

QUANTA Plex™ Celiac IgA Profile

708940

For *In Vitro* Diagnostic Use

CLIA Complexity: High

Intended Use

The QUANTA Plex™ Celiac IgA Profile is a fluorescent immunoassay for the semi-quantitative detection of IgA anti-human tissue transglutaminase (h-tTG) and anti-deamidated gliadin peptide (DGP) antibodies, the detection of an insufficient amount of IgA, in human serum. The presence of these antibodies in conjunction with other laboratory and clinical findings is an aid in the diagnosis of the gluten sensitive enteropathy celiac disease. Insufficient IgA indicates that there is not enough IgA to allow detection of IgA anti-h-tTG or anti-DGP.

Summary and Explanation of the test

Celiac disease is a chronic condition whose main features include inflammation and characteristic “flattening” of intestinal mucosa resulting in a malabsorption syndrome known as a gluten sensitive enteropathy. The exact etiology of the disease remains unknown but gliadin, the alcohol soluble fraction of wheat gluten, is clearly the toxic agent.^{1,2}

Originally, a series of intestinal biopsies was used to diagnose celiac disease. More recently, serological testing for anti-gliadin, anti-endomysial and anti-tTG antibodies has been suggested for screening patients with suspected gluten sensitive enteropathy as well as for monitoring dietary compliance.³⁻⁵ The European Society of Pediatric Gastroenterology and Nutrition has recommended the use of antibody markers such as anti-gliadin and anti-endomysial antibodies to reduce the number of intestinal biopsies needed to make a diagnosis.⁵

The endomysial antigen has been identified as the protein cross-linking enzyme tTG.⁶ Specific ELISA procedures incorporating tTG as the antigen afford a reliable, objective alternative to the traditional immunofluorescent-based assays using thin sections of primate esophagus as substrate.⁶⁻⁸ The h-tTG antigen has been produced by recombinant technology, and may have certain advantages compared with the antigen from guinea pig liver.^{7,8}

Recent work has revealed that gliadin reactive antibodies from patients with celiac disease bind a very limited number of specific epitopes on the gliadin molecule.⁹⁻¹¹ These studies further reveal that selective deamidation of gliadin by the celiac-associated enzyme tTG results in enhanced binding by anti-gliadin antibodies. Assays using deamidated gliadin peptides have higher diagnostic accuracy for celiac disease than standard anti-gliadin and even anti-tTG assays.^{12,13} Additionally, these antibodies are of interest to follow disease activity over time and for monitoring adherence to a gluten-free diet.¹³

A significant proportion of celiac disease patients are IgA deficient.¹⁴⁻¹⁷ Thus, a sensitive screening strategy for at risk populations includes testing for IgA anti-DGP and anti-h-tTG antibodies, as well as testing for selective IgA deficiency.

Dermatitis Herpetiformis (DH) is a skin disease that, as with celiac disease, is caused by ingestion of wheat protein. A majority of patients with DH have jejunal villous atrophy identical to that found in celiac disease and strict gluten-free diet improves both gut and skin lesions.^{1,2,18} Current serological methods such as the endomysial, native gliadin and tTG assays exhibit lower performance when testing for DH, with sensitivities ranging from only 60-75%¹⁸, compared to sensitivities greater than 95% for celiac disease.³⁻⁸

The QUANTA Plex™ Celiac IgA Profile is a screening test for antibodies found in people with celiac disease and the related gluten sensitive disorder dermatitis herpetiformis. It allows the simultaneous detection of IgA antibodies to both a synthetic deamidated gliadin peptide and recombinant human tTG, and at the same time detects an IgA deficiency.

Principles of the Procedure

Recombinant h-tTG and synthetic DGP are each bound to different, fluorescently “colored” beads. The two different antigen coated beads, as well as an anti-IgA-coated control bead and an IgA-coated control bead, are mixed together and put into wells of a microwell plate under conditions that preserve the antigens in their reactive state. Pre-diluted controls, a serum free control consisting of Sample Diluent with no serum added, and diluted patient sera are added to separate microwells, allowing any DGP and h-tTG antibodies present to bind to the immobilized antigens on the beads, as well as free IgA to bind to the anti-IgA bead. Then an anti-human IgA conjugated to a fluorescent probe is added to each microwell. A second incubation allows the anti-human IgA fluorescent conjugate to bind to any patient antibodies that have become attached to the antigens on the beads or captured by the anti-IgA bead, and to the IgA on the IgA bead. The samples are then measured in the Luminex™ 100 or 200 flow analyzer. This flow analyzer can discriminate the color of each bead from the others as well as measure the fluorescent intensity of the conjugate on each bead.¹⁹ The fluorescent intensity on the bead is proportional to the amount of bound conjugate, which in turn is proportional to the amount of patient antibodies bound to the antigen coated beads or the anti-IgA bead. Each antibody can be semi-quantitated by comparing the fluorescent intensity of the patient sample with that of the corresponding Calibrator. Comparing the calculated Luminex Units of the anti-IgA bead and the IgA bead can identify a selective IgA-deficient serum as described below in Interpretation of Results. These two control beads can also be used to ensure that false negative results due to operational error are detected as described in Quality Control. Note that these beads yield the same results when a person is deficient in IgA as when no serum is added to the well.

Reagents

1. Polystyrene microwell plate 12 (1 x 8) microwell strips (stamped silver in color) with holder, containing beads of 4 different “colors”. Each of the colored beads is coated with a different purified antigen (h-tTG, DGP, anti-IgA or IgA), in a foil package containing desiccants
2. QUANTA Plex™ Negative Control, 1 vial of buffer containing preservative and human serum with no human IgA antibodies to h-tTG and DGP antigens, prediluted, ready to use, 1.2mL
3. Celiac IgA Calibrator, 1 vial of buffer containing preservative and human serum IgA antibodies to h-tTG and DGP antigens, prediluted, ready to use, 1.2mL
4. Celiac IgA Positive Control, 1 vial of buffer containing preservative and human serum IgA antibodies to h-tTG and DGP antigens, prediluted, ready to use, 1.2mL

5. HRP Sample Diluent, 1 vial – colored pink, containing Tris-buffered saline, Tween 20, protein stabilizers and preservatives, 50mL (also used as the Serum Free Control)
6. Fluorescently labeled IgA Conjugate, goat anti-human IgA (alpha chain specific), 1 amber vial – lyophilized powder, containing buffer, protein stabilizers and preservatives. Refer to the Methods section for reconstitution instructions.
7. QUANTA Plex™ Conjugate Diluent, 1 vial - colored pink, containing buffer, protein stabilizers and preservatives, 7mL

Warnings

1. **WARNING:** The HRP Sample Diluent and controls contain a chemical (0.02% chloramphenicol) known to the State of California to cause cancer.
2. All human source material used in the preparation of controls for this product has been tested and found negative for antibody to HIV, HBsAg, and HCV by FDA cleared methods. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the QUANTA Plex™ Negative Control, Celiac IgA Calibrator and Celiac IgA Positive Control should be handled in the same manner as potentially infectious material.²⁰
3. Sodium Azide is used as a preservative. Sodium Azide is a poison and may be toxic if ingested or absorbed through the skin or eyes. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. Flush sinks, if used for reagent disposal, with large volumes of water to prevent azide build-up.
4. Use appropriate personal protective equipment while working with the reagents provided.
5. Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing of wastes.

Precautions

1. This product is for *In Vitro* Diagnostic Use.
2. Substitution of components other than those provided in this kit may lead to inconsistent results. All controls are kit lot number specific.
3. Adaptation of this assay for use with certain automated liquid sample processors was shown to yield equivalent results to those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
4. A variety of factors influence the assay performance. These include the starting temperature of the reagents, the ambient temperature, the accuracy and reproducibility of the pipetting technique, the reproducibility of the mixing technique, the Luminex™ 100 or 200 flow analyzer used to measure the results and the length of the incubation times during the assay. Careful attention to consistency is required to obtain accurate and reproducible results.
5. Strict adherence to the protocol is recommended.
6. Incomplete resealing of the zip-lock pouch containing microwell strips and desiccants will result in antigen degradation and poor precision.
7. Unacceptably low fluorescence may be observed following multiple uses from a single bottle of fluorescent conjugate over a period of time. It is important to follow all recommended fluorescent conjugate handling procedures to prevent this occurrence.
8. Chemical contamination of the fluorescent conjugate can result from improper cleaning or rinsing of equipment or instruments. Residues from common laboratory chemicals such as formalin, bleach, ethanol, or detergent will cause degradation of the fluorescent conjugate over time. Thoroughly rinse all equipment or instruments with distilled or deionized water after the use of chemical cleaners/disinfectants.

Storage Conditions

1. Store all the kit reagents at 2-8°C. Do not freeze. Reagents are stable until the expiration date when stored and handled as directed.
2. Unused microwell strips with antigen-coated beads should be securely resealed in the foil pouch containing desiccants and stored at 2-8°C.

Specimen Collection

This procedure should be performed with a serum specimen. Microbially contaminated, heat-treated, or specimens containing visible particulates should not be used. Grossly hemolyzed or lipemic serum should not be used.

Following collection, the serum should be separated from the clot. CLSI (formerly NCCLS) Document H18-A3 recommends the following storage conditions for samples: 1) Store samples at room temperature no longer than 8 hours. 2) If the assay will not be completed within 8 hours, refrigerate the sample at 2-8°C. 3) If the assay will not be completed within 48 hrs, or for shipment of the sample, freeze at -20°C or lower. Frozen specimens must be mixed well after thawing and prior to testing.

Procedure: Materials provided

- 1 Celiac IgA Profile microwell plate, 12 (1 x 8) microwell strips (stamped silver in color), with holder
- 1 1.2mL prediluted, ready to use QUANTA Plex™ Negative Control
- 1 1.2mL prediluted, ready to use Celiac IgA Calibrator
- 1 1.2mL prediluted, ready to use Celiac IgA Positive Control
- 1 50mL HRP Sample Diluent (also used as the Serum Free Control)
- 1 Bottle of lyophilized Fluorescent Conjugate, goat anti-human IgA
- 1 7mL QUANTA Plex™ Conjugate Diluent

Additional Materials Required But Not Provided

- Micropipet to deliver 5 and 500µL
- Disposable micropipet tips

Test tubes for patient sample dilutions, 1 to 4mL volume
Distilled or deionized water
Sheath Fluid for Luminex™ 100 or 200 flow analyzer
Luminex™ 100 or 200 flow analyzer
8-channel Electronic pipet to deliver 5, 30, 45, 50 and 60µL or Automated pipetter/diluter

Using the Luminex™ 100 or 200 Flow Analyzer

1. See the user's manual provided with the Luminex™ for detailed instructions on running the Luminex™ 100 or 200 flow analyzer and the Luminex™ Integrated System (IS) Version 2.0 or higher software program. For additional information and for troubleshooting problems with this assay, contact INOVA Diagnostics, Inc. technical service at the address or telephone number found on the last page of the Direction Insert. Brief Luminex™ 100 or 200 flow analyzer operating instructions are provided below.
2. Calibrate the Luminex™ using the Calibration and Control beads supplied by Luminex Corporation at least once per month and verify that calibration was successful. In addition, calibrate the Luminex™ if the delta calibration temperature is more than 3 degrees, if the assay controls are out of range or as needed.
3. The Luminex™ takes 30 minutes to warm-up after being turned on. When the warm-up period is completed, perform the prime, alcohol flush and wash operations recommended by the manufacturer.
4. Using IS Version software, load the "QP Celiac IgA Profile" template and ensure that all lot information is correct. If necessary, update the lot information. The parameters in the template are as follows: The bead colors are h-tTG = 27, DGP = 28, anti-IgA = 29, IgA = 30. The events per bead are 50, the sample size is 50µL, the flow rate is 60µL/minute (fast), and the gate is 7500 to 17000. The median values are used for the Median Fluorescent Intensity (MFI).
5. Input the sample names either manually or by clicking on "Load Pa List".
6. Load the plate into the XY platform of the Luminex™.
7. Run the Luminex™ by clicking the "Start Plate" button.
8. When finished for the day, perform the sanitize and soak operations prior to turning the instrument off.

Method:

Before you start

1. For programming information for automated equipment, contact INOVA Diagnostics Inc., Technical Services.
2. Turn on the Luminex™ 100 or 200 flow analyzer, ensure that it is warmed-up, and perform all daily maintenance operations as previously described. If necessary, calibrate the instrument and verify that the calibration was successful.
3. Bring all reagents and patient samples to room temperature (20-26°C) and mix them well.
4. If the anti-human IgA fluorescent conjugate has not been reconstituted, add 6mL of QUANTA Plex™ Conjugate Diluent to the amber vial containing the lyophilized powder and swirl the container for approximately 30 seconds to dissolve the contents. The reconstituted fluorescent conjugate is stable for 3 months at 2-8°C. **Do not freeze.**
5. Make sure that the sheath fluid container in the Luminex™ is filled with Sheath Fluid available from Luminex Corporation and that the waste bottle is empty.
6. Prepare a 1:101 dilution of each patient sample by adding 5µL of each serum to 500µL of HRP Sample Diluent, then vortex to thoroughly mix the solution. Diluted samples must be used within 8 hours of preparation. **DO NOT DILUTE** the QUANTA Plex™ Negative Control, the Celiac IgA Calibrator and Positive Control.
7. Determination of the presence or absence of IgA anti-h-tTG and anti-DGP antibodies using arbitrary units and detecting selective IgA deficiency, requires two wells for the Calibrator and one well each for the Negative, Positive and Serum Free Controls. It is recommended that samples be run in singleton.

Assay procedure

1. **All reagents must be brought to room temperature (20-26°C) prior to beginning the assay.** Place the required number of microwells/strips in the holder. **Immediately return unused strips to the pouch containing desiccants and seal securely to minimize exposure to water vapor and light.**
2. Add 45µL of HRP Sample Diluent to each microwell that will contain a **patient sample (and the Serum Free Control)**. Do **not** add HRP Sample Diluent to the first four microwells, as these will contain the QUANTA Plex™ Negative Control, the Celiac IgA Calibrator (in duplicate), and the Celiac IgA Positive Control. **Do not** add the diluted patient samples to the microwells at this time.
3. Maintain one of the following timing sequence when adding controls, samples, and conjugate to the microwells. Because the samples are read sequentially at a rate of approximately 1 sample every 19 seconds (or one 8-well strip in approximately 2½ minutes) by the Luminex™, the patient samples and also the conjugate must be added to the microwells at this rate to minimize any front to back assay variation. If the controls, samples or the conjugate are added one at a time, stagger each addition of these to the next microwell by 19 seconds. If controls, samples or the conjugate are added 8 at a time to a strip, stagger each addition of any of these to the next strip by 2 ½ minutes. Both of these timing schemes will take approximately 30 minutes for the addition of the samples to all 12 strips of an entire plate and will minimize front to back assay variation.
4. Vortex, then add 50µL of each of the following **prediluted** controls: The QUANTA Plex™ Negative Control to the first microwell, the Celiac IgA Calibrator to the second and third microwells, and the Celiac IgA Positive Control to the fourth microwell. An electronic pipet must be used when manually adding samples and mixing the beads. Use one of the timing sequences for adding the controls as described in step 3. **Vigorously pipet at least 30µL of the QUANTA Plex™ Negative Control, Celiac IgA Calibrator and Positive Control up and down four times in order to mix the beads and the controls in each microwell.** The 30-minute incubation time begins after adding the QUANTA Plex™ Negative Control into the **first** microwell.

5. Immediately continue the assay by adding 5 μ L HRP Sample Diluent to the fifth well (the serum free control) and 5 μ L of diluted patient serum to the appropriate microwells (note: this makes a 1:1010 final dilution of the patient serum). Maintain the same timing sequence as used in step 4. **Mix the diluted patient sample and the HRP Sample Diluent in the microwell by vigorously pipetting at least 30 μ L of the contents of the microwell up and down four times.** Continue timing the incubation for 30 minutes from the time of the addition of the QUANTA Plex™ Negative Control. Place the microwell strips at room temperature, on a level surface, and away from direct sunlight for the remainder of the incubation period.
6. At the end of the first incubation period, add 50 μ L of the fluorescent Conjugate to each microwell and **vigorously pipet at least 60 μ L of the contents of the microwell up and down four times.** Maintain the same timing sequence for adding the conjugate that was used in steps 4 and 5. The conjugate should be removed from the bottle using standard aseptic conditions and good laboratory techniques. Remove only the amount of conjugate from the bottle necessary for the assay. **TO AVOID POTENTIAL MICROBIAL AND/OR CHEMICAL CONTAMINATION, NEVER RETURN UNUSED CONJUGATE TO THE BOTTLE.** Incubate the microwell strips for 30 minutes at room temperature, on a level surface and away from direct sunlight. The incubation time begins after the first conjugate addition.
7. Within one hour after completion of the 30-minute fluorescent conjugate incubation, read the Celiac IgA Profile plate on the Luminex™ as detailed in the section above, “Using the Luminex™ 100 or 200 Flow Analyzer.”

Quality Control

1. The Celiac IgA Calibrator (in duplicate), the Celiac IgA Positive Control, the QUANTA Plex™ Negative Control and the Serum Free Control should be run with every batch of samples to ensure that all reagents and procedures have performed properly.
2. Note that since the Celiac IgA Calibrator, the Celiac IgA Positive Control and QUANTA Plex™ Negative Control are prediluted, they do not control for procedural methods associated with specimen dilutions.
3. Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations. Additional suitable control sera may be prepared by aliquoting pooled human serum specimens and storing them at $\leq -20^{\circ}$ C.
4. In order for the test results to be considered valid, all of the criteria listed below must be met. If any of these are not met, the test should be considered invalid and the assay repeated. The value in Luminex Units (LU) of the Calibrator for each antigen is found on the box label for that kit. Refer to the formula in the Calculation of Results section to determine the LUs of the QUANTA Plex™ Negative Control, Celiac IgA Positive Control and the Serum Free Control.
 - a. The value of the QUANTA Plex™ Negative Control must be < 20 LU for h-tTG and DGP. It must be between 10 and 40 LU for both the IgA and anti-IgA control beads.
 - b. The value of the Celiac IgA Positive Control must be ≥ 30 LU for h-tTG and between 30 LU and 130 LU for DGP. It must be between 10 and 40 LU for both the IgA and anti-IgA control beads.
 - c. The value of the Serum Free Control must be < 20 LU for h-tTG and DGP. For the anti-IgA bead the value must be < 5.0 LU and for the IgA bead it must be between 10 and 40 LU.
 - d. The ratio of the LUs for Anti-IgA/IgA must be >0.50 for the QUANTA Plex™ Negative Control and the Celiac IgA Positive Control and <0.50 for the Serum Free Control.
 - e. The anti-IgA and IgA control beads are meant to detect selective IgA deficiency and to ensure that false negative patient results due to operational mistakes are detected. If either of the two control beads has a calculated result of less than 5.0 LU for any particular patient, a result of “Retest” will appear in Version IS 2.0 and higher.
 - i. The possibility exists that the patient has selective IgA deficiency if the results for anti-h-tTG and DGP are negative (that is less than 20 LU) and the anti-IgA bead is less than 5 LU, while at the same time the value of the IgA control bead is greater than 20 LU, yielding a ratio of anti-IgA LU/IgA LU of <0.5 . Note: this same result may be found if patient serum was not added to the well.
 - ii. If both the anti-IgA bead and the IgA bead are less than 5.0 LU the possibility exists that conjugate was not added to the well. The patient should be re-tested to confirm the negative result.
5. The QUANTA Plex™ Negative Control, the Celiac IgA Calibrator and Positive Control, and the Serum Free Control are intended to monitor for substantial reagent failure. The user should refer to CLSI (formerly NCCLS) Document C24-A3 for additional guidance on appropriate QC practices.

Calculation of Results

The MFIs for all controls and patient samples for each antigen and the two control beads are first determined. The reactivity in LU of a given sample for each type of antigen can then be calculated by the following formula. Divide the MFI of the sample by the MFI of the Celiac IgA Calibrator for that antigen and multiply the result by the number of LUs assigned to the Celiac IgA Calibrator for that antigen. The LU value of the Calibrator for each antigen is found on the box label for that kit lot. The following example is for determining anti-h-tTG reactivity. Use the equivalent formula for the DGP Bead and the two control beads.

$$\text{Sample Value (in LU)} = \frac{\text{Sample MFI for h-tTG}}{\text{h-tTG Calibrator MFI}} \times \text{h-tTG Calibrator value (in LU)}$$

The IgA antibody reactivity for h-tTG and DGP can then be classified according to the table below.

	LU
Negative	<20
Weak Positive	20-30
Positive	>30

Reactivity is related to the quantity of autoantibody present on the bead in a non-linear fashion. While increases and decreases in patient antibody concentrations will be reflected in a corresponding rise or fall in fluorescent reactivity, the change is not proportional (i.e. a doubling of the antibody concentration will not double the reactivity). In addition, the amount of total IgA in a patient's serum affects the measured MFI. If a more accurate quantitation of patient autoantibody is required, the sample should be run on a quantitative test.

Interpretation of Results

The QUANTA Plex™ Assay is very sensitive to technique and is capable of detecting small differences in patient populations. Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

It is suggested that the results reported by the laboratory should include the statement: “The following results were obtained with the INOVA QUANTA Plex™ Celiac IgA Profile. Values obtained with different manufacturers’ assay methods may not be used interchangeably. The magnitude of the reported IgA autoantibody levels cannot always be correlated to an endpoint titer.”

To determine if the patient has a sufficient or insufficient amount of IgA in their serum, divide the LU of the anti-IgA bead by the LU of the IgA bead. If the result is less than 0.5 and the patient is negative for both anti-h-tTG and anti-DGP, the sample should be re-tested to confirm the result. If confirmed that the patient has an insufficient amount of IgA, the sample should be reflexed to IgG anti-h-tTG and IgG anti-DGP tests. If confirmation of IgA deficiency is needed, the sample should be tested on a quantitative IgA test.

Limitations of the Procedure

1. The presence of immune complexes or other immunoglobulin aggregates in the patient sample may cause an increased level of non-specific binding and produce false positives in this assay.
2. Not all celiac disease and Dermatitis Herpetiformis patients are positive for h-tTG or DGP IgA antibodies.
3. Results of this assay should be used in conjunction with clinical findings and other serological tests.
4. Failure to adequately mix the controls and/or the diluted serum samples with the preserved beads in the plate may yield higher %C.V. values than those typically found in ELISA assays.
5. After the half-hour incubation with the fluorescent conjugate, there is approximately a 10% further increase in fluorescence for every additional half-hour of incubation time.
6. The performance characteristics of this assay have not been established for matrices other than serum.
7. Failure to maintain consistent reagent addition timing may result in increased front-to-back assay variation.

Expected Values

The ability of the QUANTA Plex™ Celiac IgA Profile assay to detect anti-h-tTG and anti-DGP antibodies was evaluated by comparison to commercially available ELISA tests from INOVA Diagnostics, Inc. Results of the ELISAs and each of the QUANTA Plex™ Celiac IgA Profile tests were determined to be positive if the patient sample was greater than or equal to 20 Luminex Units or ELISA Units and negative if less than 20 LU or EU.

Normal Range

Two hundred seventy eight samples from normal blood donors were run on the QUANTA Plex™ Celiac IgA Profile test. Two hundred seventy-seven normal samples (99.6%) were negative on the h-tTG portion of the QUANTA Plex™ test. The highest sample had a value of 29 LU. The average value was 3.2 LU with a standard deviation (SD) of 1.5. The cutoff is 11 SD above the average. All but 3 of the 278 normal samples (98.9%) were negative on the DGP portion of the QUANTA Plex™ Celiac IgA Profile test. The highest sample had a value of 88 LU. The same sample was also positive on the DGP ELISA. Not including the double positive sample, the average value was 4.4 LU with a SD of 5.8. The cutoff is 2.7 SD above the average.

Clinical Sensitivity and Specificity

To determine the clinical sensitivity of the autoantibody assays, 29 samples from active celiac disease patients who were IgA sufficient were tested on the QUANTA Plex™ Celiac IgA Profile and the corresponding ELISAs. The clinical sensitivity for IgA anti-h-tTG was 93% (with a 95% Confidence Interval [CI] from 77-99%). The clinical sensitivity for IgA anti-DGP was 73% (95% CI from 53-92%). To determine the clinical specificity, 494 patients who were not diagnosed with celiac disease were tested. This group included normal blood donors, patients with rheumatic, liver, gastrointestinal and infectious diseases. The clinical specificity for anti-h-tTG was 98%, with a 95% CI of 96-99%. The clinical specificity of anti-DGP was also 98% (95% CI from 96-99%).

Clinical Sensitivity and Specificity for the IgA anti-h-tTG and DGP tests in the Celiac IgA Profile

h-tTG IgA		Diagnosis		
		Positive (CD with sufficient IgA and not on gluten free diet)	Negative (Controls not diagnosed with CD)	Total
Luminex	Positive	27	11**	38
	Negative	2*	483	485
	Total	29	494	523

*Both of these samples were negative on the tTG ELISA. **Ten of these are positive by ELISA
Sensitivity: $27/(27+2) \times 100 = 93.1\%$ ([CI] 77-99%) Specificity: $483/(11+483) \times 100 = 97.8\%$ ([CI] 96-99%)

DGP IgA		Diagnosis		
		Positive (CD with sufficient IgA and not on gluten free diet)	Negative (Controls not diagnosed with CD)	Total
Luminex	Positive	18	12**	30
	Negative	11*	482	493
	Total	29	494	523

*Ten of these samples are negative by ELISA. **Ten of these are positive by ELISA.

Sensitivity: $18/(18+11) \times 100 = 62.1\%$ ([CI] 53-92%). Specificity: $482/(12+482) \times 100 = 97.6\%$ ([CI] 96-99%)

Comparison between QUANTA Plex™ and ELISA Assays

The relative positive, negative and total percent agreement (with 95% Confidence Intervals) of the QUANTA Plex™ autoantibody assays compared to the ELISAs were calculated for all samples tested and are shown in the tables below.

h-tTG IgA		ELISA		
		Positive	Negative	Total
Luminex	Positive	174	2	176
	Negative	21	757	778
	Total	195	759	954

Positive Percent Agreement: $174/(174+21) \times 100 = 89.2\%$ (84-93%)
 Negative Percent Agreement: $757/(2+757) \times 100 = 99.7\%$ (99-100%)
 Total Percent Agreement: $(174+757)/(174+2+21+757) \times 100 = 97.6\%$

DGP IgA		ELISA		
		Positive	Negative	Total
Luminex	Positive	136	4	140
	Negative	31	783	814
	Total	167	787	954

Positive Percent Agreement: $136/(136+31) \times 100 = 81.4\%$ (75-87%)
 Negative Percent Agreement: $783/(4+783) \times 100 = 99.5\%$ (99-100%)
 Total Percent Agreement: $(136+783)/(136+4+31+783) \times 100 = 96.3\%$

Detection of insufficient amount of IgA

Sera from 5 patients known to have selective IgA deficiency were tested on the Celiac IgA Profile, as were 949 other patients as described above. Those 5 plus 21 additional patients yielded ratios on the anti-IgA LU/IgA LU of less than 0.5 and thus had insufficient IgA for the Celiac IgA Profile.

Sensitivity and Specificity for the anti-IgA LU/IgA LU Ratio in the QUANTA Plex™ Celiac IgA Profile

Positive Controls N=5	QUANTA Plex™ Ratio >0.5	QUANTA Plex™ Ratio <0.5	Clinical Sensitivity QUANTA Plex™ (95% CI)	All Negative Controls N=949	QUANTA Plex™ Ratio >0.5	QUANTA Plex™ Ratio <0.5	Clinical Specificity QUANTA Plex™ (95% CI)
Anti-IgA/IgA Ratio	0	5	100% (82-100%)**	Anti-IgA/IgA Ratio	928	21*	99% (98-100%)**

*Based on serologic parameters, 12 of these appeared to be IgA deficient celiac disease patients, 7 were highly diluted controls and 1 was known low total serum IgA. Based on an incidence of 1 in 500 IgA deficient, 2 additional samples would be expected to be positive by this incidence rate.

**Calculations based on 18 expected deficient sera.

Precision and Reproducibility

Intra-assay and Inter-assay variation. For within-assay variation, 18 samples were assayed 10 times each on a single assay. The variation in 3 to 5 representative samples for each test is listed in the table below. For between-assay variation, 20 samples were assayed in 6 tests run on 6 different days. The variation in 3 to 5 representative samples for each test is listed in the table below.

Within-assay variation			Between-assay variation		
Antigen	Average LU	% C.V.	Antigen	Average LU	% C.V.
h-tTG	22	6%	h-tTG	23	9%
h-tTG	23	7%	h-tTG	26	8%
h-tTG	31	7%	h-tTG	31	8%
h-tTG	89	9%	h-tTG	102	3%
h-tTG	255	4%	h-tTG	263	3%
DGP	17	12%	DGP	19	4%
DGP	22	8%	DGP	23	11%
DGP	25	8%	DGP	26	5%
DGP	59	4%	DGP	65	9%
DGP	38	8%	DGP	44	9%
anti-IgA	5	5%	anti-IgA	5	8%
anti-IgA	11	4%	anti-IgA	11	5%
anti-IgA	20	2%	anti-IgA	20	5%
IgA	27	5%	IgA	28	4%
IgA	10	4%	IgA	10	5%
IgA	21	2%	IgA	23	4%

References

1. Trier JS: Celiac Sprue: *N Engl J Med* **325**: 1709-1719, 1991.
2. Strober W: Gluten-sensitive enteropathy: A nonallergic immune hypersensitivity of the gastrointestinal tract. *J. Allergy Clin. Immunol.* **78**: 202-211, 1986.
3. McMillan SA, Haughton DJ, Biggart JD, et al.: Predictive value for coeliac disease of antibodies to gliadin, endomysium and jejunum in patients attending for jejunal biopsy. *BMJ* **303**: 1163-1165, 1991.
4. Valdimarsson T, Franzen L, Grodzinsky E, et al.: Is small bowel biopsy necessary in adults with suspected celiac disease and IgA anti-endomysial antibodies? 100% positive predictive value for celiac disease in adults. *Digestive Diseases and Science* **41**: 83-87, 1996.
5. Walker-Smith JA, Guandalini S, Schmitz J, et al.: Revised criteria for diagnosis of celiac disease: Report of working group of European Society of Pediatric Gastroenterology and Nutrition (ESPGAN). *Arch Diseases of Childhood* **65**: 909-911, 1990.
6. Dieterich W, Ehnis T, Bauer M, et al.: Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nature Medicine* **3**: 797-801, 1997.
7. Sardy M, Odenthal U, Karpatis S, et al.: Recombinant human tissue transglutaminase ELISA for the diagnosis of gluten-sensitive enteropathy. *Clin Chem* **45**: 2142, 1999.
8. Sblattero D, Berti I, Trevisiol C, et al.: Human recombinant tissue transglutaminase ELISA : an innovative diagnostic assay for celiac disease. *Am J Gastroenterology* **95**: 1253-1257, 2000.
9. Osman AA, Günnel T, Dietl A, et al.: B-Cell epitopes of gliadin. *Clin Exp Immunol* **121**: 248-254, 2000.
10. Aleanzi M, Demonte AM, Esper C, et al.: Celiac disease: Antibody recognition against native and selectively deamidated gliadin peptides. *Clin Chem* **47**: 2023-2028, 2001.
11. Schwertz E, Kahlenberg F, Sack U, et al.: Serologic assay based on gliadin-related nonapeptides as a highly sensitive and specific diagnostic aid in celiac disease. *Clin Chem* **50**: 2370-2375, 2004.
12. Prince HE: Evaluation of the INOVA Diagnostics enzyme-linked immunosorbent assay kits for measuring serum immunoglobulin G (IgG) and IgA to deamidated gliadin peptides. *Clin Vaccine Immunol* **13**: 150-151, 2006.
13. Sugai E, Vazquez H, Nachman F, et al.: Accuracy of testing for antibodies to synthetic gliadin-related peptides in celiac disease. *Clin Gastroenterol Hepatol* **4**: 1112-1117, 2006.
14. Collin P, Maki M, Keyrilainen O, et al.: Selective IgA deficiency and celiac disease. *Scand J Gastroenterol* **27**: 367-371, 1992.
15. Cataldo F, Marino V, Ventura A, et al.: Prevalence and clinical features of selective immunoglobulin A deficiency in coeliac disease: an Italian multicentre study. *Gut* **42**: 362-365, 1998.
16. Fernandez E, Blanco C, Garcia S, et al.: Use of low concentrations of human IgA anti-tissue transglutaminase to rule out selective IgA deficiency in patients with suspected celiac disease. *Clin Chem* **51**: 1014-1016, 2005.
17. Dahlbom I, Olsson M, Forooz NK, et al.: Immunoglobulin G (IgG) anti-tissue transglutaminase antibodies used as markers for IgA-deficient celiac disease patients. *Clin Diag Lab Immunol* **12**: 254-258, 2005.
18. Chorzelski TP, Sulej J, Tcherzewska H, et al.: IgA class endomysium antibodies in dermatitis herpetiformis and celiac disease. *Ann NY Acad Sci* **420**: 325-334, 1983.
19. Martins TB, Burlingame R, von Mühlen CA, et al.: Evaluation of multiplexed fluorescent microsphere immunoassay for detection of autoantibodies to nuclear antigens. *Clin Diagn Lab Immunol* **11**: 1054-1059, 2004.
20. Biosafety in Microbiological and Biomedical Laboratories: Centers for Disease Control/National Institutes of Health, Fifth Edition, 2007.

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Medical Technology Promedt Consulting GmbH
Altenhofstrasse 80
D-66386 St. Ingbert, Germany
Tel.: +49-6894-581020
Fax.: +49-6894-581021
www.mt-procons.com

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