes an inventive protocol according to certain aspects of the present invention, and proposes testing against a repertoire of wheat antigens and peptides so as to provide the most accurate information and confirmation of celiac disease, Crohn's disease, gluten immune reactivity/sensitivity and autoimmunity.

[0073] It should be apparent to those skilled in the art that many more modifications besides those already described are possible without departing from the inventive concepts herein. The inventive subject matter, therefore, is not to be restricted except in the scope of the appended claims. Moreover, in interpreting both the specification and the claims, all terms should be interpreted in the broadest possible manner consistent with the context. In particular, the terms "comprises" and "comprising" should be interpreted as referring to elements, components, or steps in a non-exclusive manner, indicating that the referenced elements, components, or steps may be present, or utilized, or combined with other elements, components, or steps that are not expressly referenced. Where the specification claims refers to at least one of something selected from the group consisting of A, B, C .... and N, the text should be interpreted as requiring only one element from the group, not A plus N, or B plus N, etc.

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[0074]

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Claims

1. A method of making a determination that assists in differentiating celiac disease from (a) gluten immune reactivity and sensitivity and from (b) a diagnosis related to gluten immune reactivity/sensitivity and autoimmunity, using test results derived from analyzing a blood sample obtained from a human, comprising:

   testing or having the blood sample tested for IgA against deamidated α-gliadin 33-mer and transglutaminase; and
   testing or having the blood sample tested for IgA against deamidated glutenin 21-mer
   wherein celiac disease is indicated by a positive signal for IgA against deamidated α-gliadin 33-mer, transglutaminase and deamidated glutenin 21-mer.

2. The method of claim 1, further comprising testing or having the blood sample tested for a serological pattern indicative of silent celiac disease
   where at least one of IgA and IgG test positive against at least one of α-gliadin 33-mer, deamidated α-gliadin 33- mer, α-gliadin 25-mer, α-gliadin 18-mer, α-gliadin 17-mer, γ-gliadin 15-mer, ω-gliadin 17-mer, glutenin, deamidated glutenin, gluteomorphin, prodynorphin and wheat germ agglutinin; and both IgA and IgG test negative against tranglutaminase-2, and at least one of IgA and IgG tests positive against at least one of transglutaminase-3 and tranglutaminase-6.

3. The method of claim 1, further comprising testing or having the blood sample tested for a serological pattern indicative of gluten immune reactivity and sensitivity
   where at least one of IgA and IgG test positive against at least one of α-gliadin, γ-gliadin, ω-gliadin, glutenin, deamidated glutenin, gluteomorphin, and wheat germ agglutinin;
   and both IgA and IgG test negative against deamidated α-gliadin, deamidated glutenin or transglutaminase-2.

4. The method of claim 1, further comprising testing or having the blood sample tested for a serological pattern indicative of a diagnosis related to gluten immune reactivity/sensitivity and autoimmunity
   where at least one of IgA and IgG test positive against at least one of native α-gliadin, γ-gliadin, ω-gliadin, glutenin, deamidated glutenin, gluteomorphin, and wheat germ agglutinin; at least one of IgG, IgA and IgM test positive to at least one of glutamic acid decarboxylase and cerebellar tissue; and
   IgA tests negative against deamidated α-gliadin, deamidated glutenin or transglutaminase.

Patentansprüche

1. Verfahren für eine Feststellung, die hilft, Zöliakie von (a) GlutenImmunreaktivität und -Sensitivität und (b) von einer Diagnose, die zur Gluten-Immunreaktivität/Sensitivität und Autoimmunität im Bezug steht, zu unterscheiden, und zwar unter Verwendung von Testergebnissen, die aus der Analyse einer von einem Menschen erhaltenen Blutprobe abgeleitet wurden, wobei das Verfahren Folgendes aufweist:

   Testen oder Testenlassen der Blutprobe auf IgA gegen deamidiertes α-Gliadin-33-mer und Transglutaminase; und
   Testen oder Testenlassen der Probe auf IgA gegen deamidiertes Glutenin 21-mer;
   wobei Zöliakie angezeigt wird durch ein positives Signal auf IgA gegen deamidiertes α-Gliadin-33-mer, Transglutaminase und deamidiertes Glutenin-21-mer.

2. Verfahren nach Anspruch 1, welches ferner das Testen oder Testenlassen der Blutprobe auf ein serologisches Muster bzw. eine serologische Konstellation aufweist, die auf stumme Zöliakie hinweist,

   wobei mindestens eines von IgA und IgG positiv gegen mindestens eines von Folgenden getestet wird: α-Gliadin-33-mer, deamidiertes α-Gliadin-33-mer, α-Gliadin-25-mer, α-Gliadin-18-mer, α-gliadin-17-mer, γ-Gliadin-15-mer, ω-Gliadin-17-mer, Glutenin, deamidiertes Glutenin, Gluteomorphin, Prodynorphin und Weizenkeimagglutinin; und
   wobei sowohl IgA als auch IgG negativ gegen Transglutaminase-2 getestet wird, und mindestens einer von IgA
   und IgG positiv gegen mindestens eine von Transglutaminase-3 und Transglutaminase-6 getestet wird.

3. Verfahren nach Anspruch 1, welches ferner das Testen oder Testenlassen der Blutprobe auf eine serologische Konstellation aufweist, die auf Glutenimmunreaktivität und -empfindlichkeit hinweist,
   wobei mindestens eines von IgA und IgG positiv ge-

gen mindestens eines von Folgenden getestet wird: α-Gliadin, γ-Gliadin, ω-Gliadin, Glutenin, deamidiertes Glutenin, Gluteomorphin und Weizenkeimagglutinin;
und sowohl IgA als auch IgG negativ getestet werden gegen deamidiertes α-Gliadin, deamidiertes Glutenin oder Transglutaminase-2.

4. Verfahren nach Anspruch 1, welches ferner das Testen oder das Testenlassen der Blutprobe auf eine serologische Konstellation aufweist, die eine Diagnose im Zusammenhang mit Glutenimmunreaktivität /-empfindlichkeit und Autoimmunität anzeigt,
wobei mindestens eines von IgA und IgG positiv gegenüber mindestens eines von Folgenden getestet wird: natives α-Gliadin, γ-Gliadin, ω-Gliadin, Glutenin, deamidiertes Glutenin, Gluteomorphin und Weizenkeimagglutinin; wobei mindestens eines von IgG, IgA und IgM positiv getestet wird für mindestens eines von Glutaminsäuredecarboxylase und Kleinhirngewebe; und
wobei IgA negativ gegen deamidiertes α-Gliadin, desamidiertes Glutenin oder Transglutaminase getestet wird.

Revendications

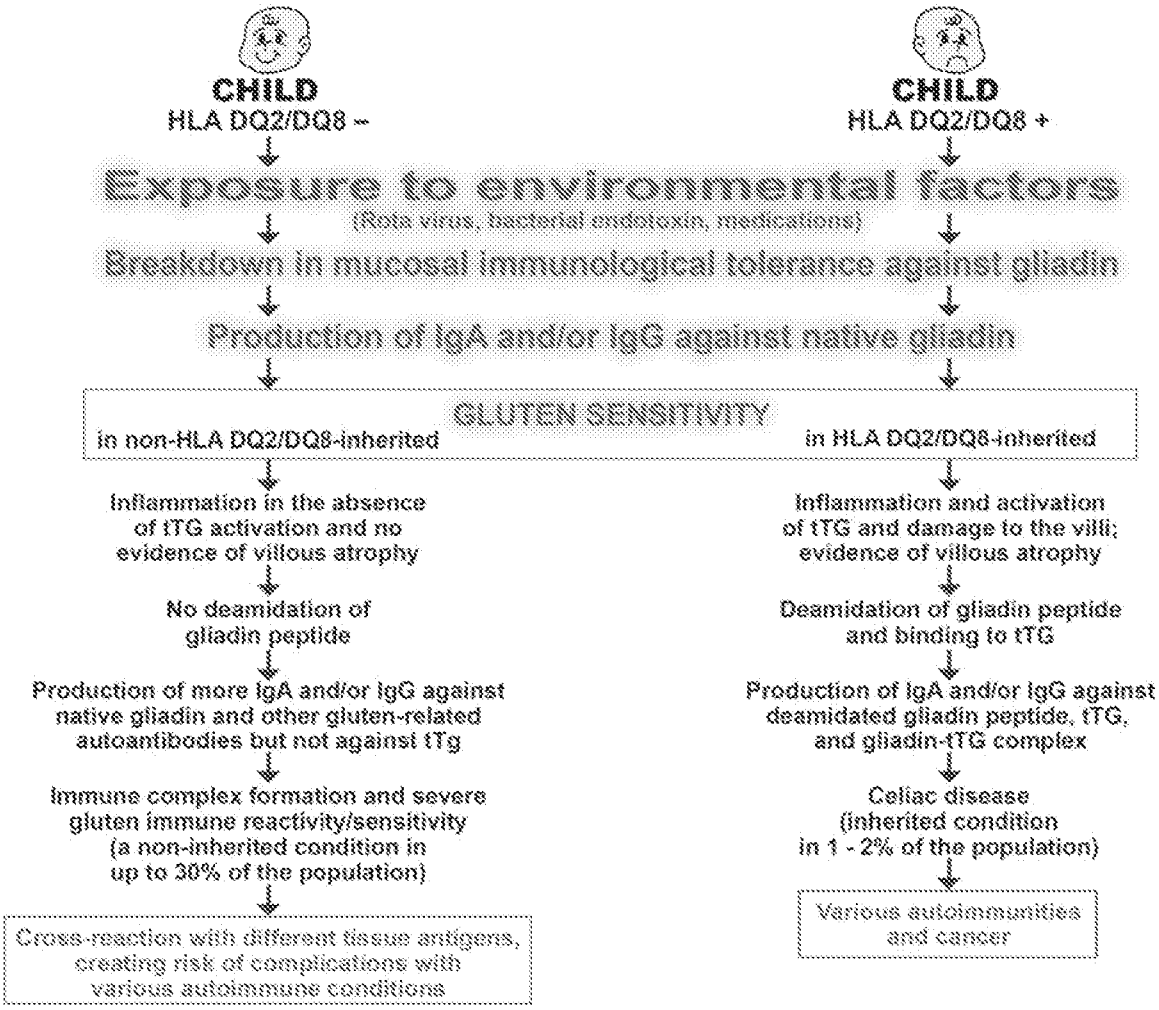
1. Procédé de détermination qui aide à différencier une maladie coeliaque (a) d'une réactivité et sensibilité immunitaires au gluten et (b) d'un diagnostic lié à la réactivité/sensibilité immunitaire et à l'auto-immunité au gluten, en utilisant des résultats de test dérivés d'une analyse d'un échantillon sanguin obtenu d'un humain, comprenant :

tester ou faire tester l'échantillon sanguin pour l'IgA par rapport à l'α-gliadine-33-mer désamidée et la transglutaminase ; et
tester ou faire tester l'échantillon sanguin pour l'IgA par rapport à la gluténine 21-mer désamidée
dans lequel la maladie coeliaque est indiquée par un signal positif pour l'IgA par rapport à l'α-gliadine-33-mer désamidée, la transglutaminase et la gluténine 21-mer désamidée.

2. Procédé selon la revendication 1, comprenant en outre tester ou faire tester l'échantillon sanguin pour un modèle sérologique indiquant une maladie coeliaque silencieuse
où au moins l'un parmi l'IgA et l'IgG est testé positif par rapport à au moins l'un parmi l'a-gliadine 33-mer, l'α-gliadine 33-mer désamidée, l'α-gliadine 25-mer, l'α-gliadine 18-mer, l'α-gliadine 17-mer, la γ-gliadine-15-mer, l'ω-gliadine 17-mer, la gluténine, la gluténine désamidée, la glutéomorphine, la prodynorphine et l'agglutinine de germe de blé; et
l'IgA et l'IgG sont toutes deux testées négatives par rapport à la transglutaminase-2, et au moins l'une parmi l'IgA et l'IgG est testée positive par rapport à au moins l'une parmi la transglutaminase-3 et la transglutaminase-6.

3. Procédé selon la revendication 1, comprenant en outre tester ou faire tester l'échantillon sanguin pour un profil sérologique indiquant une réactivité et sensibilité immunitaires du gluten
où au moins une parmi l'IgA et l'IgG est testée positive par rapport à au moins l'une parmi l'α-gliadine, la γ-gliadine, l'ω-gliadine, la gluténine, la gluténine désamidée, la glutéomorphine et l'agglutinine de germe de blé ;
et l'IgA et l'IgG sont toutes deux testées négatives par rapport à l'α-gliadine désamidée, la gluténine désamidée ou la transglutaminase-2.

4. Procédé selon la revendication 1, comprenant en outre tester ou faire tester l'échantillon sanguin pour un profil sérologique indicateur d'un diagnostic lié à la réactivité/sensibilité immunologique et à l'auto-immunité au gluten
où au moins l'une parmi l'IgA et l'IgG est testée positive contre au moins l'une parmi l'α-gliadine native, la γ-gliadine, l'ω-gliadine, la gluténine, la gluténine désamidée, la glutéomorphine et l'agglutinine de germe de blé ; au moins l'une parmi l'IgG, l'IgA et l'IgM est testée positive à au moins un parmi l'acide glutamique décarboxylase et un tissu cérébelleux ; et
l'IgA est testée négative par rapport à l'a-gliadine désamidée, la gluténine désamidée ou la transglutaminase.



Differentiation of Gluten Sensitivity from Celiac Disease

Figure 1

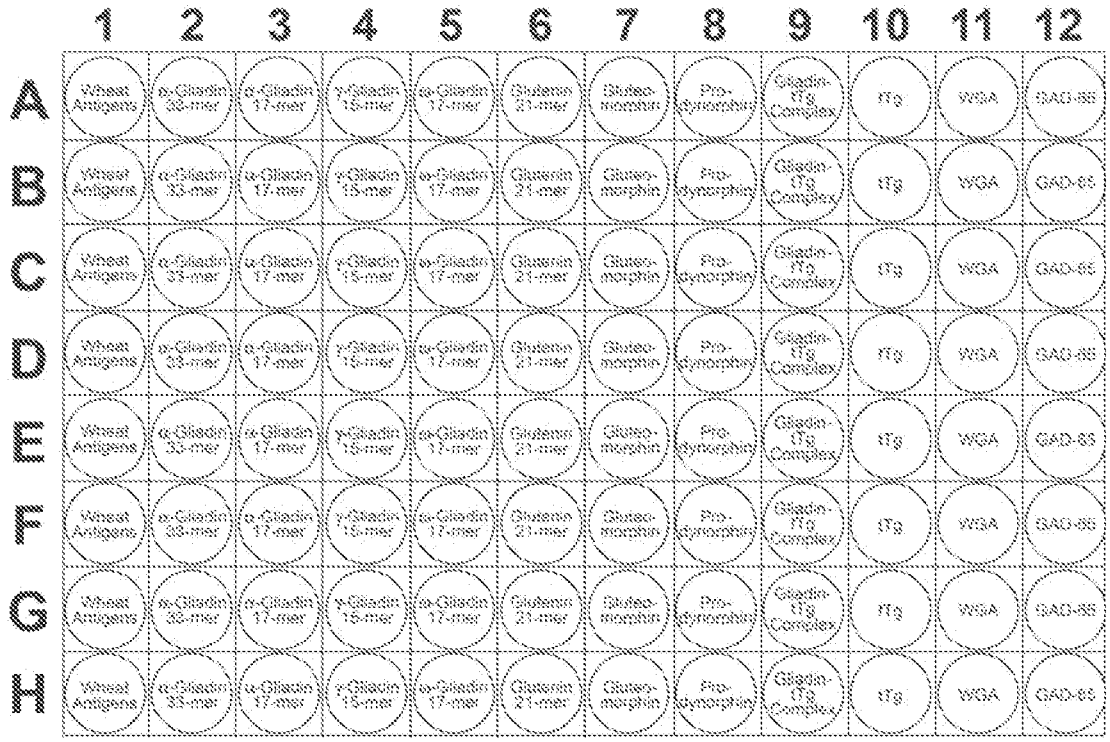


Figure 2

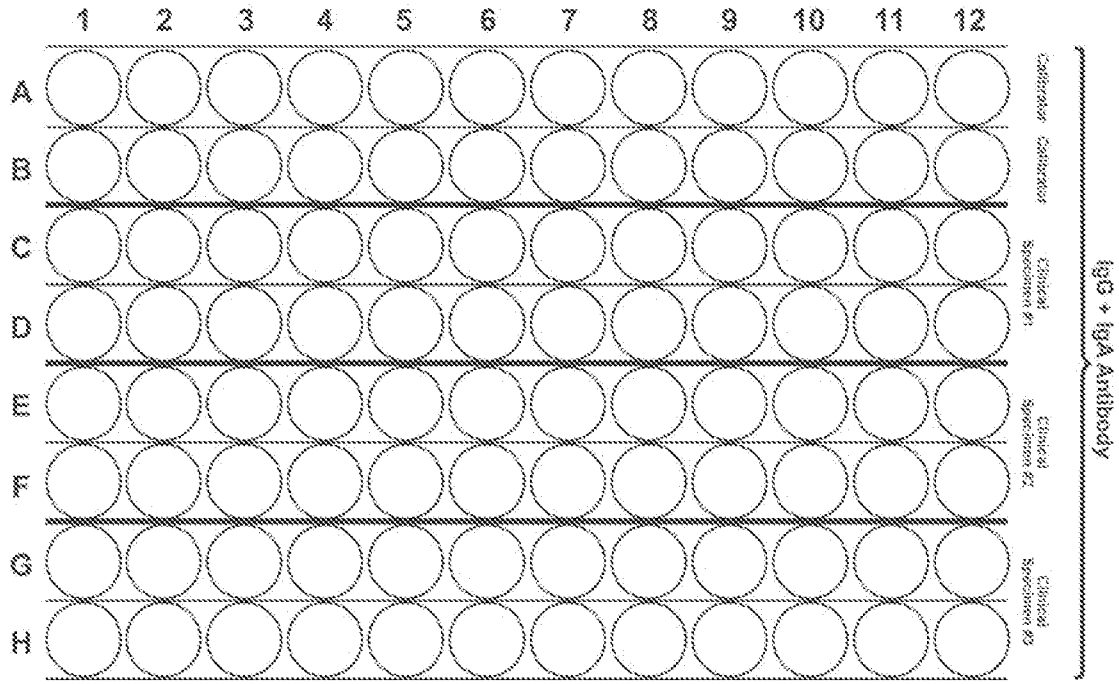
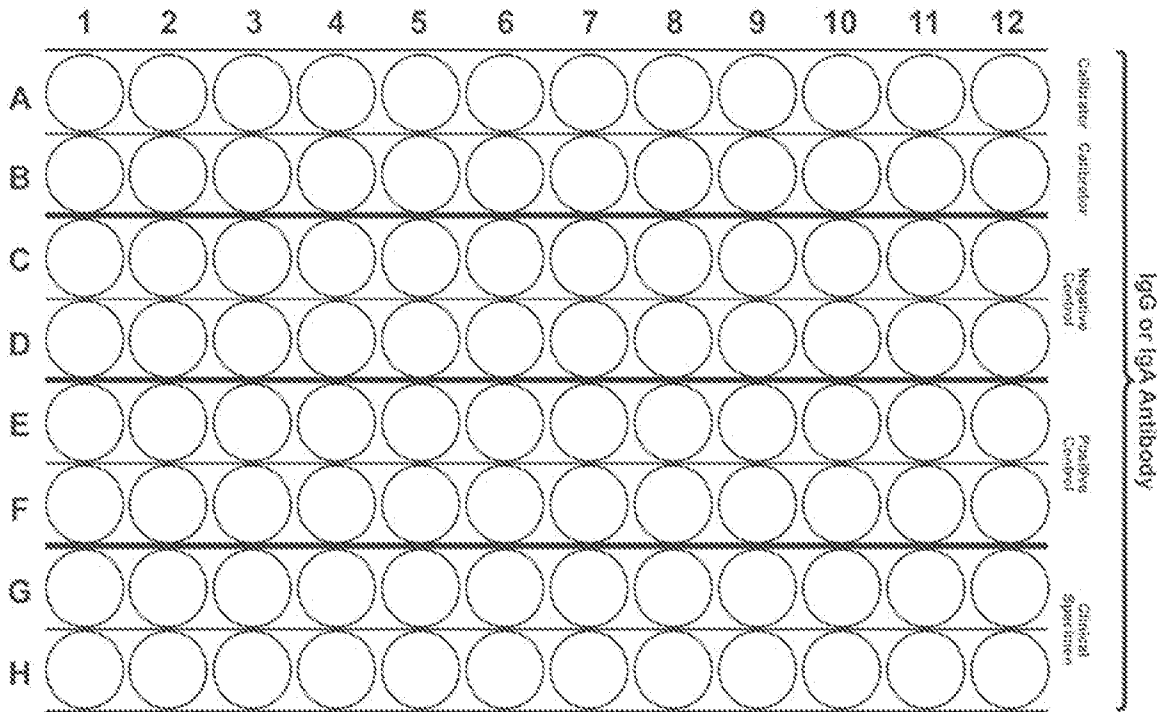


Figure 3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Calibrator
Calibrator
Negative Control
Negative Control
Positive Control
Positive Control
Clinical Specimen
Clinical Specimen
IgG or IgA Antibody

Figure 4

Table 1 - Reportable Ranges & Reference Ranges (ELISA Index)

Antigens	Reportable Range	In Range (Negative)	Equivocal	Out of Range (Positive)	Reference Range
Wheat IgG	0.3-6.4	0.24-1.0	1.01-1.3	>1.3	0.24-1.3
Wheat IgA	0.4-9.7	0.0-1.9	1.91-2.4	>2.4	0.0-2.4
α-Gliadin-33 IgG	0.3-10.0	0.16-1.1	1.11-1.4	>1.4	0.16-1.4
α-Gliadin-33 IgA	0.6-16.0	0.6-1.5	1.51-1.8	>1.8	0.6-1.8
α- Gliadin-17 IgG	0.3-5.0	0.0-1.1	1.11-1.5	>1.5	0.0-1.5
α- Gliadin-17 IgA	0.6-14.8	0.4-1.6	1.61-2.0	>2.0	0.4-2.0
γ- Gliadin-15 IgG	0.4-12.0	0.14-1.3	1.31-1.7	>1.7	0.14-1.7
γ- Gliadin-15 IgA	0.5-16.0	0.7-1.6	1.61-1.9	>1.9	0.7-1.9
ω- Gliadin IgG	0.5-1.0	0.24-1.3	1.31-1.6	>1.6	0.24-1.6
ω- Gliadin IgA	0.6-15.4	0.6-1.5	1.51-1.8	>1.8	0.6-1.8
Glutenin IgG	0.2-6.0	0.0-1.1	1.11-1.5	>1.5	0.0-1.5
Glutenin IgA	0.5-15.9	0.5-1.4	1.41-1.7	>1.7	0.5-1.7
Gluteomorphin IgG	0.3-8.0	0.12-1.2	1.21-1.5	>1.5	0.12-1.5
Gluteomorphin IgA	0.5-15.1	0.6-1.5	1.51-1.8	>1.8	0.6-1.8
Prodynorphin IgG	0.4-13.0	0.13-1.7	0.131-1.7	>1.7	0.13-1.7
Prodynorphin IgA	0.6-16.0	0.6-1.5	1.51-1.8	>1.8	0.6-1.8
Gliadin-tTg Complex IgG	0.4-13.0	0.12-1.2	1.21-1.6	>1.6	0.12-1.6
Gliadin-tTg Complex IgA	0.6-15.5	0.5-1.3	1.31-1.6	>1.6	0.5-1.6
Transglutaminase IgG	0.5-12.0	0.21-1.1	1.11-1.4	>1.4	0.21-1.4
Transglutaminase IgA	0.5-10.9	0.6-1.3	0.31-1.5	>1.5	0.6-1.5
Wheat Germ Agglutinin IgG	0.5-10.0	0.17-1.1	1.1-1.5	>1.5	0.17-1.5
Wheat Germ Agglutinin IgA	0.6-16.9	00.9-1.6	1.61-1.9	>1.9	0.9-1.9
Glutamic Acid Decarboxylase IgG	0.4-12.0	0.29-1.1	1.11-1.3	>1.3	0.29-1.3
Glutamic Acid Decarboxylase IgA	0.8-12.9	0.6-1.3	1.31-1.5	>1.5	0.6-1.5

Table 2 - IgG Antibody Pattern of 3 Patients with Celiac Disease reacting against various Wheat Antigens, Peptides and Tissue Antigens expressed as Optical Density with Calculation of Indices

	Wheat Antigens	*Alpha Gliadin 33-mer	Alpha Gliadin 17-mer	Gamma Gliadin 15-mer	Omega Gliadin 17-mer	Glutenin 21-mer	Gluteo-morphin	Pro-Dynorphin	Gliadin-tTg Complex	**tTg	Wheat Germ Agglutinin	***GAD-65
Cal 1	0.41	0.33	0.35	0.32	0.51	0.40	0.50	0.38	0.39	0.46	0.37	0.38
Cal 2	0.44	0.35	0.35	0.32	0.25	0.43	0.49	0.37	0.40	0.44	0.36	0.42
Sample 1	3.77	1.04	3.86	1.02	3.84	1.34	1.44	3.84	1.76	3.83	1.15	3.81
(OD)	3.78	1.08	3.85	1.06	3.85	1.28	1.45	3.84	1.73	3.85	1.20	3.81
Index	**8.91**	**3.11**	**11.02**	**3.26**	**10.14**	**3.19**	**2.92**	**10.32**	**4.43**	**8.55**	**3.24**	**9.52**
Sample 2	3.85	3.87	1.13	1.10	3.86	1.08	3.84	1.26	1.37	3.84	1.45	0.98
(OD)	3.85	3.86	1.11	1.17	3.84	1.09	3.84	1.31	1.36	3.84	1.33	1.04
Index	**9.09**	**11.32**	**3.21**	**3.55**	**10.15**	**2.63**	**7.76**	**3.44**	**3.46**	**8.55**	**3.83**	**2.53**
Sample 3	3.77	0.89	1.11	3.53	1.23	3.37	1.31	1.15	3.84	1.37	3.84	3.54
(OD)	3.79	0.80	1.00	3.51	1.15	3.39	1.21	1.09	3.85	1.28	3.83	3.54
Index	**8.93**	**2.46**	**3.03**	**11.05**	**3.14**	**8.23**	**2.55**	**2.99**	**9.74**	**2.95**	**10.56**	**8.85**

*Native + Deamidated Gliadin 33-mer **Transglutaminase ***Glutamic Acid Decarboxylase

$$\text{Index} = \frac{\text{Mean OD of patients}}{\text{Mean OD of calibrators}}$$

Table 3 - IgA Antibody Pattern of 3 Patients with Celiac Disease reacting against various Wheat Antigens, Peptides and Tissue Antigens expressed as Optical Density with Calculation of Indices

	Wheat Antigens	*Alpha Gliadin 33-mer	Alpha Gliadin 17-mer	Gamma Gliadin 15-mer	Omega Gliadin 17-mer	Glutenin 21-mer	Gluteo-morphin	Pro-Dynorphin	Gliadin-tTg Complex	**tTg	Wheat Germ Agglutinin	***GAD-65
Cal 1	0.36	0.37	0.36	0.36	0.31	0.41	0.43	0.39	0.39	0.37	0.41	0.48
Cal 2	0.39	0.37	0.36	0.36	0.39	0.41	0.43	0.41	0.39	0.38	0.39	0.39
Sample 1	3.00	3.64	1.23	3.82	0.47	3.82	3.83	3.39	3.83	3.84	3.87	0.83
(OD)	3.03	3.56	1.22	3.81	0.45	3.81	3.82	3.40	3.81	3.86	3.86	0.84
Index	7.97	9.69	3.43	10.67	1.31	9.42	8.88	8.51	9.84	10.29	9.69	1.93
Sample 2	3.55	0.96	3.83	0.95	3.82	1.75	1.31	3.82	3.87	3.85	1.65	3.80
(OD)	3.53	1.01	3.82	0.99	3.83	1.97	1.40	3.83	3.87	3.86	1.77	3.83
Index	9.34	2.65	10.69	2.71	10.96	4.59	3.14	9.60	9.98	10.30	4.26	8.83
Sample 3	3.81	3.84	2.99	3.83	0.99	0.40	1.85	1.33	3.83	3.85	3.81	3.48
(OD)	3.80	3.85	2.92	3.81	0.94	0.35	1.74	1.15	3.82	3.84	3.82	3.43
Index	10.05	10.36	8.27	10.68	2.76	0.92	4.16	3.11	9.86	10.26	9.56	7.99

*Native + Deamidated Gliadin 33-mer **Transglutaminase ***Glutamic Acid Decarboxylase

$$\text{Index} = \frac{\text{Mean OD of patients}}{\text{Mean OD of calibrators}}$$

Table 4 - IgG Antibody Pattern of 3 Patients with Gluten Immune Reactivity/Sensitivity/Autoimmunity reacting against various Wheat Antigens, Peptides and Tissue Antigens expressed as Optical Density with Calculation of Indices

	Wheat Antigens	*Alpha Gliadin 33-mer	Alpha Gliadin 17-mer	Gamma Gliadin 15-mer	Omega Gliadin 17-mer	Glutenin 21-mer	Gluteo-morphin	Pro-Dynorphin	Gliadin-tTg Complex	**tTg	Wheat Germ Agglutinin	***GAD-65
Cal 1	0.33	0.42	0.34	0.33	0.38	0.38	0.36	0.35	0.33	0.35	0.35	0.36
Cal 2	0.36	0.48	0.36	0.38	0.42	0.44	0.39	0.40	0.45	0.38	0.38	0.43
Sample 1	3.86	0.95	3.22	1.22	0.41	1.29	1.54	3.81	0.67	0.44	1.28	1.25
(OD)	3.87	0.88	3.26	1.26	0.33	1.34	1.36	3.84	0.64	0.41	1.31	1.20
Index	11.14	2.03	9.37	3.46	0.93	3.21	3.84	10.31	1.66	1.17	3.55	3.09
Sample 2	3.88	3.87	2.71	2.05	3.86	1.98	1.97	1.95	0.41	0.40	1.95	2.14
(OD)	3.88	3.87	2.65	2.10	3.85	2.28	2.20	2.19	0.37	0.41	2.18	2.21
Index	11.16	8.58	7.75	5.80	9.72	5.21	5.52	5.58	1.00	1.12	5.66	5.50
Sample 3	3.85	2.44	2.38	3.86	2.41	2.50	3.86	2.49	1.49	1.00	3.86	3.88
(OD)	3.85	2.36	2.33	3.86	2.40	2.40	3.86	2.40	1.58	0.96	3.86	3.87
Index	11.10	5.33	6.81	10.79	6.05	5.99	10.23	6.60	3.91	2.69	10.59	9.79

*Native + Deamidated Gliadin 33-mer **Transglutaminase ***Glutamic Acid Decarboxylase

$$\text{Index} = \frac{\text{Mean OD of patients}}{\text{Mean OD of calibrators}}$$

Table 5 - IgA Antibody Pattern of 3 Patients with Gluten Immune Reactivity/Sensitivity/Autoimmunity reacting against various Wheat Antigens, Peptides and Tissue Antigens expressed as Optical Density with Calculation of Indices

	Wheat Antigens	*Alpha Gliadin 33-mer	Alpha Gliadin 17-mer	Gamma Gliadin 15-mer	Omega Gliadin 17-mer	Glutenin 21-mer	Gluteo-morphin	Pro-Dynorphin	Gliadin-tTg Complex	**tTg	Wheat Germ Agglutinin	***GAD-65
Cal 1	0.392	0.462	0.401	0.368	0.400	0.449	0.467	0.445	0.400	0.430	0.485	0.397
Cal 2	0.421	0.462	0.414	0.379	0.418	0.453	0.479	0.481	0.392	0.402	0.455	0.404
Sample 1	2.176	0.371	0.995	3.098	0.346	2.240	2.141	2.452	0.271	0.254	2.470	0.664
(OD)	2.248	0.424	0.827	3.123	0.407	2.402	2.297	2.531	0.329	0.245	2.354	0.724
Index	5.442	0.860	2.236	8.328	0.921	5.146	4.691	5.381	0.758	0.600	5.132	1.733
Sample 2	1.763	1.442	0.260	1.332	0.377	1.073	1.007	0.994	0.195	0.343	1.101	0.743
(OD)	1.664	1.573	0.252	1.483	0.342	1.063	1.058	1.089	0.205	0.351	1.144	0.764
Index	4.215	3.263	0.628	3.768	0.879	2.368	2.183	2.249	0.505	0.834	2.388	1.881
Sample 3	3.862	0.429	0.350	0.208	0.364	0.269	0.401	0.336	0.335	0.438	3.868	0.359
(OD)	3.828	0.426	0.397	0.245	0.325	0.290	0.546	0.372	0.360	0.473	3.833	0.438
Index	9.459	0.925	0.917	0.606	0.842	0.620	1.001	0.765	0.878	1.095	8.193	0.995

*Native + Deamidated Gliadin 33-mer **Transglutaminase ***Glutamic Acid Decarboxylase

$$\text{Index} = \frac{\text{Mean OD of patients}}{\text{Mean OD of calibrators}}$$

Table 6 - IgG Antibody Pattern of 3 Patients with Crohn's Disease reacting against various Wheat Antigens, Peptides and Tissue Antigens expressed as Optical Density with Calculation of Indices

	Wheat Antigens	*Alpha Gliadin 33-mer	Alpha Gliadin 17-mer	Gamma Gliadin 15-mer	Omega Gliadin 17-mer	Glutenin 21-mer	Gluteo-morphin	Pro-Dynorphin	Gliadin-tTg Complex	**tTg	Wheat Germ Agglutinin	***GAD-65
Cal 1	0.45	0.41	0.38	0.39	0.36	0.39	0.55	0.47	0.71	0.49	0.41	0.55
Cal 2	0.38	0.33	0.44	0.44	0.37	0.40	0.54	0.56	0.60	0.49	0.48	0.46
Sample 1	3.74	1.56	0.65	2.29	0.56	2.10	0.54	0.69	3.81	0.81	1.23	1.83
(OD)	3.68	1.59	0.56	2.15	0.57	2.01	0.51	0.55	3.54	0.75	1.25	1.80
Index	8.97	4.26	1.47	5.30	1.54	5.22	0.96	1.19	5.59	1.59	2.79	3.59
Sample 2	3.87	0.47	0.72	0.55	0.59	0.49	0.52	0.44	1.04	0.76	3.83	3.87
(OD)	3.84	0.43	0.61	0.53	0.46	0.48	0.51	0.37	1.11	0.70	3.80	3.86
Index	9.31	1.22	1.62	1.29	1.43	1.23	0.94	0.78	1.63	1.49	8.62	7.63
Sample 3	3.86	3.79	3.86	3.67	3.85	3.24	3.84	3.86	1.71	3.80	3.82	3.84
(OD)	3.84	3.79	3.84	3.59	3.85	3.25	3.83	3.83	1.73	3.74	3.80	3.59
Index	9.31	10.23	9.33	8.67	10.51	8.22	7.03	7.43	2.62	7.71	8.60	7.34

*Native + Deamidated Gliadin 33-mer **Transglutaminase ***Glutamic Acid Decarboxylase

$$\text{Index} = \frac{\text{Mean OD of patients}}{\text{Mean OD of calibrators}}$$

Table 7 - IgA Antibody Pattern of 3 Patients with Crohn's Disease reacting against various Wheat Antigens Peptides and Tissue Antigens expressed as Optical Density with Calculation of Indices

	Wheat Antigens	*Alpha Gliadin 33-mer	Alpha Gliadin 17-mer	Gamma Gliadin 15-mer	Omega Gliadin 17-mer	Glutenin 21-mer	Gluteo-morphin	Pro-Dynorphin	Gliadin-tTg Complex	**tTg	Wheat Germ Agglutinin	***GAD-65
Cal 1	0.37	0.39	0.40	0.39	0.43	0.43	0.46	0.47	0.41	0.42	0.48	0.42
Cal 2	0.45	0.44	0.46	0.42	0.47	0.51	0.54	0.50	0.48	0.48	0.49	0.47
Sample 1	3.81	3.80	0.22	3.68	0.26	3.13	2.55	2.70	0.97	0.93	3.81	1.03
(OD)	3.82	3.79	0.20	3.17	0.27	3.18	2.50	2.58	1.00	0.97	3.80	1.02
Index	**9.30**	**9.08**	**0.49**	**8.50**	**0.59**	**6.68**	**5.06**	**5.44**	**2.23**	**2.13**	**7.85**	**2.32**
Sample 2	3.00	0.87	0.67	0.74	0.33	0.66	1.43	1.12	0.98	0.29	1.00	1.07
(OD)	3.43	0.85	0.64	0.70	0.32	0.66	1.12	1.03	1.00	0.31	1.02	1.10
Index	**7.85**	**2.05**	**1.52**	**1.79**	**0.72**	**1.40**	**2.56**	**2.21**	**2.23**	**0.67**	**2.08**	**2.43**
Sample 3	3.89	3.56	0.99	0.99	2.25	1.05	1.12	3.85	1.00	1.18	3.73	3.87
(OD)	3.89	3.48	0.99	0.99	2.25	1.03	1.10	3.82	0.99	1.20	3.83	3.85
Index	**9.48**	**8.43**	**2.27**	**2.45**	**4.98**	**2.20**	**2.23**	**7.90**	**2.25**	**2.65**	**7.79**	**8.69**

*Native + Deamidated Gliadin 33-mer **Transglutaminase ***Glutamic Acid Decarboxylase

$$\text{Index} = \frac{\text{Mean OD of patients}}{\text{Mean OD of calibrators}}$$

Table 8 – IgG and IgA Reactivity For Detection Of Gluten Immune Reactivity And Sensitivity, And Its Differentiation From Celiac Disease

		INDICATIONS				
Antigens and Peptides	Antibody Isotype	Current Testing for Celiac Disease	Proposed Testing for Celiac Disease	Testing Proposed for Celiac Disease in this Patent Application	Testing Proposed for Gluten Immune Reactivity and Sensitivity in this Patent Application	Testing Proposed for Gluten Immune Reactivity, Sensitivity and Autoimmunity in this Patent Application
α-Gliadin 33-Mer	IgG	+ or —	+ or —	+ or —	+	+
	IgA	+	+	+	+ or —	+ or —
Deamidated α-Gliadin 33-Mer	IgG	+ or —	+ or —	+ or —	—	—
	IgA	+	+	+	—	—
Transglutaminase	IgG	+ or —	+ or —	+ or —	+ or —	+ or —
	IgA	+	+	+	—	—
Gliadin Transglutaminase Complex	IgG	Not tested	+ or —	+ or —	+ or —	+ or —
	IgA		+	+	—	—
α-Gliadin 17-Mer	IgG	Not tested	Not tested	+ or —	+	+
	IgA			+	+ or —	+ or —
γ-Gliadin 15-Mer	IgG	Not tested	Not tested	+ or —	+	+
	IgA			+	+ or —	+ or —
ω-Gliadin 17-Mer	IgG	Not tested	Not tested	+ or —	+	+
	IgA			+	+ or —	+ or —
Glutenin 21-Mer	IgG	Not tested	Not tested	+ or —	+	+
	IgA			+	+ or —	+ or —
Deamidated Glutenin 21-Mer	IgG	Not tested	Not tested	+ or —	—	—
	IgA			+	—	—
Gluteomorphin	IgG	Not tested	Not tested	+ or —	+	+
	IgA			+	+ or —	+ or —
Prodynorphin	IgG	Not tested	Not tested	+ or —	+	+
	IgA			+	+ or —	+ or —
Wheat Germ Agglutinin	IgG	Not tested	Not tested	+ or —	+	+
	IgA			+	+ or —	+ or —
Glutamic Acid Decarboxylase	IgG	Not tested	Not tested	+ or —	+ or —	+
Cerebellar Peptide	IgG	Not tested	Not tested	+ or —	+ or —	+
Other Tissue Antigens	IgG	Not tested	Not tested	+ or —	+ or —	+

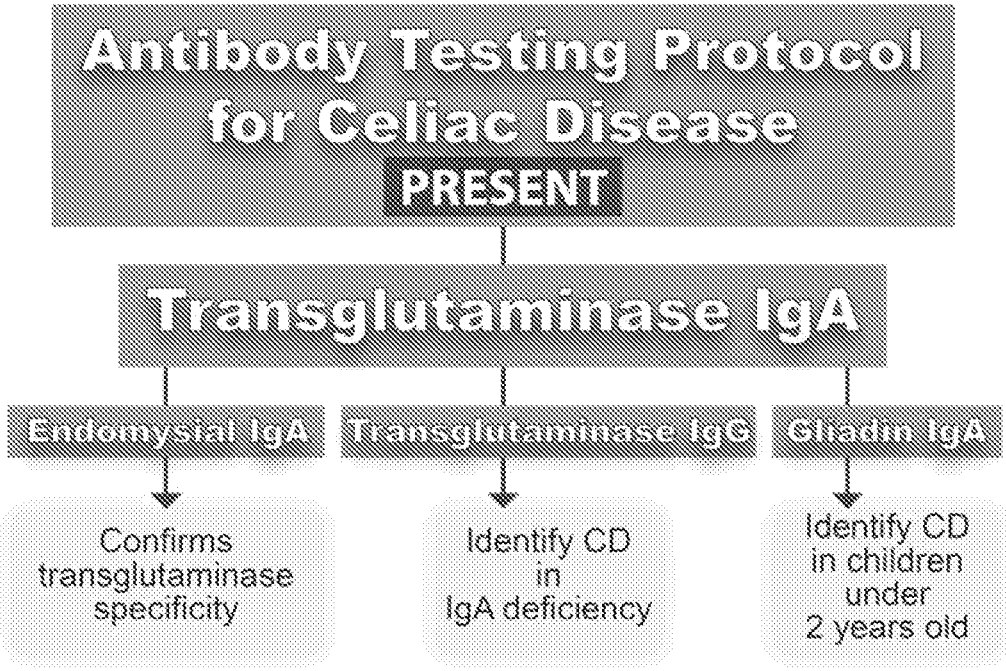

Figure 5 (Prior Art)

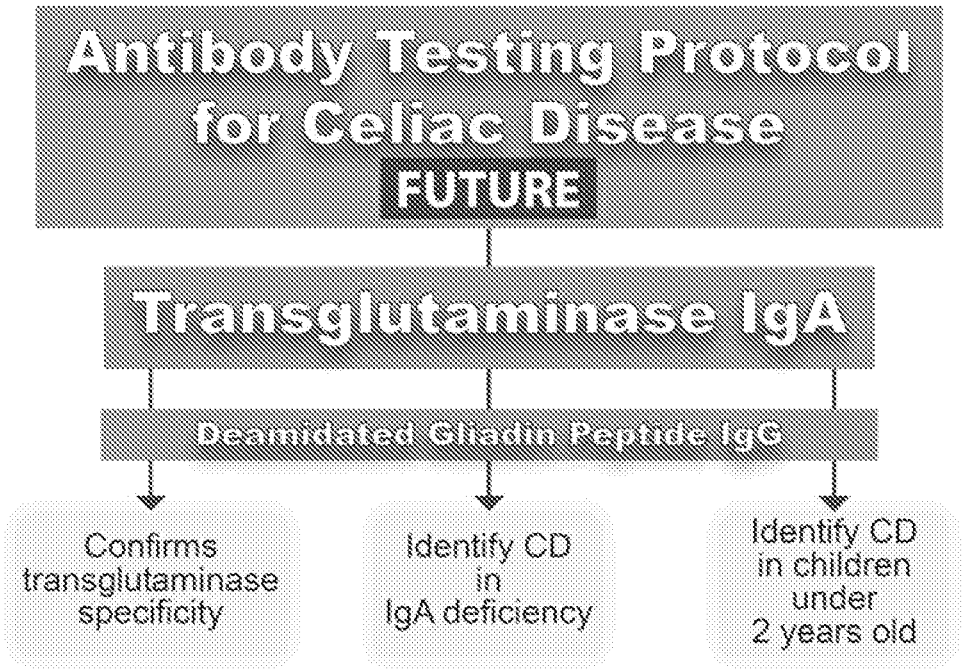

Figure 6 (Prior Art)

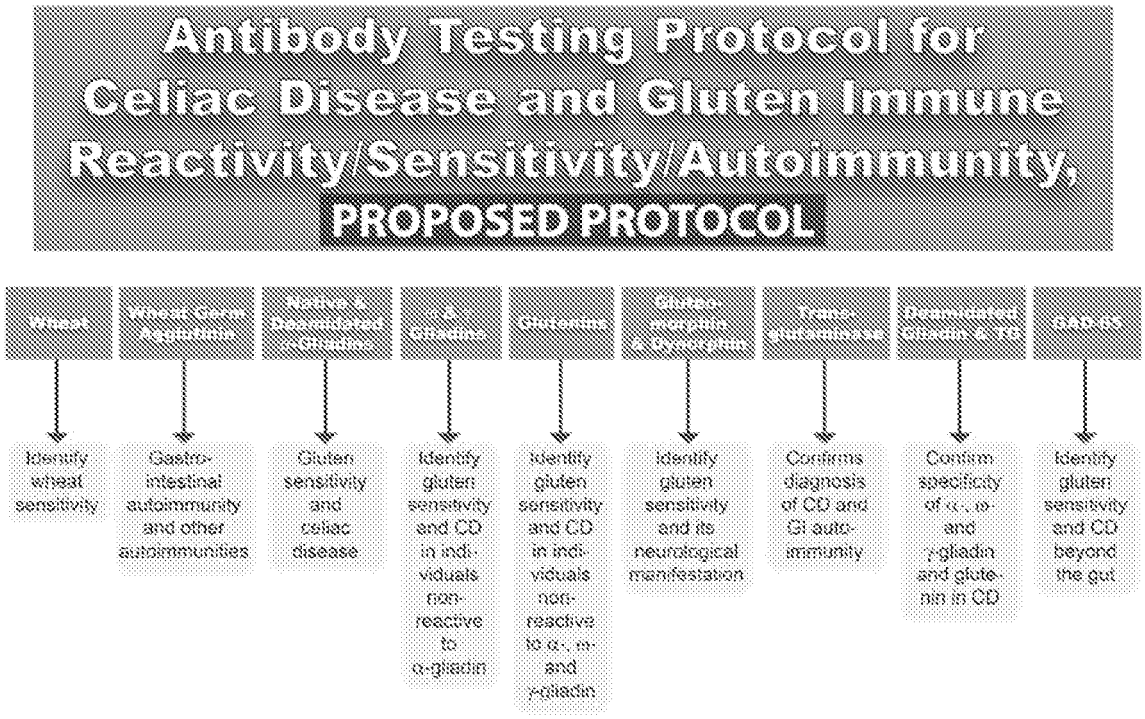
Antibody Testing Protocol for Celiac Disease and Gluten Immune Reactivity/Sensitivity/Autoimmunity,
PROPOSED PROTOCOL
Wheat
Wheat Germ Agglutinin
Native & Deamidated gliadins
α-Gliadins
Glutenins
Gluten-ataxia & Cerebellar
Tissue-transglutaminase
Deamidated Gliadin & TG
GAD-65
Identify wheat sensitivity
Gastro-intestinal autoimmunity and other autoimmunities
Gluten sensitivity and celiac disease
Identify gluten sensitivity and CD in indi-viduals non-reactive to α-gliadin
Identify gluten sensitivity and CD in indi-viduals non-reactive to α-, ω- and γ-gliadin
Identify gluten sensitivity and its neurological manifestation
Confirms diagnosis of CD and GI auto-immunity
Confirm specificity of α-, ω- and γ-gliadin and glute-nin in CD
Identify gluten sensitivity and CD beyond the gut

Figure 7

REFERENCES CITED IN THE DESCRIPTION

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专利名称(译)	用于检测谷蛋白敏感性的方法和装置及其与乳糜泻的区别		
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摘要(译)

抗体被用作生物标志物,以帮助区分面筋免疫反应性和敏感性,沉默性腹腔疾病,克罗恩氏病和其他与肠道相关的病理与典型的腹腔疾病。 在一类实施方案中,测试来自人或其他动物的血清,唾液或其他样品的抗(a)小麦抗原;(b)麦醇溶蛋白抗原;和 (c)小麦胚芽凝集素,谷氨酸吗啡,谷蛋白,脱酰胺谷蛋白,强啡肽和强啡肽中的一种或多种。 当小麦抗原和麦醇溶蛋白抗原均选自α-麦醇溶蛋白33-mer,α-麦醇溶蛋白17-mer,γ-麦醇溶蛋白15-mer,和 ω-麦醇溶蛋白17-mer和谷蛋白21-mer。 测试板和试剂盒可以有利地测试针对至少3种,5种,7种或全部α-麦醇溶蛋白,γ-麦醇溶蛋白,ω-麦醇溶蛋白,谷蛋白,小麦胚芽凝集素,谷胱甘肽,强啡肽,转谷氨酰胺酶2,转谷氨酰胺酶3, 转谷氨酰胺酶6和与麦醇溶蛋白结合的转谷氨酰胺酶。

$$\text{Index} = \frac{\text{Mean OD of patients}}{\text{Mean OD of calibrators}}$$