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DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

DEC 2 4 2003

Mr. Kevin J. Lawson
Director of Regulatory Affairs
IMMCO Diagnostics
60 Pineview Drive
Buffalo, NY 14228

Re: k032571
Trade/Device Name: ImmuLisa Anti-human tissue Transglutaminase (human tTG) IgA ELISA
Regulation Number: 21 CFR 866.5660
Regulation Name: Multiple autoantibodies immunological test system
Regulatory Class: Class II
Product Code: MVM
Dated: November 21, 2003
Received: December 10, 2003

Dear Mr. Lawson:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820).

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific information about the application of labeling requirements to your device, or questions on the promotion and advertising of your device, please contact the Office of In Vitro Diagnostic Device Evaluation and Safety at (301) 594-3084. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 443-6597 or at its Internet address http://www.fda.gov/cdrh/dsma/dsmamain.html.

Sincerely yours,

Steven Gutman

Steven I. Gutman, M.D., M.B.A.
Director
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and
Radiological Health

Enclosure

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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INDICATIONS FOR USE STATEMENT

510(k) Number (if known): K 032571

Device Name: ImmuLisa Anti-human tissue Transglutaminase (human tTG) IgA ELISA

Indications For Use: An enzyme linked immunoassay (ELISA) for the detection and semi-quantitation of IgA antibodies to human tissue transglutaminase, as an aid in diagnosing patients with Gluten Sensitive Enteropathy (celiac Disease and Dematitis herpetiformis)

(PLEASE DO NOT WRITE BELOW THIS LINE - CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)

Prescription Use ✓ OR Over-The-Counter Use _____
(Per 21 CFR 801.109) (Optional Format 1-2-96)

Mana M Chan
Division Sign-Off

Office of In Vitro Diagnostic Device Evaluation and Safety

510(k) 032571

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Food and Drug Administration
2098 Gaither Road
Rockville, Maryland 20850

KEVIN LAWSON
DIRECTOR, RA
IMMCO DIAGNOSTICS, INC.
60 PINEVIEW DR.
BUFFALO NY 14228

JAN 9 2004

Re: K032571

Categorization Notification

Regulations codified at 42 CFR 493.17 et. seq., implementing the Clinical Laboratory Improvement Amendments of 1988, require the Secretary to provide for the categorization of specific clinical laboratory test systems by the level of complexity. Based upon these regulations, the following commercially marketed test systems or assay for the analyte is categorized below:

Test System: IMMCO DIAGNOSTICS IMMULISA ANTI-HUMAN TISSUE TRANSGLUTAMINASE (HU-TTG) ANTIBODY (IGA) ELISA
Analyte : ANTI-TISSUE TRANSGLUTAMINASE (TTG)
Complexity : High
510K or PMA Number : K032571

This complexity categorization is effective as of the date of this notification. This cagegcrization will be reported on FDA's home page http://www.fda.gov/cdrh/clia. This categorization information may be provided to the user of the commercially marketed test system or assay as specified for the analyte indicated. This categorization will also be announced in a Federal Register Notice, which will provide opportunity for comment on the decision. FDA reserves the right to reevaluate and recategorize this test based upon the comments received in response to the Federal Register Notice. For questions regarding FDA's categorization procedure, contact Clara A. Sliva, Acting CLIA Coordinator, at (301)827-0496 or email at CLIA@CDRH.FDA.GOV

Sincerely yours,

Steven Gutman, M.D.

Steven I. Gutman, M.D., M.B.A.
Director
Office of In Vitro Diagnostic Device Evaluation and Safety
Center for Devices and Radiological Health

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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5/28/03 P: /DCLD/OIVD CLIA ROUTE SLIP

OIVD CLIA ROUTE SLIP

1) CST login GMcB **Date** 9-5-03
CST include copy of PI and manufacturer cover letter with CLIA Route Slip
510(k) or PMA Number: K032571
Date Received in DMC: 9-3-03
Division: IMDB **Reviewer:** AKC **Date:** 12/18/03

2) Reviewer completes section 2:
For OTC tests, collection kits **OTC CONTACT PHONE # 1-**_____

Manufacturer Immco Diagnostics
Instrument / reagent / and other (i.e. manual) if applicable Anti-Human Tissue
(Test system / Qualifier 1 / Qualifier 2) Immunlisa Transglutaminase
(hu-tTG) Antibody (IgA) ELISA

Analyte: Anti-Tissue Transglutaminase (tTG)

Rationale: IMM-057 **Total:** 15
Complexity: High **Mod or High 493.17 letter**

Complexity WAIVED (choose 1)
9 by regulation 493.15 letter ___
OTC, prescription home use section 123 letter___
Professional use version of OTC, prescription home use___
Trade Name Change Letter ____
Multiple test systems, analytes, attach supplemental sheet: Yes__ **NO** ✓
CLIA Database Sign-OFF AKC **DATE** 12/18/03
If not signed off in database, route slip will be returned to reviewer
REVIEWER SIGN-OFF AKC **DATE** 12/18/03

Reviewer: give completed CLIA route slip, final PI and Scoring Worksheet to your CLIA team rep

3) CLIA Team Review: [signature] **Date:** 12/18/03

4) CAS or RZH: [signature] **Date:** 1/9/04

5) Division Secretary: [signature] **Date:** 12/22/03
Add-to-files: Update contact information in CLIA database

GMcB 9 2004

2

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request 2014-6998; Released 10/30/14

Supplemental Information

Test System Name _____
Analyte Name: _____
Decision: MOD ____
Decision: HIGH ____
DECISION: WAIVED ____

Test System Name _____
Analyte Name: _____
Decision: MOD ____
Decision: HIGH ____
DECISION: WAIVED ____

Test System Name _____
Analyte Name: _____
Decision: MOD ____
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Test System Name _____
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Test System Name _____
Analyte Name: _____
Decision: MOD ____
Decision: HIGH ____
DECISION: WAIVED ____

Test System Name _____
Analyte Name: _____
Decision: MOD ____
Decision: HIGH ____
DECISION: WAIVED ____

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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2/16/00 P: DCLD/CLIA

Scoring Worksheet

(b) (5)

[redacted]

4

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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ImmuLisa™ Procedure at a Glance

Prepare Dilutions of Specimens

Pipette 100 µL of Specimens, Calibrators and Controls into Microwells

Incubate 60 min. at Room Temperature

Wash Microplate 4x

Pipette 100 µL of Conjugate into Microwells

Incubate 30 min. at Room Temperature

Wash Microplate 4x

Pipette 100 µL of Enzyme Substrate into Microwells

Incubate 30 min. at Room Temperature

Pipette 100 µL of Stop Solution into Microwells

Read absorbance at 405 nm

For technical assistance please contact:

IMMCO Diagnostics, Inc.
60 Pineview Drive
Buffalo, NY 14228-2120
Telephone: (716) 691-0091
Fax: (716) 691-0466
Toll Free USA/Canada: 1-800-537-TEST
E-Mail: info@immcodiagnostics.com

or your local product distributor

ImmuLisa™ Anti-human Tissue Transglutaminase (hu tTG) Antibody IgA ELISA

For in vitro diagnostic use
CLIA Complexity: High
CDC Analyte Identification Code: 0546
CDC Test System Identification Code: 28569

Code 1144 **PRODUCT INSERT** 96 Determinations

INTENDED USE

An enzyme linked immunosorbent assay (ELISA) for the detection and semi-quantitation of anti-human Tissue Transglutaminase IgA antibodies in human serum to aid in the diagnosis of patients with celiac disease and dermatitis herpetiformis.

SUMMARY AND EXPLANATION

Celiac Disease (CD) is an autoimmune gastrointestinal disorder that may occur in genetically susceptible individuals triggered by the ingestion of gluten-containing grains such as wheat, barley and rye. Of the many autoimmune disorders, CD represents one of the few where the etiological agent is known and the disease subsides and goes in remission once the etiological agent is withdrawn from the diet. *Dermatitis Herpetiformis* (DH) is an autoimmune bullous disorder related to CD that is also triggered by ingestion of gluten and responds similarly to a gluten-free diet.

CD is characterized by malabsorbtion resulting from inflammatory injury to the small intestinal mucosa and, when prolonged, can cause malnutrition. The classical symptoms of CD include diarrhea, weight loss and malnutrition.

Only a small percentage of patients with CD presents with classical symptoms. Consequently, the clinical spectrum of CD has grown much broader than in the past to include patients that do not present with classical symptoms. It is not uncommon for the initial symptoms to be non-gastrointestinal or for gastrointestinal symptoms, if present, to be mild or intermittent. Some common non-gastrointestinal manifestations include short stature, iron and folate deficiency, anemia, bone loss, aphthous stomatitis, arthralgia, dental enamel defects, etc. The need to examine a wider range of clinical presentation has led to greater numbers of individuals diagnosed with CD later in life than ever before. Adults may present with iron deficiency, macrocytic anemia and hypocalcaemia.

Studies have found the prevalence of CD to be highly variable from population to population.[1] The true prevalence has been difficult to ascertain. Disparate criteria used in diagnosis of CD are often the cause. If clinical criteria alone are used in determining prevalence, the incidence of CD is much lower as compared with incidence established by serological methods.[1,2] Using serological methods, the incidence of CD in the general population is approximately one in 200.

Diagnosis of CD based on clinical criteria thus can be misleading and may lead to serious delays in proper diagnosis. Frequently, delays in diagnosis extend 10-13 years from the first clinical presentation of symptoms.

Failure to diagnose CD early on may predispose an individual to long-term complications such as splenic atrophy and intestinal lymphoma. The incidence of lymphoma arising in the context of CD is difficult to ascertain. One study has shown incidence of lymphoma involving the gastrointestinal (GI) tract in patients with CD to range from 3.6 percent to 40 percent.[3] In another recent study,[4] CD is associated with significantly

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elevated risk for intestinal lymphoma, especially for non-Hodgkin's. A gluten-free diet (GFD) normalizes the mucosa and helps reduce the malignant potential.

Histological examination of the small intestinal biopsy remains the gold standard for diagnosing CD, but it has its own limitations. These include some patients with latent or even active CD that may have normal histopathology.[5]

The revised European Society of Paediatric Gastroenterology and Nutrition (ESPGHAN) criteria include only a single biopsy with clear-cut remission of clinical symptoms on GFD[6]. Positive serology at the time of diagnosis with disappearance on GFD contributes to the diagnosis. The various serological tests employed in the work-up of patients suspected to have CD include anti-gliadin antibody (AGA), anti-endomysial antibody (EMA), anti-reticulin antibody (ARA) and anti-tissue transglutaminase (tTG) antibody tests. Antibodies to gliadin and tTG are detected by ELISA, whereas endomysium and reticulin antibodies are detected by indirect immunofluorescence. EMA are very specific indicators of CD. However, the EMA test is an immuno-histochemical method that requires experience in reading immunofluorescence reactions. Rosario et al[7] concludes that "EmA-IgA is 100 percent sensitive and specific in active, untreated IgA-sufficient CD patients when performed by an established laboratory." EMA and tTG antibodies are also present in 60% - 70% of patients with DH.

Since identification of tTG as the endomysial antigen, ELISA methods have been described for detecting antibodies in the sera of patients with CD. The advantage of the anti-tTG antibody assay is that it is automatable and less subjective than EMA. For this reason, many laboratories have opted to use the tTG antibody method as the screening method. In these laboratories, it may be the only assay used for detection of CD cases. In various studies on the efficacy of the tTG antibody method for screening for CD, the specificity and sensitivity of this method has been found to range from 90 percent to 95 percent.[8] Human tTG has been described to improve the sensitivity of the tTG antibody assay for CD.

PRINCIPLES OF PROCEDURES

The anti-hu tTG antibody test is performed as a solid phase immunoassay (ELISA). Microwells are coated with recombinant hu tTG antigen followed by blocking the unreacted sites to reduce nonspecific binding. Controls, calibrators and patient serum samples are incubated in the antigen coated wells which allows anti-tTG antibodies present in the serum to bind. Unbound antibody and other serum proteins are removed by washing the microwells. Antibodies bound to the microwells are detected by adding enzyme labeled anti-human IgA conjugates to the wells. These enzyme conjugated antibodies bind specifically to the human immunoglobulin of the appropriate class. Unbound enzyme conjugate is removed by washing. Specific enzyme substrate (pNPP) is then added to the wells and the presence of antibodies to hu tTG is detected by a color change produced by the conversion of the pNPP substrate. The reaction is stopped and the intensity of color change, which is proportional to the concentration of antibody, is read by a spectrophotometer at 405 nm. Results are expressed in ELISA units per milliliter (EU/ml).

REAGENTS

Storage and Preparation

Store all reagents at 2-8°C. **Do not freeze.** Do not use if reagent is not clear or if a precipitate is present. All reagents must be brought to room temperature (20-25°C) prior to use. When stored at 2-8°C, the reconstituted wash buffer is stable until the kit expiration date. Reconstitute the wash buffer to 1 liter with distilled or deionized water. Coated microwell strips are for one time use only.

REFERENCES

1. Guandalini S, Gupta P. Celiac disease- A diagnostic challenge. *Clin Appl Immun Rev* 2002;2:293-305.
2. Fasano A, et al. Prevalence of celiac disease in at risk and not-at-risk groups in the United States 2003; 163:286-292.
3. Wolber R, et al. Lymphocytic gastritis in patients with celiac sprue or spruelike intestinal disease. *Gastroenterology* 1990;98:310-315.
4. Catassi, et al. Risk of non-Hodgkin lymphoma in celiac disease. *JAMA* 2002;287:1413-1419.
5. Arranz E, Ferguson A. Intestinal antibody pattern of celiac disease: Occurrence in patients with normal jejunal biopsy histology. *Gastroenterology* 1993;104:1263-72.
6. Hill ID, et al. Celiac disease: Working group report of the first world congress of pediatric gastroenterology, hepatology, and nutrition. *J. Pediatr Gastroenterol Nutr* 2002; 35:S78-S88
7. Rosario D, et al. Further studies of anti-endomysium and anti-gliadin antibodies in patients with suspected celiac disease. *J Pediatr Gastroenterol Nutr* 1998;27:191-195.
8. Vitoria JC, et al. Antibodies to gliadin, endomysium, and tissue transglutaminase for the diagnosis of celiac disease. *J Pediatr Gastroenterol Nutr* 1999;29:571-4.
9. Biosafety in Microbiological and Biomedical Laboratories. Centers for Disease Control National Institutes of Health; 1993 (HHS Pub No [CDC] 93-8395).
10. Sulkanen S, Haltunen T, Laurila K, et al. Tissue Transglutaminase autoantibody enzyme-linked immunosorbent assay in detecting celiac disease. Gastroenterology; 1998, 115:1322-1328.
11. Dietrich W, Laag E, Schopper H, et al. Autoantibodies to tissue transglutaminase as predictors of celiac disease; Gastroenterology 1998, 115:1317-1321.

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Precision:

Based on 10 replicates, the intra-assay and inter-assay Coefficient of Variation (CV) of the IgA anti-hu tTG ELISA test were calculated.

	inter-assay CV	intra-assay CV
High (199 EU/ml)	6.2%	2.9%
Medium (85 EU/ml)	8.4%	6.5%
Low (41 EU/ml)	15.6%	9.6%

Recovery:

Samples with known anti-hu tTG concentrations were mixed with appropriate dilutions of another positive sample with known amounts of anti-tTG. Anti-hu tTG antibody levels of the mixed samples were determined and from the values obtained the percent recovery calculated. The results are as follows:

	anti-hu tTG Ab. conc. added (EU/ml)	anti-hu tTG Ab. conc. obtained (EU/ml)	% Recovery
Sample 1	107.5	112	104.2
Sample 2	86	94.6	110
Sample 3	64	70.9	109.9

Precautions

For *in vitro* diagnostic use. All human derived components used have been tested for HBsAg, HCV, HIV-1 and 2 and HTLV-I and found negative by FDA licensed tests. However, human blood derivatives and patient specimens should always be considered potentially infectious. Follow good laboratory practices in storing, dispensing and disposing of these materials[9].

WARNING - Sodium azide (NaN_3) may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal of liquids, flush with large volumes of water to prevent azide buildup. Sodium azide may be toxic if ingested. If ingested, report incident immediately to laboratory director or poison control center.

Instructions should be followed exactly as they appear in this kit insert to ensure valid results. Do not interchange kit components with those from other sources other than the same lot number from IMMCO Diagnostics. Follow good laboratory practices to minimize microbial and cross contamination of reagents when handling. Do not use beyond expiration date on the label.

Materials Provided

Kit contains sufficient reagents to perform 96 determinations each.

12 x 8	Ready to use **Microplate** with individual breakaway microwells coated with hu tTG antigen.
1 x 1.5 ml	Ready to use **Positive Control*** *(red cap)*. Contains human serum positive for anti-tTG antibodies. The expected concentration range in EU/ml is printed on the label.
1 x 1.5 ml	Ready to use **Negative Control*** *(white cap)*. Contains human serum.
4 x 1.5 ml	Ready to use **set of 4 Calibrators***; Calibrator A *(green cap)*, Calibrator B *(violet cap)*, Calibrator C *(blue cap)* and Calibrator D *(yellow cap)*. Human serum containing antibodies to hu tTG. Concentrations in EU/ml are printed on the labels.
1 x 12 ml	Ready to use **anti-human Alk. Phos. Conjugate***. Color coded pink.
1 x 60 ml	Ready to use **Serum Diluent***. Color coded blue.
1 x 12 ml	Ready to use **Enzyme Substrate***. Contains pNPP. **Protect from light.**
1 x 12 ml	Ready to use **Stop Solution.**
2 vials	Powder **Wash Buffer**. Reconstitute to one liter each.
2 x	Protocol Sheets

*Contains <0.1% NaN_3

Materials Required But Not Provided

- Deionized or distilled water
- Squeeze bottle to hold diluted wash buffer
- Pipettors capable of delivering 5 µl to 1000 µl
- Disposable pipette tips
- Clean test tubes 12 x 75 mm and test tube rack
- Timer
- Absorbent paper
- Microplate reader capable of reading absorbance values at 405 nm. If dual wavelength microplate reader is available, the reference filter should be set at 600-650 nm.
- Automatic microplate washer capable of dispensing 200 µl

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Other available serological tests to aid in the Diagnosis of CD

ImmuGlo™ anti-endomysial antibody (EMA) IFA	*Code 1114*
ImmuGlo™ anti-reticulin antibody (ARA) IFA	*Code 1115*
ImmuLisa™ anti-gliadin antibody (AGA) IgA ELISA	*Code 1117A*
ImmuLisa™ anti-gliadin antibody (AGA) IgG ELISA	*Code 1117G*
ImmuLisa™ anti-gliadin antibody (AGA) screen ELISA	*Code 1117S*
ImmuLisa™ anti-tTG antibody IgA ELISA	*Code 1157*
ImmuLisa™ anti-tTG antibody IgG ELISA	*Code 1157G*

SPECIMEN COLLECTION AND HANDLING

Only serum specimens should be used in this procedure. Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of the test and should not be used. Store specimens at 2-8°C for no longer than one week. For longer storage, serum specimens should be frozen. Avoid repeated freeze and thaw of samples.

PROCEDURE

Procedural Notes

- Before starting with the assay read carefully the product insert.
- Let serum specimens and test reagents equilibrate at room temperature for at least 30 minutes before starting with the test procedure. Return all unused specimens and reagents to refrigerator immediately after use.
- All dilutions of the patient samples should be prepared prior to starting with the assay.
- Good washing technique is critical. If washing is performed manually, adequate washing is accomplished by directing a forceful stream of wash buffer with a wide tip wash bottle across the entire microplate. **An automated microplate washer is recommended.**
- Use a multichannel pipette capable of delivering 8 wells simultaneously. This speeds the process and provides for a more uniform incubation time.
- For all steps, careful control of timing is important. The start of all incubation periods begins with the completion of reagent addition.
- Addition of all samples and reagents should be performed at the same rate and in the same sequence.
- Remove required microwell strips from the pouch and carefully reseal the pouch to prevent condensation in the unused wells. Return pouch immediately to refrigerator.

Test Method

Step 1 Let all reagents and specimens equilibrate at room temperature.

Step 2 Label protocol sheet to indicate sample placement in the wells. It is good laboratory practice to run samples in duplicate.

Step 3 For a **qualitative determination** use only the Ready to Use Low Calibrator D (*vial with yellow cap*).

or For a **semi-quantitative determination** use the Ready to Use Calibrators A through D as depicted in the sample layout below.

PERFORMANCE CHARACTERISTICS

The utility of the ImmuLisa™ anti-hu tTG IgA Antibody ELISA was determined by comparing the results with:

a) another commercially available anti-hu tTG IgA ELISA method and

b) ImmuGlo™ anti-endomysial antibody immunofluorescence method.

Normal Range: The normal range was established by testing 64 serum samples from apparently healthy donors obtained from the Red Cross. The mean plus three standard deviations of the mean of this normal population was used to determine the cut-off between normal and borderline positive individuals.

Comparative Specificity and Sensitivity

A. Endomysial Antibodies vs. ImmuLisa™ hu tTG IgA Antibody ELISA: A total of 274 samples were tested for antibodies to endomysium using ImmuGlo™ anti-endomysial antibody kit and the results of anti-endomysial antibodies were compared with ImmuLisa anti-hu tTG ELISA method. The results are summarized below:

B. ImmuLisa™ anti-hu tTG IgA ELISA vs. Another Commercial anti-hu tTG IgA ELISA Method: A total of 74 samples were tested on the ImmuLisa™ anti-hu tTG IgA antibody kit and another commercially available anti-hu tTG IgA kits. The results of these studies follow:

ImmuLisa™ anti-hu tTG IgA

ImmuGlo™ EMA	Positive	Negative	Total
Positive	109	3	112
Negative	5	157	162
Total	114	160	274

Relative Agreement: 97%
Relative Sensitivity: 98%
Relative Specificity: 94%

ImmuLisa™ anti-hu tTG IgA

Other ELISA	Positive	Negative	Total
Positive	57	1	58
Negative	1	15	16
Total	58	16	74

Relative Agreement: 97%
Relative Sensitivity: 96%
Relative Specificity: 98%

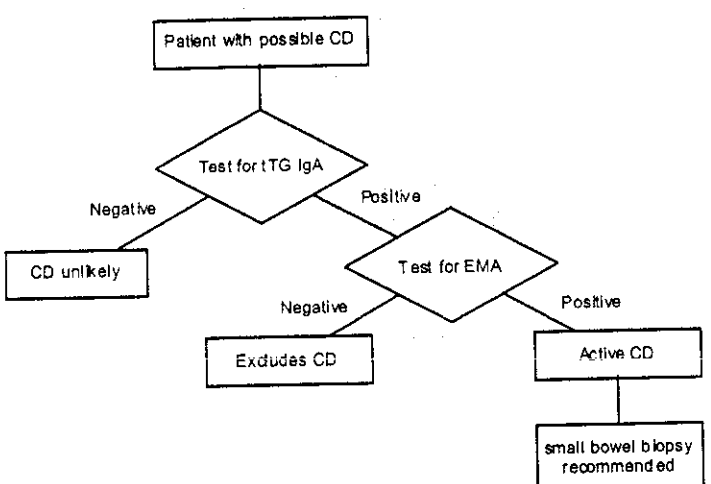
C. Cross Reactivity: A total of 65 disease controls from autoimmune vesiculobullous cases such as pemphigus were tested. None were found positive.

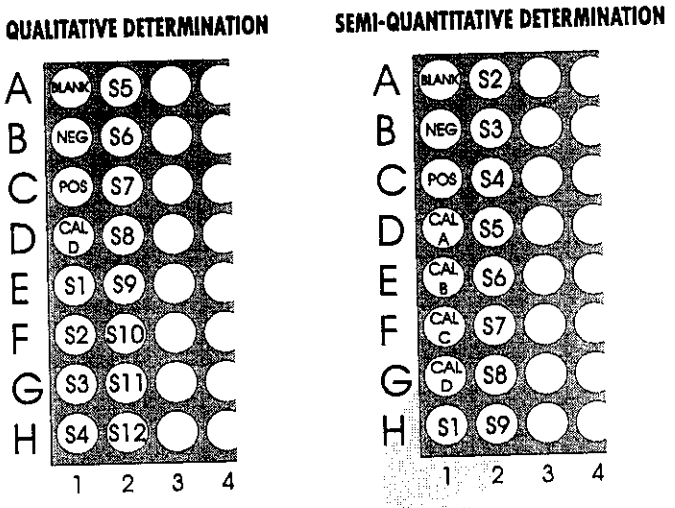
Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

As there are several antibody markers associated with CD, the following algorithm may serve as a guide in the interpretation of various serological markers seen in CD:

EMA	ARA	AGA IgG	AGA IgA	anti-tTG	Interpretation
+	+	+	+	+	CD - certain
+	+	-	+	+	CD - certain
+	+	-	-	+	CD - certain
+	-	+	+	+	CD - certain
+	-	-	-	+	CD - highly probable
-	+	+	+	+	CD - highly probable
-	-	+	+	+	CD - possible
-	-	+	-	-	CD - unlikely but may not be precluded
-	-	-	+	-	CD - unlikely but may not be precluded
-	-	-	-	-	CD - excluded

Some investigators have taken the following approach to establish the diagnosis of CD using serological methods:





Specimen Layout

Step 4 Prepare a **1:51** dilution of the patient samples by mixing **10 µl** of the patient sera with **0.5 ml** of Serum Diluent.

Step 5 Pipette **100 µl** of Ready to Use Calibrators, Positive and Negative controls and diluted patient samples to the appropriate microwells as per protocol sheet.

Note: Include one well which contains **100 µl** of the Serum Diluent as a reagent blank. Zero the ELISA reader against the reagent blank. The absorbance of the reagent blank should not be more than 0.3 when read against air.

Step 6 Incubate **60 minutes** (± 5 min) at room temperature.

Step 7 Wash **4x** with wash buffer. For manual washing, fill each microwell with reconstituted wash buffer. Discard the fluid by inverting and tapping out the contents of each well or by aspirating the liquid from each well. To blot at the end of the last wash, invert strips and tap the wells vigorously on absorbent paper towels. For automatic washers, program the washer as per manufacturer's instructions.

Step 8 Pipette **100 µl** of Conjugate into microwells.

Step 9 Incubate **30 minutes** (± 5 min) at room temperature.

Step 10 Wash all microwells as in Step 7.

Step 11 Pipette **100 µl** of Enzyme Substrate into each microwell in the same order and timing as for the Conjugate.

Step 12 Incubate **microwells 30 minutes** (± 5 min) at room temperature.

Step 13 Pipette **100 µl** of Stop Solution into each microwell using the same order and timing as for the addition of the Enzyme Substrate. Read absorbance within 1 hour of adding Stop Solution.

Step 14 Read absorbance of each microwell at **405 nm** using a single or 405/630nm dual wavelength microplate reader against the reagent blank set at zero absorbance.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request 2014-6998; Released 10/30/14

Quality Control

Calibrators, Positive and Negative Controls and a reagent blank must be run with each assay to verify the integrity and accuracy of the test. The absorbance reading of the reagent blank should be <0.3. Calibrator A should have an absorbance reading >1.0, otherwise the test must be repeated. The negative control must be <20 EU/ml. If the test is run in duplicate, take the mean of the two readings to determine the concentration of anti-hu tTG. While performing qualitative determinations, the optical density of Calibrator D must be greater than that of the negative control and less than the positive control. For semi-quantitative determinations, the positive control must give values in the range stated on the vial. We recommend borderline samples be tested with a fresh sample taken at a later date to ensure accuracy.

RESULTS

Calculations

Concentrations of the patient samples can be determined by either of two methods:

1. QUALITATIVE DETERMINATION

Results obtained by this method should be reported as positive or negative.

$$\frac{\text{Abs. of Test Sample}}{\text{Abs. of Calibrator D}} \times \text{EU/ml of Calibrator D} = \text{EU/ml Test Sample}$$

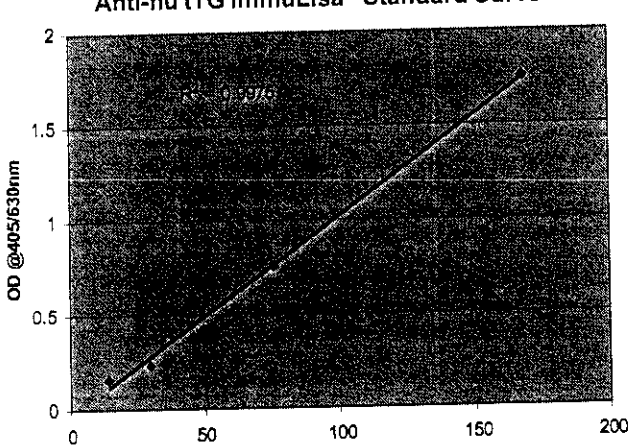
2. SEMI-QUANTITATIVE DETERMINATION

Plot absorbance of Calibrator A through D against their respective concentration on linear-linear graph paper. Plot the concentration in EU/ml on the X-axis against the absorbance on the Y-axis and draw the best fit curve. Determine the concentrations of patient samples on the curve against the corresponding absorbance value.

Calibrator

The ready to use Calibrators are included to provide semi-quantitation and must be used with each run. Patient samples containing higher antibody levels may give absorbance values greater than that of the Calibrator A. For determining accurate semi-quantitative values such serum samples should be further diluted so they fall within the range of the calibrator curve when retested. For determining EU/ml, multiply the units obtained by the dilution factor.

Anti-hu tTG ImmuLisa™ Standard Curve



Interpretation

The following serves only as a guide in the interpretation of the laboratory results. These values were determined by testing 113 adult normal blood donors. The values depicted below are the mean of the normal subjects plus 3SD. Each laboratory must determine its own normal values.

anti-hu tTG value	Interpretation
<20 EU/ml	Negative
20-25 EU/ml	Indeterminate (Borderline)
>25 EU/ml	Positive

LIMITATIONS OF THE PROCEDURE

The ImmuLisa™ anti-hu tTG assay should not be performed on grossly hemolyzed, microbially contaminated or lipemic samples. The method should be used for testing human serum samples only. Results obtained serve only as an aid in the diagnosis and should not be interpreted as diagnostic in themselves. In IgA deficient CD patients the anti-hu tTG IgA ELISA may be negative.

EXPECTED VALUES

The expected values in a normal population are negative (<20 EU/ml for adults and children). However, it has been determined that some apparently healthy, asymptomatic individuals may test positive for IgA anti-tTG antibodies.

The incidence of anti-tTG antibodies has been studied by various investigators and the findings are summarized below:

Incidence of EMA and tTG Antibodies

Study Group	No.	EMA	tTG
Dermatitis herpetiformis	44		
on gluten diet	33	33 (100%)	33 (100%)
on gluten-restricted diet	11	0 (0%)	3 (27%)
Celiac Disease	58		
on gluten diet	34	34 (100%)	31 (91%)
on gluten-restricted diet	24	0 (0%)	1 (4%)
Controls	189	0 (0%)	0 (0%)
Pemphigus	80	0 (0%)	1 (1%)
Linear IgA dermatosis	3	0 (0%)	0 (0%)
Normal	106	0 (0%)	1 (1%)

Kumar V et al. Clin Immunol 2001; 98: 378-382

The incidence and the levels of anti-tTG antibodies is dependent upon the diet status. The levels of these antibodies decrease and eventually will become negative in patients with CD who are on a gluten-free diet. Similarly, the levels of these antibodies will increase and may become positive when patients with CD who were on a gluten-free diet ingest a gluten-containing diet[10,11].

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request 2014-6998; Released 10/30/14

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

DEC 2 4 2003

Mr. Kevin J. Lawson
Director of Regulatory Affairs
IMMCO Diagnostics
60 Pineview Drive
Buffalo, NY 14228

Re: k032571
Trade/Device Name: ImmuLisa Anti-human tissue Transglutaminase (human tTG) IgA ELISA
Regulation Number: 21 CFR 866.5660
Regulation Name: Multiple autoantibodies immunological test system
Regulatory Class: Class II
Product Code: MVM
Dated: November 21, 2003
Received: December 10, 2003

Dear Mr. Lawson:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820).

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request 2014-6998; Released 10/30/14

This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific information about the application of labeling requirements to your device, or questions on the promotion and advertising of your device, please contact the Office of In Vitro Diagnostic Device Evaluation and Safety at (301) 594-3084. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 443-6597 or at its Internet address http://www.fda.gov/cdrh/dsma/dsmamain.html.

Sincerely yours,

Steven Gutman

Steven I. Gutman, M.D., M.B.A.
Director
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and
Radiological Health

Enclosure

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request 2014-6998; Released 10/30/14

INDICATIONS FOR USE STATEMENT

510(k) Number (if known): K 032571

Device Name: ImmuLisa Anti-human tissue Transglutaminase (human tTG) IgA ELISA

Indications For Use: An enzyme linked immunoassay (ELISA) for the detection and semi-quantitation of IgA antibodies to human tissue transglutaminase, as an aid in diagnosing patients with Gluten Sensitive Enteropathy (celiac Disease and Dematitis herpetiformis)

(PLEASE DO NOT WRITE BELOW THIS LINE - CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)

Prescription Use ✓ (Per 21 CFR 801.109) OR Over-The-Counter Use _____ (Optional Format 1-2-96)

Mana M Chan
Division Sign-Off

Office of In Vitro Diagnostic Device Evaluation and Safety

510(k) 032571

3

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request 2014-6998; Released 10/30/14

DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service

Food and Drug Administration
Center for Devices and
Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
9200 Corporate Blvd.
Rockville, Maryland 20850

October 15, 2003

IMMCO DIAGNOSTICS, INC.
60 PINEVIEW DR.
BUFFALO, NY 14228
ATTN: KEVIN LAWSON

510(k) Number: K032571
Product: IMMULISA ANTI-HUMAN TISSUE TRANSGLUTAMINASE

We are holding your above-referenced Premarket Notification (510(k)) for 30 days pending receipt of the additional information that was requested by the Office of Device Evaluation. Please remember that all correspondence concerning your submission MUST cite your 510(k) number and be sent in duplicate to the Document Mail Center (HFZ-401) at the above letterhead address. Correspondence sent to any address other than the one above will not be considered as part of your official premarket notification submission. Also, please note the new Blue Book Memorandum regarding Fax and E-mail Policy entitled, "Fax and E-Mail Communication with Industry about Premarket Files Under Review. Please refer to this guidance for information on current fax and e-mail practices at www.fda.gov/cdrh/ode/a02-01.html.

The deficiencies identified represent the issues that we believe need to be resolved before our review of your 510(k) submission can be successfully completed. In developing the deficiencies, we carefully considered the statutory criteria as defined in Section 513(i) of the Federal Food, Drug, and Cosmetic Act for determining substantial equivalence of your device. We also considered the burden that may be incurred in your attempt to respond to the deficiencies. We believe that we have considered the least burdensome approach to resolving these issues. If, however, you believe that information is being requested that is not relevant to the regulatory decision or that there is a less burdensome way to resolve the issues, you should follow the procedures outlined in the "A Suggested Approach to Resolving Least Burdensome Issues" document. It is available on our Center web page at: http://www.fda.gov/cdrh/modact/leastburdensome.html

If after 30 days the requested information, or a request for an extension of time, is not received, we will discontinue review of your submission and proceed to delete your file from our review system. Pursuant to 21 CFR 20.29, a copy of your 510(k) submission will remain in the Office of Device Evaluation. If you then wish to resubmit this 510(k) notification, a new number will be assigned and your submission will be considered a new premarket notification submission.

Please remember that the Safe Medical Devices Act of 1990 states that you may not place this device into commercial distribution until you receive a decision letter from FDA allowing you to do so.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request 2014-6998; Released 10/30/14

If you have procedural or policy questions, please contact the Division of Small Manufacturers International and Consumer Assistance (DSMICA) at (301) 443-6597 or at their toll-free number (800) 638-2041, or contact me at (301) 594-1190.

Sincerely yours,

Marjorie Shulman
Supervisor Consumer Safety Officer
Premarket Notification Section
Office of Device Evaluation
Center for Devices and
Radiological Health

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request 2014-6998; Released 10/30/14

DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service

Food and Drug Administration
Center for Devices and
Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
9200 Corporate Blvd.
Rockville, Maryland 20850

September 04, 2003

IMMCO DIAGNOSTICS, INC.
60 PINEVIEW DR.
BUFFALO, NY 14228
ATTN: KEVIN LAWSON

510(k) Number: K032571
Received: 03-SEP-2003
Product: IMMULISA ANTI-HUMAN TISSUE TRANSGLUTAMINASE (HU-TTG) ANTIBODY

The Food and Drug Administration (FDA), Center for Devices and Radiological Health (CDRH), has received the Premarket Notification you submitted in accordance with Section 510(k) of the Federal Food, Drug, and Cosmetic Act (Act) for the above referenced product. We have assigned your submission a unique 510(k) number that is cited above. Please refer prominently to this 510(k) number in any future correspondence that relates to this submission. We will notify you when the processing of your premarket notification has been completed or if any additional information is required. YOU MAY NOT PLACE THIS DEVICE INTO COMMERCIAL DISTRIBUTION UNTIL YOU RECEIVE A LETTER FROM FDA ALLOWING YOU TO DO SO.

The Act, as amended by the Medical Device User Fee and Modernization Act of 2002 (MDUFMA)(Public Law 107-250), authorizes FDA to collect user fees for premarket notification submissions. (For more information on MDUFMA, you may refer to our website at http://www.fda.gov/oc/mdufma).

Please remember that all correspondence concerning your submission MUST be sent to the Document Mail Center (DMC)(HFZ-401) at the above letterhead address. Correspondence sent to any address other than the one above will not be considered as part of your official premarket notification submission. Also, please note the new Blue Book Memorandum regarding Fax and E-mail Policy entitled, "Fax and E-Mail Communication with Industry about Premarket Files Under Review". Please refer to this guidance for information on current fax and e-mail practices at www.fda.gov/cdrh/ode/a02-01.html.

You should be familiar with the manual entitled, "Premarket Notification 510(k) Regulatory Requirements for Medical Devices" available from DSMICA. If you have other procedural or policy questions, or want information on how to check on the status of your submission, please contact DSMICA at (301) 443-6597 or its toll-free number (800) 638-2041, or at their Internet address http://www.fda.gov/cdrh/dsmamain.html or me at (301)594-1190.

Sincerely yours,

Marjorie Shulman
Supervisory Consumer Safety Officer
Office of Device Evaluation
Center for Devices and Radiological Health

115

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service

Food and Drug Administration
Center for Devices and
Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
9200 Corporate Blvd.
Rockville, Maryland 20850

August 20, 2003

IMMCO DIAGNOSTICS, INC.
60 PINEVIEW DR.
BUFFALO, NY 14228
ATTN: KEVIN LAWSON

510(k) Number: K032571
Received: 20-AUG-2003
Product: IMMULISA ANTI-HUMAN TRANSGLUTAMINASE
User Fee ID Number: E

The Food and Drug Administration (FDA) Center for Devices and Radiological Heath (CDRH), has received the Premarket Notification you submitted in accordance with Section 510(k) of the Federal Food, Drug, and Cosmetic Act (Act) for the above referenced product. We have assigned your submission a unique 510(k) number that is cited above. Please refer prominently to this 510(k) number in any future correspondence that relates to this submission. YOU MAY NOT PLACE THIS DEVICE INTO COMMERCIAL DISTRIBUTION UNTIL YOU RECEIVE A LETTER FROM FDA ALLOWING YOU TO DO SO.

The Act, as amended by the Medical Device User Fee and Modernization Act of 2002 (MDUFMA) (Public Law 107-250), specifies that a submission shall be considered incomplete and shall not be accepted for filing until fees have been paid (Section 738(f)). Our records indicate that you have not submitted the user fee payment information and therefore your 510(k) cannot be filed and has been placed on hold. The payment information we need in order to begin the review of your 510(k) includes, the user fees cover sheet with the payment ID faxed to the Office of Financial Management at (301) 827-9213 and a check mailed to:

By Regular Mail	By Private Courier (e.g., Fed Ex, UPS, etc.)
Food and Drug Administration P.O. Box 956733 St. Louis, MO 63195-6733.	U.S. Bank 956733 1005 Convention Plaza St. Louis, MO 63101 (314) 418-4983

The check should be made out to the Food and Drug Administration referencing the payment identification number, and a copy of the User Fee Cover sheet should be included with the check. A copy of the Medical Device User Fee Cover Sheet should also be faxed to CDRH at (301) 594-2977 referencing the 510(k) number if you have not already sent it in with your 510(k) submission. After the FDA has been notified of the receipt of your user fee payment, your 510(k) will be filed and the review will begin. If payment has not been received within 30 days, your 510(k) will be deleted from the system. Additional information on user fees and how to submit your user fee payment may be found at http://www.fda.gov/oc/mdufma.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request 2014-6998; Released 10/30/14

Please note that since your 510(k) has not been reviewed, additional information may be required during the review process and the file may be placed on hold once again. If you are unsure as to whether or not you need to file an application with FDA or what type of application to file, you should first telephone the Division of Small Manufacturers, International and Consumer Assistance (DSMICA), for guidance at (301)443-6597 or its toll-fee number (800)638-2041, or contact them at their Internet address http://www.fda.gov/cdrh/dsmamain.html, or you may submit a 513(g) request to the Document Mail Center at the address above. If you have any questions concerning the contents of this letter, you may contact me at (301) 594-1190.

Sincerely yours,

Marjorie Shulman
Consumer Safety Officer
Office of Device Evaluation
Center for Devices and
Radiological Health

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request 2014-6998; Released 10/30/14

K032571

August 14, 2003
Food and Drug Adminstration
Office of Device Evaluation
Center for Devices and Radiological Health
Document Mail Center (HFZ-440)
Attn: Rufina Carlos
9200 Corporate Blvd.
Rockville, MD 20850

FDA/CDRH/ODE/PMO
2003 AUG 20 P 12: 45

Re: ImmuLisa Anti-Human Tissue Transglutaminase (hu-tTG) Antibody IgA ELISA
510(k) submission

Dear Ms. Carlos:

Enclosed you will find two copies of the 510(k) submission for our device listed below:

Submitter:	IMMCO Diagnostics, Inc. 60 Pineview Drive Buffalo, NY 14228
Contact:	Kevin J. Lawson Address same as above (716) 691-0091 ext. 110 kevinlawson@immcodiagnostics.com
Proprietary Name:	ImmuLisa Anti-Human Tissue Transglutaminase (hu- tTG) Antibody IgA ELISA
Classification Name:	SYSTEM, TEST, ANTIBODIES, ANTI-TISSUE TRANSGLUTAMINASE IGA
FDA Registration No.:	1315336
Manufactured by IMMCO:	same registration number
Device Class:	2
Intended Use:	An enzyme linked immunoassay (ELISA) for the detection and semi-quantitation of IgA antibodies to Human Tissue Transglutaminase (hu-tTG), as an aid towards the diagnosis of Celiac Disease

A 510(k) statement is contained within.

Please review this device at your earliest opportunity to enable us to market the device as soon as possible. Thank you for your attention to this matter.

Sincerely,

Kevin Lawson

Kevin Lawson
Director of Regulatory Affairs

SK 30

TM
II

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request 2014-6998; Released 10/30/14

TABLE OF CONTENTS

Truthful and Accurate Statement	3
Compliance with Special Controls or Performance Standards	4
Proposed Labels, Labeling and Advertisement	5
Information and Data Supportive of Substantial Equivalency Claim	6
General Description	6
Principles of Procedures	7
Similarities with Other Commercially Available Devices	8
Differences from Other Commercially Available ELISA Devices	9
Comparison Summary Table	9
Performance Comparison	10
Manufacturing Information	14
Quality Information	16
510(k) Statement	19
Indications for Use Statement	20
Appendix A	21
Appendix B	22
Appendix C	23
Appendix D	24
Appendix E	25
Appendix F	26

119

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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COMPLIANCE WITH SPECIAL CONTROLS
OR PERFORMANCE STANDARDS

To date, no special controls or performance standards which affect this system have been finalized under Section 514 of the Act.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request 2014-6998; Released 10/30/14

PROPOSED LABELS, LABELING AND ADVERTISEMENT

ImmuLisa™ anti-hu tTG (IgA) antibody ELISA 96 Tests Code: 1144

for the detection and semi-quantitative determination of anti-human tissue transglutaminase (tTG) IgA antibodies in human serum; consult PRODUCT INSERT!

para la detección y semi-cuantificación de anticuerpos anti-hu tTG en suero humano; consultar el MANUAL DE INSTRUCCIONES!

zum Nachweis und semiquantitative Bestimmung von anti-hu tTG Antikörpern in Humanserum; ARBEITSANLEITUNG beachten!

pour la recherche et le dosage semi-quantitatif des anticorps anti-hu tTG dans le serum humain; Consultez le MODE D'EMPLOI!

per la individuazione e la determinazione semi-quantitativa di anticorpi anti-hu tTG nel siero umano; consulare il MANUALE!

para pesquisa e semi-quantificacion dos anticorpos anti-hu tTG em soro humano; consulte a BULA!

12 x 8	**96 breakaway microwells**	96 micropocillos separables	Mikroplatte mit 96 Einzelvertiefungen
4 x 1.5 ml	***calibrators A-D, ready to use**	*calibradores A-D, listo para uso	*Kalibratoren A-D, gebrauchsfertig
1 x 1.5 ml	***positive control, ready to use**	*control positivo, listo para uso	*Positive Kontrolle, gebrauchsfertig
1 x 1.5 ml	***negative control, ready to use**	*control negativo, listo para uso	*Negative Kontrolle, gebrauchsfertig
1 x 12 ml	***alk. phos. conjugate**	*conjugado fos. alc.	*Alkalische Phosphatase Konjugat
1 x 60 ml	***serum diluent**	*tampón de dilución para muestras	*Gepuffertes Probendiluent
1 x 12 ml	***enzyme substrate**	*substrato enzimático	*Enzym Substrat
1 x 12 ml	**stop solution**	solución de parada	Stopplösung
2	**wash buffer for 1 litre/vial**	tampón de lavado para 1 litro/vial	Waschpuffer für je 1 Liter
12 x 8	barettes de 96 micropuits individuels	microstrips da 96 pozzetti individuali	96 micropoços partilhad
4 x 1.5 ml	*étalons A-D, prêt à l'emploi	*calibratori A-D, pronto al uso	*calibradores A-D, pronto para uso
1 x 1.5 ml	*contrôle positif, prêt à l'emploi	*controllo positivo, pronto al uso	*controle positivo, pronto para uso
1 x 1.5 ml	*contrôle négatif, prêt à l'emploi	*controllo negativo, pronto al uso	*controle negativo, pronto para uso
1 x 12 ml	*conjugué phos. alc.	*coniugato fos. alc.	*conjugado fos. alc.
1 x 60 ml	*diluant pour échantillons	*diluente per campioni	*diluente de amostras
1 x 12 ml	*substrat enzymatique	*substrato enzimatico	*substrato enzime
1 x 12 ml	solution d'arrêt	soluzione bloccante	solução de parada
2	tampon de lavage pour 1 litre/flacon	tampone di lavaggio per 1 litro/fiala	tampão de lavagem do 1 litro/viale

LO1144-rev0703

*<0.1% NaN_3 **+2-8°C** For *in vitro* diagnostic use

ImmuLisa™ **Code: 1144-1**

in vitro diagnostic use

IMMCO DIAGNOSTICS Buffalo, NY 14228-2120

anti-hu tTG antibody microplate

12 x 8	**96 breakaway microwells**	*consult PRODUCT INSERT!*
12 x 8	**96 micropocillos separables**	*consular el MANUAL DE INSTRUCCIONES!*
12 x 8	**Mikroplatte mit 96 Einzelvertiefungen**	*ARBEITSANLEITUNG beachten!*
12 x 8	**barettes de 96 micropuits individuels**	*consultez le MODE D'EMPLOI!*
12 x 8	**microstrips da 96 pozzetti individuali**	*consulare il MANUALE!*
12 x 8	**96 micropoços partilhad**	*consulte a BULA!*

+2-8°C

Lot/ch.-B XXXXXX

Exp/verw.bis/d.pér./cad/scad XXX XX

LI1144-1-0703

ImmuLisa™ *Consult PRODUCT INSERT* **Code: 2306**

in vitro diagnostic use

serum diluent 60 ml *< 0.1% NaN3*

tampón de dilución para muestras
gepuffertes Probendiluent
diluant pour échantillons
diluente per campioni
diluente de amostras

+2-8°C

Lot/ch.-B: XXXXXX

Exp/verw.bis/d.pér./cad/scad: XXX XX

LI2306-0703

ImmuLisa™ *PROTECT FROM LIGHT* **Code: 2110**
Consult PRODUCT INSERT in vitro diagnostic use

anti-human IgA alk. phos. conjugate - 12 ml
conjugado fos. alc.
alkalische phosphatase
Konjugat
conjugue phos. alc.
coniugato fos. alc.
conjugado fos. alc.

< 0.1% NaN3 **+2-8°C**

Lot/ch.-B: XXXXXX

Exp/verw.bis/d.pér./cad/scad: XXX XX

ImmuLisa™ *PROTECT FROM LIGHT* *Consult PRODUCT INSERT* **Code: 2513**
in vitro Diagnostic Use

enzyme substrate - 12 ml
substrato enzimatico
Enzym Substrat
substrat enzymatique
substrato enzimatico
substrato enzime

< 0.1% NaN3 **+2-8°C**

Lot/ch.-B: XXXXXX

Exp/verw.bis/d.pér./cad/scad: XXX XX

LI2513-0499

ImmuLisa™ *Consult PRODUCT INSERT* **Code: 2310**
in vitro Diagnostic Use

stop solution - 12 ml
solución de parada
Stopplösung
solution d'arrêt
soluzione bloccante
solção de parada

+2-8°C

Lot/ch.-B XXXXXX

Exp/verw.bis/d.pér./cad/scad: XXX XX

LI2310-0499

ImmuLisa™ **Code: 1144-2**
in vitro diagnostic use

anti-hu tTG (IgA) pos ctrl - 1.5 ml
control positivo, listo para uso
Positive Kontrolle, gebrauchsfertig
contrôle positif, prêt à l'emploi
controllo positivo, pronto al uso
controle positivo, pronto para uso
ready for use *XXX EU/ml*

<0.1% NaN3 **+2-8°C** *Lot/ch.-B* XXXXXX

Exp/verw.bis/d.pér./cad/scad: XXX XX

ImmuLisa™ **Code: 2254-1**
in vitro diagnostic use

negative control - 1.5 ml
control negativo, listo para uso
Negative Kontrolle, gebrauchsfertig
contrôle negatif, prêt à l'emploi
controllo negativo, pronto al uso
controle negativo, pronto para uso

<0.1% NaN3 **ready for use** **+2-8°C** *Lot/ch.-B:* XXXXXX

Exp/verw.bis/d.pér./cad/scad: XXX XX

LI2254-1-0801

ImmuLisa™ **Code: 1144-calA**
in vitro diagnostic use

anti-hu tTG (IgA) calib.A - 1.5ml
calibrador A, listo para uso
Kalibrator A, gebrauchsfertig
étalon A, prêt à l'emploi
calibratore A, pronto al uso
calibrador A, pronto para uso

<0.1% NaN3 ready for use *XXX EU/ml* **+2-8°C** *Lot/ch.-B:* XXXXXX

Exp/verw.bis/d.pér./cad/scad: XXX XX

ImmuLisa™ **Code: 1144-calB**
in vitro diagnostic use

anti-hu tTG (IgA) calib.B - 1.5ml
calibrador B, listo para uso
Kalibrator B, gebrauchsfertig
étalon B, prêt à l'emploi
calibratore B, pronto al uso
calibrador B, pronto para uso

<0.1% NaN3 ready for use *XXX EU/ml* **+2-8°C** *Lot/ch.-B:* XXXXXX

Exp/verw.bis/d.pér./cad/scad: XXX XX

ImmuLisa™ **Code: 1144-calC**
in vitro diagnostic use

anti-hu tTG (IgA) calib.C - 1.5ml
calibrador C, listo para uso
Kalibrator C, gebrauchsfertig
étalon C, prêt à l'emploi
calibratore C, pronto al uso
calibrador C, pronto para uso

<0.1% NaN3 ready for use *XXX EU/ml* **+2-8°C** *Lot/ch.-B:* XXXXXX

Exp/verw.bis/d.pér./cad/scad: XXX XX

ImmuLisa™ **Code: 1144-calD**
in vitro diagnostic use

anti-hu tTG (IgA) calib.D - 1.5ml
calibrador D, listo para uso
Kalibrator D, gebrauchsfertig
étalon D, prêt à l'emploi
calibratore D, pronto al uso
calibrador D, pronto para uso

<0.1% NaN3 ready for use *XXX EU/ml* **+2-8°C** *Lot/ch.-B:* XXXXXX

Exp/verw.bis/d.pér./cad/scad: XXX XX

121

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INFORMATION AND DATA SUPPORTIVE
OF SUBSTANTIAL EQUIVALENCY CLAIM

GENERAL DESCRIPTION

Celiac Disease (CD) is an autoimmune gastrointestinal disorder that may occur in genetically susceptible individuals triggered by the ingestion of gluten-containing grains such as wheat, barley and rye. Of the many autoimmune disorders, CD represents one of the few disorders where the etiological agent is known and the disease subsides and goes in remission once the etiological agent is withdrawn from the diet.

CD is characterized by malabsorbtion resulting from inflammatory injury to the small intestinal mucosa and, when prolonged, can cause malnutrition. The classical symptoms of CD include diarrhea, weight loss and malnutrition.

Only a small percentage of patients with CD, however, presents with classical symptoms. Consequently, the clinical spectrum of CD has grown much broader than in the past to include patients that do not present with classical symptoms. It is not uncommon for the initial symptoms to be non-gastrointestinal or for gastrointestinal symptoms, if present, to be mild or intermittent.

Some of the common non-gastrointestinal manifestations include short stature, iron and folate deficiency, anemia, bone loss, aphthous stomatitis, arthralgia, dental enamel defects, etc. The need to examine a wider range of clinical presentation has led to greater numbers of individuals diagnosed with CD later in life than ever before. Adults may present with iron deficiency, macrocytic anemia and hypocalcaemia.

Studies have found the prevalence of CD to be highly variable from population to population (Guandalini S, Gupta P. Celiac disease- A diagnostic challenge. *Clin Appl Immun Rev* 2002;2:293-305). The true prevalence has been difficult to ascertain. The disparate criteria used in the diagnosis of CD are often the cause. If only the clinical criteria are used in determining prevalence, the incidence of CD is much lower as compared with incidence established by serological methods (Guandalini S, Gupta P. Celiac disease- A diagnostic challenge. *Clin Appl Immun Rev* 2002;2:293-305; Fasano A, et al. Prevalence of celiac disease in at risk and not-at-risk groups in the United States 2223; 163:286-292). Using serological methods the incidence of CD in the general population is approximately one in 200.

Diagnosis of CD based on clinical criteria thus can be misleading and may lead to serious delays in proper diagnosis. Frequently, delays in diagnosis extend 10-13 years from the first clinical presentation of symptoms.

Failure to diagnose CD early on may predispose an individual to long-term complications such as splenic atrophy and intestinal lymphoma. The incidence of lymphoma arising in the context of CD is difficult to ascertain. One study has shown incidence of lymphoma involving the gastrointestinal (GI) tract in patients with CD to range from 3.6 percent to 40 percent (. Wolber R, et al. Lymphocytic gastritis in patients with celiac sprue or spruelike intestinal disease. *Gastroenterology* 1990;98:310-315). In another recent study, CD is associated with significantly elevated risk for intestinal lymphoma, especially for non-Hodgkin's (Catassi, et al. Risk of non-Hodgkin lymphoma in celiac disease. *JAMA* 2002;287:1413-1419). A gluten-free diet (GFD) normalizes the mucosa and helps reduce the malignant potential.

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Histological examination of the small intestinal biopsy remains the gold standard for diagnosing CD, but it has its own limitations. Of these include some patients with latent or even active CD that may have normal histopathology (Arranz E, Ferguson A. Intestinal antibody pattern of celiac disease: Occurrence in patients with normal jejunal biopsy histology. *Gastroenterology* 1993;104:1263-72).

The revised European Society of Paediatric Gastroenterology and Nutrition (ESPGAN) criteria include only a single biopsy with clear-cut remission of clinical symptoms on GFD (Hill ID, et al. Celiac disease: Working group report of the first world congress of pediatric gastroenterology, hepatology, and nutrition. *J. Pediatr Gastroenterol Nutr* 2002; 35:S78-S88). Positive serology at the time of diagnosis with disappearance on GFD contributes to the diagnosis. The various serological tests employed in the work-up of patients suspected to have CD include anti-gliadin antibody (AGA), anti-endomysial antibody (EMA), anti-reticulin antibody (ARA) and anti-tissue transglutaminase (tTG) antibody tests. Antibodies to gliadin and tTG are detected by ELISA, whereas endomysium and reticulin antibodies are detected by indirect immunofluorescence. EMA are very specific indicators of CD. However the EMA test is an immuno-histochemical method and requires experience in reading immunofluorescence reactions. Rosario et al (*J Pediatr Gastroenterol Nutr* 1998;27:191-195) concludes that "EmA-IgA is 100 percent sensitive and specific in active, untreated IgA-sufficient CD patients when performed by an established laboratory."

Since identification of tTG as the endomysial antigen, ELISA methods have been described for detecting antibodies in the sera of patients with CD. The advantage of the anti-tTG antibody assay is that it is automatable and less subjective than EMA. For this reason, many laboratories have opted to use the tTG antibody method as the screening method. In these laboratories, it may be the only assay used for detection of CD cases. In various studies on the efficacy of the tTG antibody method for screening for CD, the specificity and sensitivity of this method has been found to range from 90 percent to 95 percent (Vitoria JC, et al. Antibodies to gliadin, endomysium, and tissue transglutaminase for the diagnosis of celiac disease. *J Pediatr Gastroenterol Nutr* 1999;29:571-4). Human tTG has been described to improve the sensitivity of the tTG antibody assay for CD.

PRINCIPLES OF PROCEDURES

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123

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SIMILARITIES WITH OTHER COMMERCIALLY AVAILABLE DEVICES

The ImmuLisa anti-human tTG antibody IgA ELISA test kit is substantially equivalent in principle and methodology and performance to the currently marketed IMMCO ImmuLisa anti-tTG antibody IgA ELISA antibody test kit (K992878) and INOVA anti-tTG antibody kit (K011566) for the detection of IgA antibodies to tissue Transglutaminase. Copies of the package inserts for each assay have been included in the appendix of this submission. In addition, the ImmuLisa hu tTG antibody kit is also equivalent to the Immuflour EMA test kit (K912551)

Similarities among the IMMCO ImmuLisa anti hu tTG antibody IgA ELISA, the ImmuLisa anti-tTG antibody ELISA and the INOVA anti-tTG antibody IgA ELISA kits are listed below:

- All three kits are enzyme linked immunosorbant assays (ELISA) based assays.
- All three kits detect IgA autoantibodies to tTG in human sera
- All three kits allow semiquantitation of antibodies to tTG.
- All three kits follow the same basic procedure for testing antibodies in patient sera.
- All three kits provide pre-diluted controls.
- All three kits use enzyme substrate that changes color when hydrolyzed by the enzyme to a color product. The color change is directly proportional to the concentration of the antibody in patient's serum and hence is the basis for quantitation.
- All three kits use stop solution to stop the enzymatic reaction and further color change.
- All kits are recommended to be stored at 2-8°C.
- All three kits have very similar labeling content.

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DIFFERENCES FROM OTHER COMMERCIALLY AVAILABLE ELISA DEVICES

- The IMMCO kit has a set of four calibrators and a positive and negative control, while the INOVA kit has high positive, low positive and negative controls, but no calibrators.
- The enzyme conjugate in the IMMCO kit is alkaline phosphatase labeled, while the INOVA kit conjugate is horseradish peroxidase labeled.
- The wash buffer in the IMMCO kits is powder to be reconstituted to one liter in water, while INOVA provides a 40x liquid concentrate to be diluted before use.

COMPARISON SUMMARY TABLE

	IMMCO anti-tTG	IMMCO anti-human tTG	INOVA anti-human tTG
Methodology	ELISA	ELISA	ELISA
Detection of antibodies	tTG	tTG	tTG
Screening dilution	1:51	1:51	1:101
Quantitation	Semi	Semi	Semi
Conjugate	alkaline phosphatase	alkaline phosphatase	horseradish peroxidase
Conjugate specificity	IgA	IgA	IgA
Substrate	p-NPP	p-NPP	TMB
Positive control	yes	yes	yes
Negative control	yes	yes	yes
Calibrators	yes	yes	no
Serum diluent	yes	yes	yes
Wash buffer	yes	yes	yes
Stop solution	yes	yes	yes
Absorbance	405nm	405nm	450nm
Storage	2-8°C	2-8°C	2-8°C

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PERFORMANCE COMPARISON

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126

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Precision Study:

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MANUFACTURING INFORMATION

Information provided following FDA guidelines for calibration and quality control labeling for in vitro diagnostic use devices.

The IMMCO anti-human tissue Transglutaminase antibody assay is manufactured according to FDA QSR guidelines and in accordance with its registered ISO9000 quality system.

As new lots of calibrators are developed, studies are performed to calibrate values against original calibrators. Each lot of calibrator is also tested using normal human sera, clinical samples and internal standards as in the initial Cutoff Study described in **Performance Comparison: Controls and Calibrators**, above.

QUALITY INFORMATION

Information provided following FDA guidelines for calibration and quality control labeling for in vitro diagnostic use devices.

Quality control studies are run on new microplate lots. Inter- and intra-plate CV is determined selecting plates from the beginning, middle and end of production and accepted or rejected according to manufacturing acceptance criteria. New lots of calibrators and conjugate are subjected to additional quality control and tested in conjunction with each plate lot. Each calibrator vial is labeled with the EU value of its contents based on these studies.

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INTERPRETATION OF RESULTS

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QUALITY CONTROL IN PERFORMING TEST

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LIMITATION OF PROCEDURE

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[redacted]

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EXPECTED VALUES

The expected values in a normal population are negative (<20 EU/ml). However, it has been determined that some apparently healthy, asymptomatic individuals may test positive for IgA anti-human tTG antibodies. It has been suggested that all anti-tTG antibody positive samples need to be confirmed by the immunofluorescence EMA test (Fasano A, et al. Prevalence of celiac disease in at risk and not-at-risk groups in the United States 2003; 163:286-292).

The incidence of anti-tTG antibodies has been studied by various investigators and the findings of the specificity and sensitivities of the various studies are summarized below:

Reported sensitivities and specificities of various IgA t-TG antibody assays

Method	Sensitivity (%)	Specificity (%)
Guinae pig tTG		
Dieterich et al. (1998)	98	95
Sulkanen et al (1998)	95	94
Lampasona et al (1998)	84	92
Bazzigaluppi et al. (1999)	85	91
Troncone et al (1999)	92	98
Biagi et al (1999)	95	90
Lock et al (1999)	85	97
Piaggio et al (1999)	100	100
Amin et al (1999)	87	97
Miller et al (1999)	100	97
Koop et al (2000)	90	76
Sblattero et al (2000)	84	98
Hansson et al (2000)	95	96
Human erythrocyte tTG		
Bazzigaluppi et al (1999)	97	96
Sardy et al (1999)	98	98
Sblattero et al (2000)	92	99
Reombinant tTG		
Lampassona et al (1998)	98	97
Seissler et al (1999)	96	100

Adapted from Leon F et al Scand J. Gastroenterol 2001;36:849-53.

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The incidence and the levels of anti-tTG antibodies are dependent upon the diet status. The levels of these antibodies decrease and eventually will become negative in patients with CD who are on a gluten-free diet. Similarly, the levels of these antibodies will increase and may become positive when patients with CD who were on a gluten-free diet ingest a gluten-containing diet (Sulkanen S, Haltunen T, Laurila K, et al. Tissue Transglutaminase autoantibody enzyme-linked immunosorbent assay in detecting celiac disease. Gastroenterology; 1998, 115: 1322-1328. Dietrich W, Laag E, Schopper H, et al. Autoantibodies to tissue transglutaminase as predictors of celiac disease; Gastroenterology 1998, 115: 1317-1321.).

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INDICATIONS FOR USE STATEMENT

510(k) Number (if known): K032571

Device Name: ImmuLisa Anti-human tissue Transglutaminase (human tTG) IgA ELISA

Indications For Use: An enzyme linked immunoassay (ELISA) for the detection and semi-quantitation of IgA antibodies to human tissue transglutaminase, as an aid in diagnosing patients with Gluten Sensitive Enteropathy (celiac Disease and Dematitis herpetiformis)

(PLEASE DO NOT WRITE BELOW THIS LINE - CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)

Prescription Use ✓ (Per 21 CFR 801.109) OR Over-The-Counter Use _____ (Optional Format 1-2-96)

Division Sign-Off

Office of In Vitro Diagnostic Device Evaluation and Safety

510(k)_____

135

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APPENDIX A

IMMCO Anti-hu tTG Antibody ELISA Product Insert

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ImmuLisa™

Anti-human Tissue Transglutaminase Antibody (hu tTG) ELISA

For *in vitro* diagnostic use
CLIA Complexity: High
CDC Analyte Identification Code: 0546
CDC Test System Identification Code: 28569

Code 1144 **PRODUCT INSERT** 96 Determinations

INTENDED USE

An enzyme linked immunosorbent assay (ELISA) for the detection and semi-quantitation of anti-human Tissue Transglutaminase IgA antibodies in human serum to aid in the diagnosis of patients with celiac disease and dermatitis herpetiformis.

SUMMARY AND EXPLANATION

Celiac Disease (CD) is an autoimmune gastrointestinal disorder that may occur in genetically susceptible individuals triggered by the ingestion of gluten-containing grains such as wheat, barley and rye. Of the many autoimmune disorders, CD represents one of the few where the etiological agent is known and the disease subsides and goes in remission once the etiological agent is withdrawn from the diet.

CD is characterized by malabsorbtion resulting from inflammatory injury to the small intestinal mucosa and, when prolonged, can cause malnutrition. The classical symptoms of CD include diarrhea, weight loss and malnutrition.

Only a small percentage of patients with CD presents with classical symptoms. Consequently, the clinical spectrum of CD has grown much broader than in the past to include patients that do not present with classical symptoms. It is not uncommon for the initial symptoms to be non-gastrointestinal or for gastrointestinal symptoms, if present, to be mild or intermittent.

Some common non-gastrointestinal manifestations include short stature, iron and folate deficiency, anemia, bone loss, aphthous stomatitis, arthralgia, dental enamel defects, etc. The need to examine a wider range of clinical presentation has led to greater numbers of individuals diagnosed with CD later in life than ever before. Adults may present with iron deficiency, macrocytic anemia and hypocalcaemia.

Studies have found the prevalence of CD to be highly variable from population to population.[1] The true prevalence has been difficult to ascertain. Disparate criteria used in diagnosis of CD are often the cause. If clinical criteria alone are used in determining prevalence, the incidence of CD is much lower as compared with incidence established by serological methods.[1,2] Using serological methods, the incidence of CD in the general population is approximately one in 200.

Diagnosis of CD based on clinical criteria thus can be misleading and may lead to serious delays in proper diagnosis. Frequently, delays in diagnosis extend 10-13 years from the first clinical presentation of symptoms.

Failure to diagnose CD early on may predispose an individual to long-term complications such as splenic atrophy and intestinal lymphoma. The incidence of lymphoma arising in the context of CD is difficult to ascertain. One study has shown incidence of lymphoma involving the gastrointestinal (GI) tract in patients with CD to range from 3.6 percent to 40 percent.[3] In another recent study,[4] CD is associated with significantly elevated risk for intestinal lymphoma, especially for non-Hodgkin's lymphoma, even after

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(GFD) normalizes the mucosa and helps reduce the malignant potential.

Histological examination of the small intestinal biopsy remains the gold standard for diagnosing CD, but it has its own limitations. These include some patients with latent or even active CD that may have normal histopathology.[6]

The revised European Society of Paediatric Gastroenterology and Nutrition (ESPGHAN) criteria include only a single biopsy with clear-cut remission of clinical symptoms on GFD[7]. Positive serology at the time of diagnosis with disappearance on GFD contributes to the diagnosis. The various serological tests employed in the work-up of patients suspected to have CD include anti-gliadin antibody (AGA), anti-endomysial antibody (EMA), anti-reticulin antibody (ARA) and anti-tissue transglutaminase (tTG) antibody tests. Antibodies to gliadin and tTG are detected by ELISA, whereas endomysium and reticulin antibodies are detected by indirect immunofluorescence. EMA are very specific indicators of CD. However, the EMA test is an immuno-histochemical method that requires experience in reading immunofluorescence reactions. Rosario et al[8] concludes that "EmA-IgA is 100 percent sensitive and specific in active, untreated IgA-sufficient CD patients when performed by an established laboratory."

Since identification of tTG as the endomysial antigen, ELISA methods have been described for detecting antibodies in the sera of patients with CD. The advantage of the anti-tTG antibody assay is that it is automatable and less subjective than EMA. For this reason, many laboratories have opted to use the tTG antibody method as the screening method. In these laboratories, it may be the only assay used for detection of CD cases. In various studies on the efficacy of the tTG antibody method for screening for CD, the specificity and sensitivity of this method has been found to range from 90 percent to 95 percent.[9] Human tTG has been described to improve the sensitivity of the tTG antibody assay for CD.

PRINCIPLES OF PROCEDURES

The anti-hu tTG antibody test is performed as a solid phase immunoassay (ELISA). Microwells are coated with recombinant hu tTG antigen followed by blocking the unreacted sites to reduce nonspecific binding. Controls, calibrators and patient serum samples are incubated in the antigen coated wells which allows anti-tTG antibodies present in the serum to bind. Unbound antibody and other serum proteins are removed by washing the microwells. Antibodies bound to the microwells are detected by adding enzyme labeled anti-human IgA conjugates to the wells. These enzyme conjugated antibodies bind specifically to the human immunoglobulin of the appropriate class. Unbound enzyme conjugate is removed by washing. Specific enzyme substrate (pNPP) is then added to the wells and the presence of antibodies to hu tTG is detected by a color change produced by the conversion of the pNPP substrate. The reaction is stopped and the intensity of color change, which is proportional to the concentration of antibody, is read by a spectrophotometer at 405 nm. Results are expressed in ELISA units per milliliter (EU/ml).

REAGENTS

Storage and Preparation

Store all reagents at 2-8°C. **Do not freeze.** Do not use if reagent is not clear or if a precipitate is present. All reagents must be brought to room temperature (20-25°C) prior to use. When stored at 2-8°C, the reconstituted wash buffer is stable until the kit expiration date. Reconstitute the wash buffer to 1 liter with distilled or deionized water. Do not use tap water for reagents or buffers.

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120

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Precautions

For *in vitro* diagnostic use. All human derived components used have been tested for HBsAg, HCV, HIV-1 and 2 and HTLV-I and found negative by FDA licensed tests. However, human blood derivatives and patient specimens should always be considered potentially infectious. Follow good laboratory practices in storing, dispensing and disposing of these materials[10].

WARNING - Sodium azide (NaN_3) may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal of liquids, flush with large volumes of water to prevent azide buildup. Sodium azide may be toxic if ingested. If ingested, report incident immediately to laboratory director or poison control center.

Instructions should be followed exactly as they appear in this kit insert to ensure valid results. Do not interchange kit components with those from other sources other than the same catalog number from IMMCO Diagnostics. Follow good laboratory practices to minimize microbial and cross contamination of reagents when handling. Do not use beyond expiration date on the label.

Materials Provided

Kit contains sufficient reagents to perform 96 determinations each.

12 x 8	Ready to use **Microplate** with individual breakaway microwells coated with hu tTG antigen.
1 x 1.5 ml	Ready to use **Positive Control*** *(red cap)*. Contains human serum positive for anti-tTG antibodies. The expected concentration range in EU/ml is printed on the label.
1 x 1.5 ml	Ready to use **Negative Control*** *(white cap)*. Contains human serum.
4 x 1.5 ml	Ready to use **set of 4 Calibrators***; Calibrator A *(green cap)*, Calibrator B *(violet cap)*, Calibrator C *(blue cap)* and Calibrator D *(yellow cap)*. Human serum containing antibodies to hu tTG. Concentrations in EU/ml are printed on the labels.
1 x 12 ml	Ready to use **anti-human Alk. Phos. Conjugate***. Color coded pink.
1 x 60 ml	Ready to use **Serum Diluent***. Color coded blue.
1 x 12 ml	Ready to use **Enzyme Substrate***. Contains pNPP. **Protect from light.**
1 x 12 ml	Ready to use **Stop Solution**.
2 vials	Powder **Wash Buffer**. Reconstitute to one liter each.
1 x extra	Frame Holder
2 x	Protocol Sheets

*Contains <0.1% NaN_3

Materials Required But Not Provided

- Deionized or distilled water
- Squeeze bottle to hold diluted wash buffer
- Pipettors capable of delivering 5 μl to 1000 μl
- Disposable pipette tips
- Clean test tubes 12 x 75 mm and test tube rack
- Timer
- Absorbent paper
- Microplate reader capable of reading absorbance values at 405 nm. If dual wavelength microplate reader is available, the reference filter should be set at 600-650 nm.
- Automatic microplate washer capable of dispensing 200 µl

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139

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Other available serological tests to aid in the Diagnosis of CD

ImmuGlo™ anti-endomysial antibody (EMA) IFA	*Code 1114*
ImmuGlo™ anti-reticulin antibody (ARA) IFA	*Code 1115*
ImmuLisa™ anti-gliadin antibody (AGA) IgA ELISA	*Code 1117A*
ImmuLisa™ anti-gliadin antibody (AGA) IgG ELISA	*Code 1117G*
ImmuLisa™ anti-gliadin antibody (AGA) screen ELISA	*Code 1117S*
ImmuLisa™ anti-tTG antibody IgA ELISA	*Code 1157*
ImmuLisa™ anti-tTG antibody IgG ELISA	*Code 1157G*

SPECIMEN COLLECTION AND HANDLING

Only serum specimens should be used in this procedure. Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of the test and should not be used. Store specimens at 2-8°C for no longer than one week. For longer storage, serum specimens should be frozen. Avoid repeated freeze and thaw of samples.

PROCEDURE

Procedural Notes

- Before starting with the assay read carefully the product insert.
- Let serum specimens and test reagents equilibrate at room temperature for at least 30 minutes before starting with the test procedure. Return all unused specimens and reagents to refrigerator immediately after use.
- All dilutions of the patient samples should be prepared prior to starting with the assay.
- Good washing technique is critical. If washing is performed manually, adequate washing is accomplished by directing a forceful stream of wash buffer with a wide tip wash bottle across the entire microplate. **An automated microplate washer is recommended.**
- Use a multichannel pipette capable of delivering 8 wells simultaneously. This speeds the process and provides for a more uniform incubation time.
- For all steps, careful control of timing is important. The start of all incubation periods begins with the completion of reagent addition.
- Addition of all samples and reagents should be performed at the same rate and in the same sequence.
- Remove required microwell strips from the pouch and carefully reseal the pouch to prevent condensation in the unused wells. Return pouch immediately to refrigerator.

Test Method

Step 1 Let all reagents and specimens equilibrate at room temperature.

Step 2 Label protocol sheet to indicate sample placement in the wells. It is good laboratory practice to run samples in duplicate.

Step 3 For a **qualitative determination** use only the Ready to Use Low Calibrator D (*vial with yellow cap*).

or For a **semi-quantitative determination** use the Ready to Use Calibrators A through D as depicted in the sample layout below.

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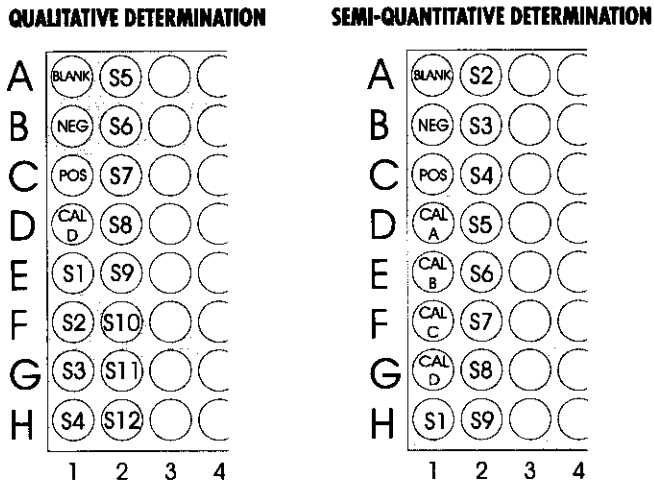

Specimen Layout

Step 4 Prepare a **1:51** dilution of the patient samples by mixing **10 µl** of the patient sera with **0.5 ml** of Serum Diluent.

Step 5 Pipette **100 µl** of Ready to Use Calibrators, Positive and Negative controls and diluted patient samples to the appropriate microwells as per protocol sheet.

Note: Include one well which contains **100 µl** of the Serum Diluent as a reagent blank. Zero the ELISA reader against the reagent blank. The absorbance of the reagent blank should not be more than 0.3 when read against air.

Step 6 Incubate **60 minutes** (± 5 min) at room temperature.

Step 7 Wash **4x** with wash buffer. For manual washing, fill each microwell with reconstituted wash buffer. Discard the fluid by inverting and tapping out the contents of each well or by aspirating the liquid from each well. To blot at the end of the last wash, invert strips and tap the wells vigorously on absorbent paper towels. For automatic washers, program the washer as per manufacturer's instructions.

Step 8 Pipette **100 µl** of Conjugate into microwells.

Step 9 Incubate **30 minutes** (± 5 min) at room temperature.

Step 10 Wash all microwells as in Step 7.

Step 11 Pipette **100 µl** of Enzyme Substrate into each microwell in the same order and timing as for the Conjugate.

Step 12 Incubate **microwells 30 minutes** (± 5 min) at room temperature.

Step 13 Pipette **100 µl** of Stop Solution into each microwell using the same order and timing as for the addition of the Enzyme Substrate. Read absorbance within 1 hour of adding Stop Solution.

Step 14 Read absorbance of each microwell at **405 nm** using a single or 405/630nm dual wavelength microplate reader against the reagent blank set at zero absorbance.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Quality Control

Calibrators, Positive and Negative Controls and a reagent blank must be run with each assay to verify the integrity and accuracy of the test. The absorbance reading of the reagent blank should be <0.3. Calibrator A should have an absorbance reading ≥1.0, otherwise the test must be repeated. The negative control must be <20 EU/ml. If the test is run in duplicate, take the mean of the two readings to determine the concentration of anti-hu tTG. While performing qualitative determinations, the optical density of Calibrator D must be greater than that of the negative control and less than the positive control. For semi-quantitative determinations, the positive control must give values in the range stated on the vial. We recommend borderline samples be tested with a fresh sample taken at a later date to ensure accuracy.

RESULTS

Calculations

Concentrations of the patient samples can be determined by either of two methods:

1. QUALITATIVE DETERMINATION

Results obtained by this method should be reported as positive or negative.

$$\frac{\text{Abs. of Test Sample}}{\text{Abs. of Calibrator D}} \times \text{EU/ml of Calibrator D} = \text{EU/ml Test Sample}$$

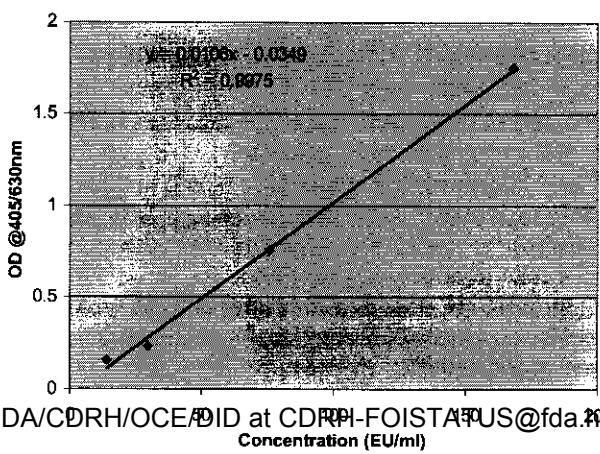
2. SEMI-QUANTITATIVE DETERMINATION

Plot absorbance of Calibrator A through D against their respective concentration on linear-linear graph paper. Plot the concentration in EU/ml on the X-axis against the absorbance on the Y-axis and draw the best fit curve. Determine the concentrations of patient samples on the curve against the corresponding absorbance value.

Calibrator

The ready to use Calibrators are included to provide semi-quantitation and must be used with each run. Patient samples containing higher antibody levels may give absorbance values greater than that of the Calibrator A. For determining accurate semi-quantitative values such serum samples should be further diluted so they fall within the range of the calibrator curve when retested. For determining EU/ml, multiply the units obtained by the dilution factor.

Anti-hu tTG ImmuLisa™ Standard Curve



Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

142

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Interpretation

The following serves only as a guide in the interpretation of the laboratory results. These values were determined by testing 113 adult normal blood donors. The values depicted below are the mean of the normal subjects plus 3SD. Each laboratory must determine its own normal values.

anti-hu tTG value	Interpretation
<20 EU/ml	Negative
20-25 EU/ml	Indeterminate (Borderline)
>25 EU/ml	Positive

LIMITATIONS OF THE PROCEDURE

The ImmuLisa™ anti-hu tTG assay should not be performed on grossly hemolyzed, microbially contaminated or lipemic samples. The method should be used for testing human serum samples only. Results obtained serve only as an aid in the diagnosis and should not be interpreted as diagnostic in themselves. In IgA deficient CD patients the anti-hu tTG IgA ELISA may be negative.

EXPECTED VALUES

The expected values in a normal population are negative (<20 EU/ml for adults and children). However, it has been determined that some apparently healthy, asymptomatic individuals may test positive for IgA anti-tTG antibodies.

The incidence of anti-tTG antibodies has been studied by various investigators and the findings are summarized below:

Incidence of EMA and tTG Antibodies

Study Group	No.	EMA	tTG
Dermatitis herpetiformis	44		
on gluten diet	33	33 (100%)	33 (100%)
on gluten-restricted diet	11	0 (0%)	3 (27%)
Celiac Disease	58		
on gluten diet	34	34 (100%)	31 (91%)
on gluten-restricted diet	24	0 (0%)	1 (4%)
Controls	189	0 (0%)	0 (0%)
Pemphigus	80	0 (0%)	1 (1%)
Linear IgA dermatosis	3	0 (0%)	0 (0%)
Normal	106	0 (0%)	1 (1%)

Kumar V et al; Clin Immunol 2001; 98: 378-382

The incidence and the levels of anti-tTG antibodies is dependent upon the diet status. The levels of these antibodies decrease and eventually will become negative in patients with CD who are on a gluten-free diet. Similarly, the levels of these antibodies will increase and may become positive when patients with CD who were on a gluten-free diet ingest a gluten-containing diet[11,12].

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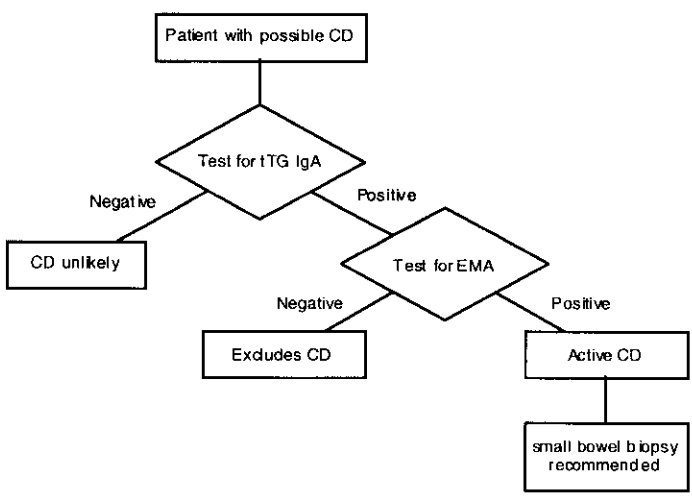
143

As there are several antibody markers associated with CD, the following algorithm may serve as a guide in the interpretation of various serological markers seen in CD:

EMA	ARA	AGA IgG	AGA IgA	anti-tTG	Interpretation
+	+	+	+	+	CD - certain
+	+	-	+	+	CD - certain
+	+	-	-	+	CD - certain
+	-	+	+	+	CD - certain
+	-	-	-	+	CD - highly probable
-	+	+	+	+	CD - highly probable
-	-	+	+	+	CD- possible
-	-	+	-	-	CD - unlikely but may not be precluded
-	-	-	+	-	
-	-	-	-	-	CD - excluded

Some investigators have taken the following approach to establish the diagnosis of CD using serological methods:



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PERFORMANCE CHARACTERISTICS

The utility of the ImmuLisa™ anti-hu tTG IgA Antibody ELISA was determined by comparing the results with:

a) another commercially available anti-hu tTG IgA ELISA method and

b) ImmuGlo™ anti-endomysial antibody immunofluorescence method.

Normal Range: The normal range was established by testing 64 serum samples from apparently healthy donors obtained from the Red Cross. The mean plus three standard deviations of the mean of this normal population was used to determine the cut-off between normal andborderline positive individuals.

Comparative Specificity and Sensitivity

A. Endomysial Antibodies vs. ImmuLisa™ hu tTG IgA Antibody ELISA: A total of 274 samples were tested for antibodies to endomysium using ImmuGlo™ anti-endomysial antibody kit and the results of anti-endomysial antibodies were compared with ImmuLisa anti-hu tTG ELISA method. The results are summarized below:

B. ImmuLisa™ anti-hu tTG IgA ELISA vs. Another Commercial anti-hu tTG IgA ELISA Method: A total of 74 samples were tested on the ImmuLisa™ anti-hu tTG IgA antibody kit and another commercially available anti-hu tTG IgA kits. The results of these studies follow:

ImmuLisa™ anti-hu tTG IgA

ImmuGlo™ EMA	Positive	Negative	Total
Positive	109	3	112
Negative	5	157	162
Total	114	160	274

Relative Agreement: 97%
Relative Sensitivity: 98%
Relative Specificity: 94%

ImmuLisa™ anti-hu tTG IgA

Other ELISA	Positive	Negative	Total
Positive	57	1	58
Negative	1	15	16
Total	58	16	74

Relative Agreement: 97%
Relative Sensitivity: 96%
Relative Specificity: 98%

C. Cross Reactivity: A total of 65 disease controls from autoimmune vesiculobullous cases such as pemphigus were tested. None were found positive.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Precision:

Based on 10 replicates, the intra-assay and inter-assay Coefficient of Variation (CV) of the IgA anti-hu tTG ELISA test were calculated.

	inter-assay CV	intra-assay CV
High (199 EU/ml)	6.2%	2.9%
Medium (85 EU/ml)	8.4%	6.5%
Low (41 EU/ml)	15.6%	9.6%

Recovery:

Samples with known anti-hu tTG concentrations were mixed with appropriate dilutions of another positive sample with known amounts of anti-tTG. Anti-hu tTG antibody levels of the mixed samples were determined and from the values obtained the percent recovery calculated. The results are as follows:

	anti-hu tTG Ab. conc. added (EU/ml)	anti-hu tTG Ab. conc. obtained (EU/ml)	% Recovery
Sample 1	107.5	112	104.2
Sample 2	86	94.6	110
Sample 3	64	70.9	109.9

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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REFERENCES

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2. Fasano A, et al. Prevalence of celiac disease in at risk and not-at-risk groups in the United States 2003; 163:286-292.
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11. Sulkanen S, Haltunen T, Laurila K, et al. Tissue Transglutaminase autoantibody enzyme-linked immunosorbent assay in detecting celiac disease. Gastroenterology; 1998, 115:1322-1328.
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ImmuLisa™ Procedure at a Glance

Prepare Dilutions of Specimens

Pipette 100 µL of Specimens, Calibrators and Controls into microwells

Incubate 60 min. at Room Temperature

Wash Microplate 4x

Pipette 100 µL of Conjugate into microwells

Incubate 30 min. at Room Temperature

Wash Microplate 4x

Pipette 100 µL of Enzyme Substrate into microwells

Incubate 30 min. at Room Temperature

Pipette 100 µL of Stop Solution into microwells

Read absorbance at 405 nm

For technical assistance please contact:

IMMCO Diagnostics, Inc.
60 Pineview Drive
Buffalo, NY 14228-2120
Telephone: (716) 691-0091
Fax: (716) 691-0466
Toll Free USA/Canada: 1-800-537-TEST
E-Mail: info@immcodiagnostics.com

or your local product distributor

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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APPENDIX B

IMMCO Anti-tTG Antibody ELISA Product Insert

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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IMMCO DIAGNOSTICS

ImmuLisa™

Anti-Tissue Transglutaminase Antibody (tTG) IgA ELISA

For *in vitro* Diagnostic Use
CLIA Complexity: High
CDC Analyte Identification Code: 0546
CDC Test System Identification Code: 28569

PRODUCT INSERT

Code 1157 96 Determinations

INTENDED USE

An enzyme linked immunosorbent assay (ELISA) for the detection and semi-quantitation of anti-tissue Transglutaminase IgA antibodies in human serum to aid in the diagnosis of patients with *celiac disease* and *dermatitis herpetiformis*.

SUMMARY AND EXPLANATION

Celiac disease (CD) is a chronic digestive disorder caused by an autoimmune reaction against gluten, a protein found in wheat. It is characterized by damage to the absorptive villi and hyperplasia of the crypts in the small intestine. Clinical presentation of CD most commonly reflects disturbed small bowel function: diarrhea, weight loss and anemia from malabsorption of iron and folic acid. Active CD increases risks of gastrointestinal malignancy[1], infertility[2], osteoporosis[3] and epilepsy and other neurological syndromes[4]. CD is treated with a gluten free diet.

CD is common in the European Caucasian population. As the disease is genetically mediated and the European and American Caucasian population share a common ancestry, actual prevalence of CD in the United States is suspected to be greater than reported in the past. Serological tests have been developed to aid in diagnosis of CD (i.e., anti-endomysial antibody (EMA), anti-reticulin antibody IFA's and anti-gliadin antibody ELISA). Recently, Dietrich et al[5] identified the antigen associated with EMA antigen to be tissue transglutaminase (tTG). This made it possible to create an ELISA based test with specificities for EMA antibodies that would be suitable for screening and less subjective than typical immunofluorescence methods.

Transglutaminases (EC 2.3.2.13) are a diverse family of Ca^{+2} dependent enzymes with distinct genes, structures, and biological functions[6]. These enzymes are ubiquitous and highly conserved across species. Transglutaminases catalyze the covalent cross-linking of specific proteins through the formation of isopeptide bonds between α-carboxyl groups of glutamine residues in one polypeptide and ε-NH_2 groups of lysine residues in another[7]. The resulting polymer network is stable and resistant to proteolysis, increasing the resistance of tissue to chemical, enzymatic, and mechanical disruption. Of all the human transglutaminases, tTG is most widely distributed. Although primarily an intracellular enzyme, tTG can be secreted from cells and accumulates in the extracellular matrix[8]. tTG may have a role in stabilizing the extracellular matrix and in anchoring cells to the basement membrane, functioning as a highly efficient biological glue. It is now thought that tTG, in addition to providing the focus of the autoimmune response in CD, autoantibodies against tTG may actually disrupt or interfere with its normal functioning, resulting in the histological and pathological changes seen in active and silent CD. Irrespective of the mechanism, tTG is an important analyte for the diagnosis of CD[5,9,10,11].

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

150

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PRINCIPLES OF PROCEDURES

The anti-tTG antibody test is performed as a solid phase immunoassay (ELISA). Microwells are coated with tTG antigen followed by blocking the unreacted sites to reduce nonspecific binding. Controls, calibrators and patient serum samples are incubated in the antigen coated wells which allows anti-tTG antibodies present in the serum to bind. Unbound antibody and other serum proteins are removed by washing the microwells. Antibodies bound to the microwells are detected by adding enzyme labeled anti-human IgA conjugates to the wells. These enzyme conjugated antibodies bind specifically to the human immunoglobulin of the appropriate class. Unbound enzyme conjugate is removed by washing. Specific enzyme substrate (pNPP) is then added to the wells and the presence of antibodies to tTG is detected by a color change produced by the conversion of the pNPP substrate. The reaction is stopped and the intensity of color change, which is proportional to the concentration of antibody, is read by a spectrophotometer at 405 nm. Results are expressed in ELISA units per milliliter (EU/ml).

REAGENTS

Storage and Preparation

Store all reagents at 2-8°C. **Do not freeze.** Do not use if reagent is not clear or if a precipitate is present. All reagents must be brought to room temperature (20-25°C) prior to use. When stored at 2-8°C, the reconstituted wash buffer is stable until the kit expiration date. Reconstitute the wash buffer to 1 liter with distilled or deionized water. Coated microwell strips are for one time use only.

Precautions

For *in vitro* diagnostic use. All human derived components used have been tested for HBsAg, HCV, HIV-1 and 2 and HTLV-I and found negative by FDA licensed tests. However, human blood derivatives and patient specimens should always be considered potentially infectious. Follow good laboratory practices in storing, dispensing and disposing of these materials[12].

WARNING - Sodium azide (NaN_3) may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal of liquids, flush with large volumes of water to prevent azide buildup. Sodium azide may be toxic if ingested. If ingested, report incident immediately to laboratory director or poison control center.

Instructions should be followed exactly as they appear in this kit insert to ensure valid results. Do not interchange kit components with those from other sources other than the same catalog number from IMMCO Diagnostics. Follow good laboratory practices to minimize microbial and cross contamination of reagents when handling. Do not use beyond expiration date on the label.

Materials provided

Kit contains sufficient reagents to perform 96 determinations each.

12 x 8 Ready to use **Microplate** with individual breakaway microwells coated with tTG antigen.

1 x 1.5 ml Ready to use **Positive Control*** *(red cap)*. Contains human serum positive for anti-tTG. The expected concentration range in EU/ml is printed on the label.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

1 x 1.5 ml	Ready to use **Negative Control*** *(white cap)*. Contains human serum.
4 x 1.5 ml	Ready to use **set of 4 Calibrators***; Calibrator A *(green cap)*, Calibrator B *(violet cap)*, Calibrator C *(blue cap)* and Calibrator D *(yellow cap)*. Human serum containing antibodies to tTG. Concentrations in EU/ml are printed on the labels.
1 x 12 ml	Ready to use **anti-human IgA Alk. Phos. Conjugate***. Color coded pink.
1 x 60 ml	Ready to use **Serum Diluent***. Color coded blue.
1 x 12 ml	Ready to use **Enzyme Substrate***. Contains pNPP. **Protect from light.**
1 x 12 ml	Ready to use **Stop Solution**.
2 vials	Powder **Wash Buffer**. Reconstitute to one liter each.
1 x extra	Frame Holder
2 x	Protocol Sheets

*Contains <0.1% NaN_3

Materials Required But Not Provided

- Deionized or distilled water
- Squeeze bottle to hold diluted wash buffer
- Pipettors capable of delivering 5 µl to 1000 µl
- Disposable pipette tips
- Clean test tubes 12 x 75 mm and test tube rack
- Timer
- Absorbent paper
- Microplate reader capable of reading absorbance values at 405 nm. If dual wavelength microplate reader is available, the reference filter should be set at 600-650 nm.
- Automatic microplate washer capable of dispensing 200 µl

Other available serological tests to aid in the Diagnosis of CD

ImmuGlo™ anti-endomysial antibody (EMA) IFA	*Code 1114*
ImmuGlo™ anti-reticulin antibody (ARA) IFA	*Code 1115*
ImmuLisa™ anti-gliadin antibody (AGA) IgG ELISA	*Code 1117G*
ImmuLisa™ anti-gliadin antibody (AGA) IgA ELISA	*Code 1117A*
ImmuLisa™ anti-gliadin antibody (AGA) screen ELISA	*Code 1117S*
ImmuLisa™ anti-tissue Transglutaminase (IgG) antibody ELISA	*Code 1157G*

SPECIMEN COLLECTION AND HANDLING

Only serum specimens should be used in this procedure. Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of the test and should not be used. Store specimens at 2-8°C for no longer than one week. For longer storage, serum specimens should be frozen. Avoid repeated freeze and thaw of samples.

PROCEDURE

Procedural Notes

- Before starting with the assay read carefully the product insert.
- Let serum specimens and test reagents equilibrate at room temperature for at least 30 minutes before starting with the test procedure. Return all unused specimens and reagents to refrigerator immediately after use.
- All dilutions of the patient samples should be prepared prior to starting with the assay.

- Good washing technique is critical. If washing is performed manually, adequate washing is accomplished by directing a forceful stream of wash buffer with a wide tip wash bottle across the entire microplate. **An automated microplate washer is recommended.**
- Use a multichannel pipette capable of delivering 8 wells simultaneously. This speeds the process and provides for a more uniform incubation time.
- For all steps, careful control of timing is important. The start of all incubation periods begins with the completion of reagent addition.
- Addition of all samples and reagents should be performed at the same rate and in the same sequence.
- Remove required microwell strips from the pouch and carefully reseal the pouch to prevent condensation in the unused wells. Return pouch immediately to refrigerator.

Test Method

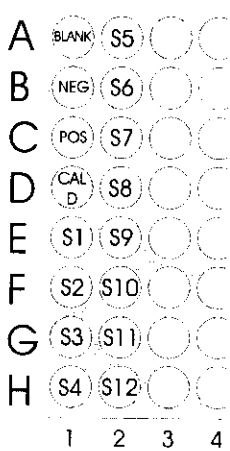
Step 1 Let all reagents and specimens equilibrate at room temperature.

Step 2 Label protocol sheet to indicate sample placement in the wells. It is good laboratory practice to run samples in duplicate.

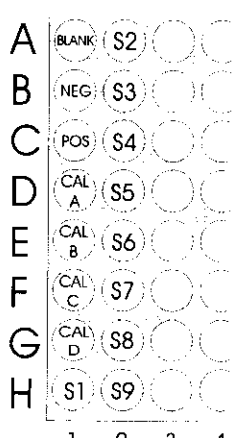
Step 3 For a **qualitative determination** use only the Ready to Use Low Calibrator D (*vial with yellow cap*).

or For a **semi-quantitative determination** use the Ready to Use Calibrators A through D as depicted in the sample layout below.

QUALITATIVE DETERMINATION



SEMI-QUANTITATIVE DETERMINATION



Step 4 Prepare a **1:51** dilution of the patient samples by mixing **10 µl** of the patient sera with **0.5 ml** of Serum Diluent.

Step 5 Pipette **100 µl** of Ready to Use Calibrators, Positive and Negative controls and diluted patient samples to the appropriate microwells as per protocol sheet.

Note: Include one well which contains **100 µl** of the Serum Diluent as a reagent blank. Zero the ELISA reader against the reagent blank. The absorbance of the reagent blank should not be more than 0.3 when read against air.

Step 6 Incubate **60 minutes** (± 5 min) at room temperature.

Step 7 Wash **4x** with wash buffer. For manual washing, fill each microwell with reconstituted wash buffer. Discard the fluid by inverting and tapping out the contents of each well or by aspirating the liquid from each well. To blot at the end of the last wash, invert strips and tap the wells vigorously on absorbent paper towels. For automatic washers, program the washer as per manufacturer's instructions.

Step 8 Pipette **100 µl** of Conjugate into microwells.

Step 9 Incubate **30 minutes** (± 5 min) at room temperature.

Step 10 Wash all microwells as in Step 7.

Step 11 Pipette **100 µl** of Enzyme Substrate into each microwell in the same order and timing as for the Conjugate.

Step 12 Incubate **microwells 30 minutes** (± 5 min) at room temperature.

Step 13 Pipette **100 µl** of Stop Solution into each microwell using the same order and timing as for the addition of the Enzyme Substrate. Read absorbance within 1 hour of adding Stop Solution.

Step 14 Read absorbance of each microwell at **405 nm** using a single or 405/630nm dual wavelength microplate reader against the reagent blank set at zero absorbance.

Quality Control

Calibrators, Positive and Negative Controls and a reagent blank must be run with each assay to verify the integrity and accuracy of the test. The absorbance reading of the reagent blank should be <0.3. The Calibrator A should have an absorbance reading of not less than 1.0, otherwise the test must be repeated. The negative control must be <20 EU/ml. If the test is run in duplicate, take the mean of the two readings to determine the concentration of anti-tTG IgA. While performing qualitative determinations, the optical density of the Calibrator D must be greater than that of the negative control and lesser than the absorbance of the positive control. For semi-quantitative determinations, the positive control must give values in the range stated on the vial. We recommend that borderline samples be tested with a fresh sample taken at a later date to ensure accuracy.

RESULTS

Calculations

The concentrations of the patient samples can be determined by either of two methods:

1. <u>QUALITATIVE DETERMINATION</u>

Results obtained by this method should be reported as positive or negative.

$$\frac{\textbf{Abs. of Test Sample}}{\textbf{Abs. of Calibrator D}} \times \textbf{EU/ml of Calibrator D} = \textbf{EU/ml Test Sample}$$

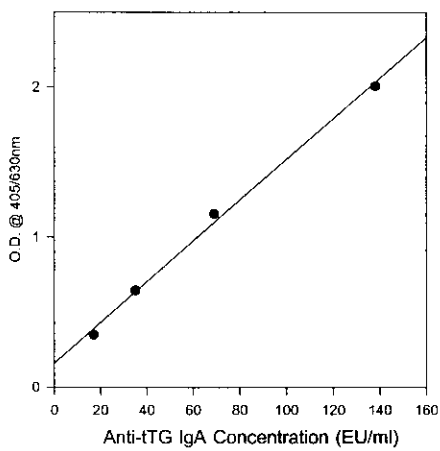
2. SEMI-QUANTITATIVE DETERMINATION

Plot absorbance of Calibrator A through D against their respective concentration on a linear-linear graph paper. Plot the concentration in EU/ml on the X-axis against the absorbance on the Y-axis and draw the best fit curve. Determine the concentrations of the patient samples from the curve against its corresponding absorbance value.

Calibrator

The ready to use Calibrators are included to provide semi-quantitation and must be used with each run. Patient samples containing higher antibody levels may give absorbance values greater than that of the Calibrator A. For determining accurate semi-quantitative values such serum sample should be further diluted so they fall within the range of the calibrator curve when retested. For determining EU/ml, multiply the units obtained by the dilution factor.

Anti-tTG IgA ImmuLisa™ Standard Curve



Interpretation

The following serves only as a guide in the interpretation of the laboratory results. These values were determined by testing 113 adult normal blood donors. The values depicted below are the mean of the normal subjects plus 3SD. Each laboratory must determine its own normal values.

anti-tTG value	Interpretation
<20 EU/ml	Negative
20-25 EU/ml	Indeterminate (Borderline)
>25 EU/ml	Positive

Records processed under FOIA Request 2014-6998; Released 10/30/14

LIMITATIONS OF THE PROCEDURE

The ImmuLisa™ anti-tTG IgA assay should not be performed on grossly hemolyzed, microbially contaminated or lipemic samples. The method should be used for testing human serum samples only. Results obtained serve only as an aid in the diagnosis and should not be interpreted as diagnostic in themselves. In IgA deficient CD patients anti-tTG may be negative.

EXPECTED VALUES

The expected values in a normal population are negative (<20 EU/ml for adults and children). However, it has been determined that some apparently healthy, asymptomatic individuals may test positive for IgA anti-tTG antibodies.

The incidence of anti-tTG antibodies has been studied by various investigators and the findings are summarized below:

Incidence of anti-tTG Antibodies in Celiac Disease And Dermatitis Herpetiformis

Study Group	Disease (Sensitivity)	Control (Specificity)
Celiac Disease		
Dietrich W et al[10]	104/106 (98%)	6/14 (96%)
Sulkanen S et al[9]	129/136 (95%)	13/207 (94%)
Dermatitis Herpetiformis		
Dietrich W et al[13]	43/61 (70%)	2/84 (98%)

The incidence and the levels of anti-tTG antibodies is dependent upon the diet status. The levels of these antibodies decrease and eventually will become negative in patients with CD who are on a gluten-free diet. Similarly, the levels of these antibodies will increase or the anti-tTG antibodies become positive when patients with CD who were on a gluten-free diet ingests a gluten-containing diet[9,10].

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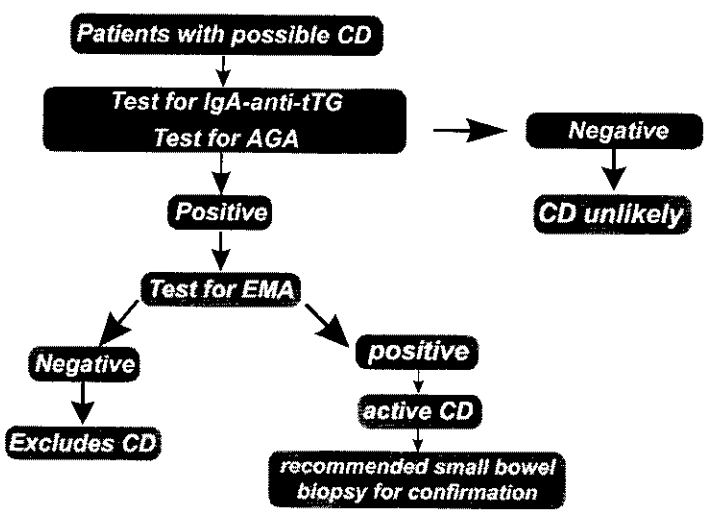
As there are several antibody markers associated with CD, the following algorithm may serve as a guide in the interpretation of various serological markers seen in CD:

EMA	ARA	AGA IgG	AGA IgA	anti-tTG	Interpretation
+	+	+	+	+	CD - certain
+	+	-	+	+	CD - certain
+	+	-	-	+	CD - certain
+	-	+	+	+	CD - certain
+	-	-	-	+	CD - highly probable
-	+	+	+	+	CD - highly probable
-	-	+	+	+	CD- possible
-	-	+	-	-	CD - unlikely but may not be precluded
-	-	-	+	-	
-	-	-	-	-	CD - excluded

Some investigators have taken the following approach to establish the diagnosis of CD using serological methods:



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PERFORMANCE CHARACTERISTICS

The utility of the ImmuLisa™ anti-tTG IgA Antibody ELISA was determined by comparing the results with:

a) another commercially available anti-tTG IgA ELISA method and

b) ImmuGlo™ anti-endomysial antibody immunofluorescence method.

Normal Range: A total of 213 samples were tested. Of these, 64 were normal blood donors and 149, obtained from a reference lab, tested negative for endomysial antibodies. Of these 213 samples tested for antibodies to tTG, only 3 were tested positive, providing a 99% specificity of the assay.

Comparative Specificity and Sensitivity

A. Endomysial Antibodies vs. ImmuLisa™ tTG IgA Antibody ELISA: A total of 244 samples were tested for antibodies to endomysium using ImmuGlo™ anti-endomysial antibody kit and the results of anti-endomysial antibodies were compared with ImmuLisa anti-tTGIgA ELISA method. The results are summarized below:

		ImmuLisa™ anti-tTG IgA		
		Positive	Negative	Total
ImmuGlo™ EMA	Positive	86	9	95
	Negative	1	148	149
	Total	87	157	244

Relative Agreement: 96%
Relative Sensitivity: 91%
Relative Specificity: 99%

B. ImmuLisa™ anti-tTG ELISA vs. Another Commercial anti-tTG IgA ELISA Method: A total of 137 samples were tested on ImmuLisa™ anti-tTG IgA antibody kit and another commercially available anti-tTG IgA kits. The results were as follows:

		ImmuLisa™ anti-tTG IgA		
		Positive	Negative	Total
Other ELISA	Positive	77	5	82
	Negative	1	54	55
	Total	78	59	137

Relative Agreement: 95%
Relative Sensitivity: 91%
Relative Specificity: 98%

C. Cross Reactivity: A total of 30 samples, 10 each positive for antinuclear, anti-basement membrane zone (BMZ) and anti-intercellular (IC) antibodies were tested for anti-tTG IgA antibodies. None were found positive.

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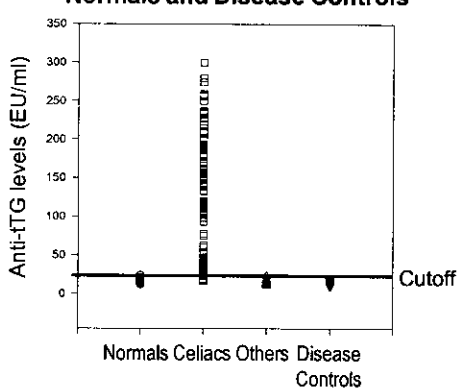
158

The following table and graph summarize the distribution of anti-tTG IgA antibodies in celiac disease, *dermatitis herpetiformis*, disease controls and normals using the ImmuLisa test system.

Incidence of Anti-tTG IgA Autoantibodies

Disease Group	No. of Cases	No. Pos	% Pos
Celiac Disease (CD)			
Gluten Diet (untreated)	43	39	91
Gluten free diet	63	13	21
Dermatitis Herpetiformis (DH)	66	45	68
Insulin Dependent Diabetes	136	18	13
Other Disorders			
Pemphigus	72	1	2
Pemphigoid	70	7	10
Thyroid Disorders	80	1	1
ANA Positive	51	0	0
AMA Positive	10	0	0
Normals			
Adults	91	3	5
Children	111	5	5

Distribution of Anti-tTG IgA in Celiacs, Normals and Disease Controls



Precision:

Based on 10 replicates, the intra-assay and inter-assay Coefficient of Variation (CV) of the anti-tTG IgA ELISA test were calculated.

	Inter-assay CV	Intra-assay CV
High (186 EU/ml)	4.9%	5.3%
Medium (118 EU/ml)	4.9%	2.3%
Low (51 EU/ml)	7.4%	9.2%

Recovery:

Samples with known anti-tTG IgA concentrations were mixed with appropriate dilutions of another positive sample with known amounts of anti-tTG IgA. Anti-tTG IgA antibody levels of the mixed samples were determined and from the values obtained the percent recovery calculated. The results are as follows:

	anti-tTGA-IgA Ab. conc. added (EU/ml)	anti-tTGA-IgA Ab. conc. obtained (EU/ml)	% Recovery
Sample 1	151	162	107
Sample 2	126	140	111

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ImmuLisa™ Procedure at a Glance

Prepare Dilutions of Specimens

↓

Pipette 100 µL of Specimens, Calibrators and Controls into microwells

↓

Incubate 60 min. at Room Temperature

↓

Wash Microplate 4x

↓

Pipette 100 µL of Conjugate into microwells

↓

Incubate 30 min. at Room Temperature

↓

Wash Microplate 4x

↓

Pipette 100 µL of Enzyme Substrate into microwells

↓

Incubate 30 min. at Room Temperature

↓

Pipette 100 µL of Stop Solution into microwells

↓

Read absorbance at 405 nm

For technical assistance please contact:

IMMCO DIAGNOSTICS

IMMCO Diagnostics, Inc.
60 Pineview Drive
Buffalo, NY 14228-2120
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or your local product distributor

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Document No. PI4157

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APPENDIX C

INOVA *t*TG Product Insert

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QUANTA Lite™ h-tTG IgA (HumanTissue Transglutaminase) 708760

For *In Vitro* Diagnostic Use
CLIA Complexity: High

Intended Use

QUANTA Lite™ h-tTG IgA is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of IgA antibodies to tissue transglutaminase (endomysium) in human serum. Detection of these antibodies is an aid in diagnosis of certain gluten sensitive enteropathies such as celiac disease and dermatitis herpetiformis.

Summary and Explanation of the test

Celiac disease and dermatitis herpetiformis, the two recognized forms of gluten sensitive enteropathy (GSE) are characterized by chronic inflammation of the intestinal mucosa and flattening of the epithelium or positive "villous atrophy".[1,2] Intolerance to gluten, the protein of wheat, rye and barley causes GSE. Patients with celiac disease may suffer from diarrhea, gastrointestinal problems, anemia, fatigue, psychiatric problems and other diverse side effects or they may be asymptomatic.[2] Dermatitis herpetiformis is a skin disease associated with GSE. All GSE patients have an increased risk of lymphoma.[3] A gluten-free diet controls GSE and associated risks.

The finding in the 1950's that gluten causes celiac disease made treatment possible by placing patients on a gluten-free diet. After the development of a method for taking small bowel biopsies orally, physicians were able to check for villous atrophy. While positive villous atrophy may occur in other disorders, GSE can be proven by following the original criteria of the European Society for Pediatric Gastroenterology and Nutrition (ESPGAN). These criteria call for approximately a year of arduous studies with: a) an initial positive gut biopsy, b) 6 months on a gluten-free diet, c) a negative second gut biopsy, d) a gluten challenge for 6 months e) a positive third gut biopsy.

The development of serum tests for three different antibodies of the IgA isotype[3] made it possible to generate more rapid, revised ESPGAN criteria for celiac disease as reported in 1990.[2] These tests include IgA endomysial antibodies (EMA),[4] IgA antigliadin antibodies (AGA) and R1 antireticulin antibodies (ARA). The revised ESPGAN criteria call for: a) a single positive gut biopsy and b) the demonstration of at least two of the three IgA class antibodies mentioned above. Since then, several studies have demonstrated that IgA EMA tests have over 99% specificity for GSE and a greater sensitivity than ARA or AGA tests.[5-13] Since the IgA EMA disappear when patients with celiac disease or dermatitis herpetiformis adhere to a gluten-free diet, tests for these antibodies also aid in checking on the adherence of patients to their diet.[4,5,12]

Recently, the endomysial antigen has been identified as the protein cross-linking enzyme known as tissue transglutaminase (tTG).[14,15] Antigen specific ELISA procedures incorporating tTG afford a reliable, objective alternative to the traditional immunofluorescent-based assays incorporating thin sections of primate esophagus as substrate. The ELISA procedure can be adapted to both large and small numbers of patient samples. Within the last two years the human tTG antigen has been produced by recombinant technology. Recombinant human antigen may have certain advantages compared with the more traditional guinea pig liver antigen.[16,17,20]

Two more recent developments in celiac disease serology are the use of native human tissue transglutaminase isolated from red blood cells and the use of tests capable of detecting IgG class tissue transglutaminase. Use of native human red blood cell tTG rather than guinea pig or recombinantly derived human antigen affords the advantage of ease of purification and results in preparations free from bacterial, insect or other contaminating proteins.[21, 22, 23, 24] The human antigen tTG ELISA assays that are presented in the literature appear to work well in comparison to the more traditional guinea pig antigen based tests.[16, 17, 20-23]

IgG class gliadin antibody kits have been in use for some time now primarily to aid in assessing IgA deficient individuals. Several investigators have demonstrated the clinical utility of detecting IgG class antibodies to tTG.[17,20,21] In one study test sensitivity was increased from 91.5% for IgA antibody alone to 98.5% when both IgA and IgG results were considered.[17]

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Principles of the Procedure

Native human tissue transglutaminase (h-tTG) antigen isolated from fresh red blood cells is bound to the wells of a polystyrene microwell plate under conditions that will preserve the antigen in its native state. Pre-diluted controls and diluted patient sera are added to separate wells, allowing any h-tTG IgA antibodies present to bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled anti-human IgA conjugate is added to each well. A second incubation allows the enzyme labeled anti-human IgA to bind to any patient antibodies, which have become attached to the microwells. After washing away any unbound enzyme labeled anti-human IgA, the remaining enzyme activity is measured by adding a chromogenic substrate and measuring the intensity of the color that develops. The assay can be evaluated spectrophotometrically by measuring and comparing the color intensity that develops in the patient wells with the color in the control wells.

Reagents

1. Polystyrene microwell ELISA plate coated with a purified h-tTG antigen, (12-1 x 8 wells), with holder in foil package containing desiccants
2. ELISA Negative Control, 1 vial of buffer containing preservative and human serum with no human antibodies to h-tTG IgA, prediluted, 1.2mL
3. h-tTG IgA ELISA Low Positive, 1 vial of buffer containing preservative and human serum antibodies to h-tTG IgA, prediluted, 1.2mL
4. h-tTG IgA ELISA High Positive, 1 vial of buffer containing preservative and human serum antibodies to h-tTG IgA, prediluted, 1.2mL
5. HRP Sample Diluent, 1 vial – colored pink containing Tris-buffered saline, Tween 20, protein stabilizers and preservative, 50mL
6. HRP Wash Concentrate, 1 vial of 40x concentrate - colored red containing Tris-buffered saline and Tween 20, 25mL. Refer to the Methods Section for dilution instructions.
7. HRP IgA Conjugate, (goat), anti-human IgA, 1 vial – colored yellow containing buffer, protein stabilizers and preservative, 10mL
8. TMB Chromogen, 1 vial containing stabilizers, 10mL
9. HRP Stop Solution, 0.344M Sulfuric Acid, 1 vial – colorless, 10mL

Warnings

1. WARNING: This product contains a chemical (0.02% chloramphenicol) in the sample diluent, controls, and conjugate 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 h-tTG IgA ELISA Low Positive, h-tTG IgA ELISA High Positive and ELISA Negative Control should be handled in the same manner as potentially infectious material.[25]
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. The HRP conjugate contains a dilute poisonous/corrosive chemical, which may be toxic if ingested in large amounts. To prevent possible chemical burns, avoid contact with skin and eyes.
5. TMB Chromogen contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
6. The HRP Stop Solution consists of a dilute sulfuric acid solution. Avoid exposure to bases, metals, or other compounds, which may react with acids. Sulfuric acid is a poison and corrosive, which may be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
7. Use appropriate personal protective equipment while working with the reagents provided.
8. 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 system may lead to inconsistent results.
3. Incomplete or inefficient washing and insufficient liquid removal from the ELISA well strips will cause

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poor precision and/or high background.

4. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from 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.
5. 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 thoroughness of washing and liquid removal from the wells of the ELISA strips, the photometer 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.
6. Strict adherence to the protocol is recommended.
7. Incomplete resealing of the zip-lock pouch containing microwell strips and desiccants will result in antigen degradation and poor precision.
8. Unacceptably low absorbencies may be observed following **two** or more uses from a single bottle of HRP conjugate over a period of time. It is important to follow all recommended HRP conjugate handling procedures to prevent this occurrence.
9. Chemical contamination of the HRP 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 HRP conjugate over time. Thoroughly rinse all equipment or instruments after the use of chemical cleaners/disinfectants.

Storage Conditions

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.
Unused antigen coated microwell strips should be resealed securely in the foil pouch containing desiccants and stored at 2-8°C.
Diluted wash buffer is stable for 1 week at 2-8°C.

Specimen Collection

This procedure should be performed with a serum specimen. Addition of azide or other preservatives to the test samples may adversely affect the results. Microbially contaminated, heat-treated, or specimens containing visible particulate should not be used. Grossly hemolyzed or lipemic serum or specimens should be avoided.

Following collection, the serum should be separated from the clot. NCCLS Document H18-A 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 h-tTG IgA ELISA microwell plate, (12-1 x 8 wells), with holder
1 1.2mL prediluted ELISA Negative Control
1 1.2mL prediluted h-tTG IgA ELISA Low Positive
1 1.2mL prediluted h-tTG IgA ELISA High Positive
1 50mL HRP Sample Diluent
1 25mL HRP Wash Concentrate, 40x concentrate
1 10mL HRP IgA Conjugate, (goat), anti-human IgA
1 10mL TMB Chromogen
1 10mL HRP Stop Solution, 0.344M Sulfuric Acid

Additional Materials Required But Not Provided

Micropipets to deliver 5, 100, 200-300, and 500µL
Disposable micropipet tips
Test tubes for patient sample dilutions, 4mL volume
Distilled or deionized water

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1L container for diluted HRP Wash Concentrate
Microwell plate reader capable of measuring OD at 450nm (and 620nm for dual wavelength readings).

Method

Before you start

1. Bring all reagents and samples to room temperature (20-26°C) and mix well.
2. Dilute the HRP Wash Concentrate 1:40 by adding the contents of the HRP Wash Concentrate bottle to 975mL of distilled or deionized water. If the entire plate will not be run within this period, a smaller quantity can be prepared by adding 2.0mL of the concentrate to 78mL of distilled or deionized water for every 16 wells that will be used. The diluted buffer is stable for 1 week at 2-8°C.
3. Prepare a 1:101 dilution of each patient sample by adding 5µL of sample to 500µL of HRP Sample Diluent. Diluted samples must be used within 8 hours of preparation. **DO NOT DILUTE** the h-tTG IgA ELISA Low Positive, h-tTG IgA ELISA High Positive and ELISA Negative Control.
4. Determination of the presence or absence of h-tTG IgA using arbitrary units requires two wells for each of the three controls and one or two wells for each patient sample. It is recommended that samples be run in duplicate.

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.
2. Add 100µL of the prediluted h-tTG IgA ELISA Low Positive, the h-tTG IgA ELISA High Positive, the ELISA Negative Control and the diluted patient samples to the wells. Cover the wells and incubate for 30 minutes at room temperature on a level surface. The incubation time begins after the last sample addition.
3. Wash step: Thoroughly aspirate the contents of each well. Add 200-300µL of the diluted HRP Wash buffer to all wells then aspirate. Repeat this sequence twice more for a total of three washes. Invert the plate and tap it on absorbent material to remove any residual fluid after the last wash. It is important to completely empty each well after each washing step. Maintain the same sequence for the aspiration as was used for the sample addition.
4. Add 100µL of the HRP IgA Conjugate to each well. Conjugate should be removed from the bottles 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 wells for 30 minutes as in step 2.
5. Wash step: Repeat step 3.
6. Add 100µL of TMB Chromogen to each well and incubate in the dark for 30 minutes at room temperature.
7. Add 100µL of HRP Stop Solution to each well. Maintain the same sequence and timing of HRP Stop Solution addition as was used for the TMB Chromogen. Gently tap the plate with a finger to thoroughly mix the wells.
8. Read the absorbance (OD) of each well at 450nm within one hour of stopping the reaction. If bichromatic measurements are desired, 620nm can be used as a reference wavelength.

Quality Control

1. The h-tTG IgA ELISA Low Positive, the h-tTG IgA ELISA High Positive and the ELISA Negative Control should be run with every batch of samples to ensure that all reagents and procedures perform properly.
2. Note that since the h-tTG IgA ELISA Low Positive, the h-tTG IgA ELISA High Positive and the ELISA Negative Control are prediluted, they do not control for procedural methods associated with dilution of specimens.
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 at $\leq$ -20° 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.

a. The absorbance of the prediluted h-tTG IgA ELISA High Positive must be greater than the absorbance of the prediluted h-tTG IgA ELISA Low Positive, which must be greater than the absorbance of the prediluted ELISA Negative Control.
b. The prediluted h-tTG IgA ELISA High Positive must have an absorbance greater than 1.0 while the prediluted ELISA Negative Control absorbance cannot be over 0.2.
c. The h-tTG IgA ELISA Low Positive absorbance must be more than twice the ELISA Negative Control or over 0.25.
d. The ELISA Negative Control and h-tTG IgA ELISA High Positive are intended to monitor for substantial reagent failure. The h-tTG IgA ELISA High Positive will not ensure precision at the assay cutoff.
e. The user should refer to NCCLS Document C24-A for additional guidance on appropriate QC practices.

Calculation of Results

The average OD for each set of duplicates is first determined. The reactivity for each sample can then be calculated by dividing the average OD of the sample by the average OD of the h-tTG IgA ELISA Low Positive. The result is multiplied by the number of units assigned to the h-tTG IgA ELISA Low Positive found on the label.

$$\text{Sample Value (units)} = \frac{\text{Sample OD}}{\text{h-tTG IgA ELISA Low Positive OD}} \times \text{h-tTG IgA ELISA Low Positive (units)}$$

Reactivity is related to the quantity of antibody present in a non-linear fashion. While increases and decreases in patient antibody concentrations will be reflected in a corresponding rise or fall in reactivity, the change is not proportional (i.e. a doubling of the antibody concentration will not double the reactivity). If a more accurate quantitation of patient antibody is required, serial dilutions of the patient sample should be run and the last dilution to measure positive in the assay should be reported as the patient's antibody titer.

Interpretation of Results

The ELISA assay is very sensitive to technique and is capable of detecting even small differences in patient populations. The values shown below are suggested values only. Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

The sample can then be classified as negative, weak positive, moderate positive or strong positive according to the table below.

	Units
Negative	<20
Weak Positive	20 – 30
Moderate to Strong Positive	>30

1. A positive result indicates the presence of h-tTG IgA antibodies and suggests the possibility of certain gluten sensitive enteropathies such as celiac disease and dermatitis herpetiformis.
2. A negative result indicates no h-tTG IgA antibody or levels below the negative cut-off of the assay.
3. It is suggested that the results reported by the laboratory should include the statement: "The following results were obtained with the INOVA QUANTA Lite™ h-tTG IgA ELISA. h-tTG IgA values obtained with different manufacturers' assay methods may not be used interchangeably. The magnitude of the reported IgA levels cannot be correlated to an endpoint titer."

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. A negative h-tTG IgA result in an untreated patient does not rule out gluten-sensitive enteropathy. This finding can often be explained by selective IgA deficiency, a relatively frequent finding in celiac disease. INOVA offers a h-tTG IgG kit for testing these patients (QUANTA Lite™ h-tTG IgG).

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3. Results of this assay should be used in conjunction with clinical findings and other serological tests.
4. The assay performance characteristics have not been established for matrices other than serum.

Expected Values

The ability of the QUANTA Lite™ h-tTG (human tissue transglutaminase) IgA ELISA to detect IgA tissue transglutaminase antibodies was evaluated by comparison to another test for detection of IgA tTG antibody as well as by comparing results with actual clinical diagnosis in two external clinical evaluations and one internal study.

Normal Range

The normal range study was performed in the research laboratory at INOVA Diagnostics. A total of 185 samples were tested. A total of 102 males were tested. Age ranged from 6-60 years with an average age of 34. A total of 83 females were tested ranging in age from 7-63 years with an average age of 31.

When these 185 samples were tested with the QUANTA Lite™ h-tTG IgA kit, only one sample (0.5%) was positive. This one sample fell right above the cutoff of 20 units. Other than this one weakly positive sample, the next highest reactive sample had a value of 11.5 units and most values were in single digits. The average value for this group of normal samples was 4.4 units.

Relative Sensitivity and Specificity

To determine the sensitivity and specificity of the assay, a total of 257 samples were tested by both the QUANTA Lite™ h-tTG IgA and another commercial test for tTG antibody. The results are summarized below:

		h-tTG +	h-tTG -		
guinea pig liver tTG	+	51	4*	Relative Sensitivity	92.7%
	-	17**	185	Relative Specificity	91.6%
				Relative Efficiency	91.8%

* 2 of these were endomysial and gliadin IgA negative.

** Most of these were known or suspected celiacs who on previous testing had weak or negative serologies.

Clinical Sensitivity and Specificity

The QUANTA Lite™ h-tTG IgA (tissue transglutaminase) antibody kit as evaluated in one external clinical studies and one internal clinical study. The following table summarizes the findings of both the external and internal clinical studies.

Patient Group	No.	No. Pos	(%)
Normals	185	1*	(0.5%)
Celiac	24	24	(100%)
Celiac (pediatric)	11	10	(91%)
Celiac IgA deficient	16	0	
Ulcerative colitis	14	0	
Crohn's disease	6	1**	(17%)
Pernicious anemia	6	0	
Chronic active hepatitis	9	0	
Misc. connective tissue autoantibody positives	55	0	

* This 1 sample was weakly positive at 20 units.

** This 1 sample was weakly positive at 23 units and was also weakly positive at 22 units on the QUANTA Lite™ tTG kit using guinea pig liver antigen.

Cross-Reactivity

To further assess the specificity of the QUANTA Lite™ h-tTG IgA kit, a total of 90 patients with other gastrointestinal problems as well as patients with a variety of high titered connective tissue disease associated autoantibodies were tested. A total of 14 sera from patients with ulcerative colitis, 6 with Crohn's disease, 6 with pernicious anemia, 9 with chronic active hepatitis were tested. Also tested were a group of sera from a collection of sera used to manufacture other INOVA autoantibody ELISA kits. A total of 55 sera

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were tested including 8 positive for SS-A, 6 positive for SS-B, 9 positive for Sm, 11 positive for RNP, 13 positive for Scl-70 and 8 positive for Jo-1.

Of these 90 samples, only one sample from a patient with Crohn's disease was positive. This patient was only weakly positive at 23 units for IgA h-tTG. This same sample, when tested on the guinea pig based tTG , was found to also be positive at 22 units. Other than this one weakly positive sample, none of the other samples were above 8 units.

Precision and Reproducibility

The precision and reproducibility of the assay was measured by running six replicates each of a strong positive, weak positive and a negative sample in six separate assays. The mean reactivity of the strong positive was 132.6 units, the weak positive was 25.0 units while the mean value for the negative was 13.4. The standard deviation and coefficient of variation for each sample are summarized below.

	Negative		Strong Positive		Weak Positive	
	SD	CV	SD	CV	SD	CV
Overall	1.05	7.8%	3.76	2.8%	0.66	2.6%
Within Run	0.65	4.8%	3.01	2.3%	0.69	2.8%
Between Run	0.78	5.8%	3.21	2.4%	0.44	1.8%

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QUANTA Lite™ h-tTG IgA (HumanTissue Transglutaminase) 708760

Nur für "*In-Vitro* Diagnostik"

Verwendungszweck

QUANTA Lite™ h-tTG IgA ist ein „Enzyme-linked Immunosorbent Assay" (ELISA) für die semi-quantitative Bestimmung von IgA Antikörper gegen Gewebetransglutaminase (Endomysium) im Humanserum. Die Bestimmung dieser Antikörper gegen h-tTG IgA unterstützt mit anderen serologischen Nachweisen und der Beurteilung des klinischen Bildes die Diagnostik des gluteninduzierten Enteropathien wie Zöliakie und Dermatitis herpetiformis.

Informationen zum Test

Zöliakie und Dermatitis herpetiformis, zwei Formen der gluteninduzierten Enteropathie (GSE), sind charakterisiert durch chronische Entzündungen der intestinalen Mucosa und durch eine Zottenatrophie. [1,2] Intoleranz gegenüber Gluten, ein Protein, das in Weizen, Roggen und Gerste vorkommt, verursacht GSE. Die Erkrankung kann asymptomatisch verlaufen oder es können folgende Symptome auftreten: Diarrhöe, Malabsorption, Anämie, Müdigkeit, psychiatrische Probleme und bei Kindern sogar eine Wachstumsretardation.[2] Dermatitis herpetiformis ist eine Hauterkrankung assoziert mit GSE. Alle GSE Patienten haben ein erhöhtes Risiko, an einem Lymphom zu erkranken. [3] GSE wird durch eine glutenfreie Diät behandelt, wodurch sich die Risiken minimieren lassen.

In den 50er Jahren konnte nachgewiesen werden, dass Gluten Zöliakie verursacht und somit war ein Behandlung ermöglicht, nämlich die Patienten einer gluten-freien Diät zu unterziehen. Mit Hilfe der oralen Darmbiopsie war es den Ärzten möglich, das Ausmaß der Zottenatrophie zu beurteilen. Während eine positive Zottenatrophie auch bei anderen Krankheiten vorkommen kann, wird GSE mit Hilfe nach-folgender Kriterien der European Society for Pediatric Gastroenterology and Nutrition (ESPGAN) festgelegt. Diese Kriterien erfordern aufwendige Studien von annähernd einem Jahr:
a) eine zu Beginn positive Darmbiopsie, b) sechs Monate glutenfreie Diät, c) eine zweite negative Darmbiopsie, d) eine Provokation mit Gluten über sechs Monate, e) eine dritte positive Darmbiopsie.

Die Entwicklung von Serumtesten für drei verschiedene Antikörper der Immunglobulinklasse IgA[3] ermöglichte 1990 eine überarbeitete Version der ESPGAN Kriterien für Zöliakie.[2] Diese Teste beinhalten die Bestimmung von endomysialen IgA Antikörpern (EMA),[4] Anti-Gliadin-Antikörpern IgA (AGA) sowie R1 Anti-Retikulin Antikörper (ARA). Die überarbeiteten ESPGAN Kriterien fordern:
a) eine einzige positive Darmbiopsie, b) den positiven Nachweis von zumindest zwei der drei oben erwähnten IgA Antikörper Seither haben verschiedene Studien gezeigt, daß IgA-EMA Tests bei GSE eine Spezifität von über 99% erzielen und eine größere Sensitivität als ARA oder AGA Teste erbringen.[5-13] IgA-EMA Antikörper verschwinden, sobald sich Patienten mit Zöliakie oder Dermatitis herpetiformis an eine glutenfreie Diät halten. Deshalb werden diese Antikörper auch dazu verwendet, um den Patienten auf die Einhaltung seiner Diät zu überprüfen.[4,5,12]

Vor kurzem konnte das endomysiale Antigen als Gewebe-Transglutaminase (tTG) identifiziert werden.[14,15] Antigen-spezifische ELISA-Teste unter Verwendung von tTG bieten eine zuverlässige, objektive Alternative zum herkömmlichen Immunfluoreszenz-Test. Bei diesem Test werden dünne Schnitte vom Primaten-Ösophagus als Substrat verwendet. Der ELISA Test kann sowohl bei einer großen als auch kleinen Anzahl von Patientenproben angewendet werden. Seit den letzten beiden Jahren wird das humane tTG-Antigen rekombinant hergestellt. Rekombinantes humanes Antigen hat bestimmte Vorteile im Vergleich zum traditionellen Meerschweinchenleber-Antigen.[16,17,20]

Zwei kurz zurückliegende Entwicklungen in der Zöliakie-Serologie sind die Verwendung nativen humanen Gewebetransglutaminase, aus roten Blutzellen isoliert, und die Verwendung von Tests, mit deren Hilfe IgG Gewebetransglutaminase nachgewiesen werden kann. Die Verwendung von nativem roten Blutzell-tTG anstatt Meerschweinchen- oder rekombinantem humanem Antigen hat den Vorteil leichterer Reinigung und Präparationen, die frei von bakteriologischen, Insekten- oder anderen Verunreinigungsproteinen sind.[21,22,23,24] Die humanen Antigen tTG ELISA Tests, die in der Literatur beschrieben sind, scheinen besser zu funktionieren als die traditionellen Meerschweinchenleber-Antigen Tests.[16,17,20-23]

Gliadin Antikörperkits des IgG Isotyps werden schon seit geraumer Zeit primär zur Abklärung von IgA-Defizienzen verwendet. Mehrere Forschergruppen haben die klinische Nützlichkeit von IgG-Antikörpern

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gegen tTG.[17,20,21] In einer dieser Studien wurde die Testsensitivität von 91,5% für IgA-Antikörper alleine auf 98,5% erhöht, wenn sowohl IgA als auch IgG berücksichtigt wurden.[17]

Testprinzip

Natives humanes Gewebetransglutaminase (h-tTG) Antigen, isolatiert aus frischen roten Blutzellen, wird so beschichtet, daß das Antigen in seiner ursprünglichen Konfiguration erhalten bleibt. Vorverdünnte Kontrollen und verdünnte Patientenseren werden in verschiedene Kavitäten pipettiert. Die vorhandenen h-tTG IgA Antikörper binden an das Antigen. Der Rest der Probe/Kontrolle wird durch Waschen entfernt. Enzymmarkiertes anti-humanes IgA wird in die Kavitäten pipettiert und bindet während einer zweiten Inkubation an den Patienten-Antikörper. Nachdem in einem weiteren Waschschritt das restliche Konjugat entfernt worden ist, wird ein Chromogen-Substrat zugegeben. Die Intensität der entstandenen Farbreaktion wird nach dem Abstoppen mit dem Mikrotiterplatten-Photometer gemessen. Die quantitative Auswertung erfolgt durch einen Vergleich der Extinktionswerte der Patienten mit dem Wert eines Kalibrators.

Inhalt der Testpackung

1. Mikrotiter-ELISA-Platte beschichtet mit hochgereinigtem h-tTG Antigen, (12-1 x 8 Kavitäten), mit Streifenhalter in Folienverpackung und Trockenmittel.
2. ELISA Negative Kontrolle, 1 Flasche Puffer mit Stabilisator und Humanserum ohne humane Antikörper gegen h-tTG IgA, gebrauchsfertig vorverdünnt, 1,2 mL
3. h-tTG IgA ELISA Low Positive (Kalibrator), 1 Flasche Puffer mit Stabilisator und Humanserum mit Antikörpern gegen h-tTG IgA, gebrauchsfertig vorverdünnt, 1,2 mL
4. h-tTG IgA ELISA High Positive (positive Kontrolle), 1 Flasche Puffer mit Stabilisator und Humanserum mit Antikörpern gegen h-tTG IgA, gebrauchsfertig vorverdünnt, 1,2 mL
5. HRP Probenverdünner, 1 Flasche – rosa gefärbt mit Tris-gepufferter Kochsalzlösung, Tween 20, Proteinstabilisatoren und Konservierungsmittel, 50 mL
6. HRP Waschkonzentrat, 1 Flasche mit 40fachem Konzentrat – rot gefärbt mit gepufferter Kochsalzlösung und Tween 20, 25 mL. Zur Verdünnung bitte das entsprechende Kapitel in der Anleitung beachten.
7. HRP IgA Konjugat, (Ziege), anti-humanes IgA, 1 Flasche – gelb gefärbt mit Puffer, Proteinstabilisatoren und Konservierungsmittel, 10 mL
8. TMB Chromogen, 1 Flasche mit Stabilisatoren, 10 mL
9. HRP Stopplösung, 0,344M Schwefelsäure, 1 Flasche – farblos, 10 mL

Hinweise

1. VORSICHT: Probenverdünner, Kontrollen und Konjugat enthalten 0,02% Chloramphenicol. Es ist im US-Bundesstaat Kalifornien und allgemein bekannt, daß dieser Stoff Krebs verursachen kann.
2. Alle Reagenzien für die Herstellung dieses Tests wurden auf Antikörper gegen HIV, HBsAg und HCV getestet und für negativ befunden. Dennoch sollten alle humanen Kontrollen wie potentiell infektiöses Humanserum oder Blutproben behandelt werden.[25]
3. NaN_3 wird als Stabilisator verwendet. NaN_3 ist ein Giftstoff und kann bei Einnahme toxische Reaktionen verursachen. Vorsichtig handhaben und Kontakt mit Augen und Haut vermeiden! Den Kontakt mit Metall, basischen Stoffen oder anderen Komponenten, die mit Säure reagieren können, vermeiden. Bei der Entsorgung von Reagenzien ist daher mit viel Leitungswasser nachzuspülen, um Ansammlungen im Abwassersystem zu verhindern.
4. Das HRP Konjugat enthält eine verdünnte Chemikalie, die bei Einnahme toxisch wirken kann. Daher den Kontakt mit Haut und Augen vermeiden.
5. Das TMB Chromogen enthält ein Reizmittel, das bei Inhalation, Einnahme oder Absorbtion durch die Haut gesundheitliche Schäden verursachen kann. Kontakt mit Haut und Augen vermeiden.
6. Die HRP Stopplösung besteht aus verdünnter Schwefelsäure. Den Kontakt mit Basen, Metallen und anderen Stoffen, die mit Säure reagieren können, vermeiden. Schwefelsäure ist ein Giftstoff und kann bei Einnahme toxische Reaktionen hervorrufen. Den Kontakt mit Haut und Augen vermeiden.
7. Die vorgeschriebene persönliche Schutzausrüstung während der Arbeit mit Reagenzien tragen.
8. Verschüttete Reagenzien sofort beseitigen. Bei der Entsorgung von Abfällen alle Umweltvorschriften beachten.

Vorsichtsmaßnahmen

1. Dieser Test ist für "*In-Vitro* Diagnostik".

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2. Die Verwendung anderer als im Testkit vorhandenen Komponenten kann zu widersprüchlichen Ergebnissen führen.
3. Unvollständiges Waschen und ungenügendes Entfernen der Flüssigkeiten aus den ELISA Kavitäten führt zu einer schlechten Präzision und zu hohen Hintergrund-Extinktionen.
4. Die Adaptation dieses Testsystems auf automatische Probenverarbeitung und andere Instrumentierung, ganz oder teilweise, kann unterschiedliche Ergebnisse zur manuellen Durchführung ergeben. Es liegt in der Verantwortung eines jeden Labors, die automatische Bearbeitung so zu überprüfen, daß Testergebnisse innerhalb akzeptabler Bereiche erzielt werden.
5. Eine Reihe von Faktoren beeinflusst das Testergebnis. Hierzu zählen die Genauigkeit und Reproduzierbarkeit der Pipettierung, der Typ des verwendeten Photometers, die Temperatur der Reagenzien, die Umgebungs-Temperatur, die Gründlichkeit des Waschens und der Entfernung der Flüssigkeiten aus den Vertiefungen der ELISA-Streifen, und die Einhaltung der Inkubationszeiten. Es ist deshalb sehr wichtig, für gleichbleibende Bedingungen zu sorgen.
6. Die strikte Einhaltung der Testprozedur wird empfohlen. Jede Änderung im Protokoll erfolgt auf Risiko des Anwenders.
7. Das unvollständige Verschließen der Mikrotiterkavitäten und des Trockenmittels führt zu Antigenabbau und schlechter Präzision.
8. Eine unakzeptabel niedrige Absorption kann beobachtet werden, wenn eine Flasche HRP Konjugat bei **zwei-** oder mehrfachem Gebrauch über einen längeren Zeitraum benutzt wird. Daher ist es wichtig, die Hinweise zum Umgang mit dem HRP Konjugat genau zu beachten.
9. Chemische Kontamination des HRP Konjugates kann durch unzureichendes Reinigen oder Spülen der Ausrüstung oder der Instrumente verursacht werden. Rückstände gebräuchlicher Laborchemikalien wie z.B. Formalin, Bleichmittel, Ethanol oder Spülmittel führen zum Abbau des HRP Konjugates im Verlauf der Zeit. Das gründliche Spülen der gesamten Ausrüstung und Instrumentierung nach Verwendung chemischer Reinigungsmittel ist daher unbedingt erforderlich.

Lagerung

1. Lagerung aller Kit-Reagenzien bei 2-8°C. Nicht einfrieren. Die Reagenzien sind stabil bis zum Ende des Haltbarkeitsdatums bei vorschriftsmäßiger Lagerung und Handhabung.
2. Nicht verwendete Mikrotiterstreifen wieder in die Originalverpackung geben, luftdicht verschließen und in den Kühlschrank zurücklegen.
3. Der verdünnte Waschpuffer ist bei 2-8°C eine Woche stabil.

Proben

Die Testdurchführung sollte mit Serumproben erfolgen. Werden Azide oder andere Stabilisatoren zu den Serumproben gegeben, können die Ergebnisse nachteilig beeinflusst werden. Mikrobakteriell kontaminierte, hämolytische, lipämische oder durch Hitzeeinwirkung inaktivierte Proben sollten nicht verwendet werden.

Nach der Blutentnahme ist das Serum vom Blut zu trennen. Das NCCLS Dokument H18-A empfiehlt die folgenden Lagerungsbedingungen für Patientenproben: 1) Proben bei Raumtemperatur nicht länger als 8 Stunden lagern. 2) Kann die Testdurchführung nicht innerhalb von 8 Stunden erfolgen, die Proben bei 2-8°C kühl lagern. 3) Kann die Testdurchführung nicht innerhalb von 48 Stunden erfolgen ist die Probe bei -20°C oder niedriger einzufrieren. Eingefrorene Proben müssen nach dem Auftauen und vor der Testung gut geschüttelt werden.

Testdurchführung

In der Testpackung vorhandenes Material

1 h-tTG IgA ELISA Mikrotiterplatte, (12-1 x 8 Kavitäten), mit Streifenhalter
1 1,2 mL vorverdünnte ELISA Negative Kontrolle
1 1,2 mL vorverdünnte h-tTG IgA ELISA Low Positive Kontrolle
1 1,2 mL vorverdünnte h-tTG IgA ELISA High Positive Kontrolle
1 50 mL HRP Probenverdünner
1 25 mL HRP Waschkonzentrat, 40faches Konzentrat
1 10 mL HRP IgA Konjugat (von der Ziege), anti-humanes IgA
1 10 mL TMB Chromogen
1 10 mL HRP Stopplösung, 0,344M Schwefelsäure

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Zusätzliches benötigtes Material

Pipetten für 5, 100, 200-300 und 500 µL
Einmal-Pipettenspitzen
Eppendorf-Reaktionsgefäße für die Serumverdünnung, 4 mL Volumen
Distilliertes oder deionisiertes Wasser
1 L Gefäß für verdünntes HRP Waschkonzentrat
Reader für Mikrotiterplatten mit 450 nm Filter (und 620 nm für eine bichromatische Messung)

Methode

Testvorbereitung

1. Alle Reagenzien und Proben auf Raumtemperatur bringen (20-26°C) und gründlich mischen.
2. Den gesamten Inhalt (25 mL) des Waschkonzentrats mit 975 mL Aqua dest. mischen (1:40 Verdünnung). Die verdünnte Pufferlösung ist bei 2-8°C eine Woche stabil. Soll nicht die gesamte Mikrotiterplatte auf einmal verwendet werden, so können für einen Ansatz von 16 Kavitäten 2 mL Konzentrat mit 78 mL Aqua dest. gemischt werden.
3. Serumproben 1:101 verdünnen, indem 5 µL Serum mit 500 µL gebrauchsfertigem Probenverdünner gemischt werden. Verdünnte Proben sollen innerhalb von 8 Stunden getestet werden. Die h-tTG IgA ELISA Low Positive Kontrolle, die h-tTG IgA ELISA High Positive Kontrolle und die ELISA Negative Kontrolle **nicht verdünnen.**
4. Jeder Test benötigt zwei Kavitäten für jede der drei Kontrollen sowie eine oder zwei Kavitäten für die Patientenprobe (Es wird empfohlen, alle Proben in Doppelbestimmung anzusetzen, bis die erforderliche Präzision und Reproduzierbarkeit erreicht sind).

Testdurchführung

1. **ALLE REAGENZIEN UND PATIENTENPROBEN AUF RAUMTEMPERATUR (20-26°C) BRINGEN.** Die entsprechende Anzahl Mikrotiterkavitäten abbrechen. Die Kavitäten im Rahmen befestigen. **Die unbenutzten Kavitäten wieder in die Originalverpackung geben, luftdicht verschließen und in den Kühlschrank zurücklegen, um Verdunstung zu minimieren.**
2. Je 100 µL der **gebrauchsfertigen** h-tTG IgA ELISA Low Positive Kontrolle, der h-tTG IgA ELISA High Positive Kontrolle, der ELISA Negative Kontrolle und der verdünnten Patientenproben in die entsprechenden Kavitäten pipettieren. Streifen abdecken und bei Raumtemperatur 30 Minuten inkubieren. Die Inkubationszeit beginnt nach Zugabe der letzten Probe.
3. Waschen: Den Inhalt aller Kavitäten absaugen. Die Kavitäten vollständig (200-300 µL) mit **verdünntem** HRP Waschpuffer füllen und dann gründlich absaugen. Diesen Waschschritt noch zweimal wiederholen (Insgesamt: drei Waschschritte). Anschließend die Platte auf saugfähigem Papier ausklopfen, um restliche Waschflüssigkeit zu entfernen. Die Waschschritte sind in der selben Reihenfolge wie die Pipettierschritte durchzuführen.
4. 100 µL des HRP IgA Konjugates in jede Kavität geben. Sterile Pipetten verwenden! Nur das benötigte Volumen an Konjugat aus der Flasche entnehmen. **UNBENUTZTES KONJUGAT NICHT IN DIE FLASCHE ZURÜCKPIPETTIEREN. KONTAMINATIONSGEFAHR!!** Abgedeckte Streifen bei Raumtemperatur 30 Minuten inkubieren, wie in 2. beschrieben.
5. Waschen: Schritt Nr. 3 wiederholen.
6. 100 µL TMB Chromogen in jede Kavität geben. 30 Minuten **im Dunkeln** bei Raumtemperatur inkubieren.
7. 100 µL HRP Stopplösung in jede Kavität pipettieren. Bei der Hinzugabe der Stopplösung dieselbe Reihenfolge und Zeitplan wie bei der Hinzugabe des TMB Chromogens einhalten. Die Mikrotiterplatte vorsichtig schütteln.
8. Die optische Dichte (OD) jeder Kavität bei 450 nm innerhalb einer Stunde nach Abstoppen der Reaktion ablesen. Es wird eine bichromatische Messung mit 620 nm als Referenzwellenlänge empfohlen.

Qualitätskontrolle

1. Die h-tTG IgA ELISA Low Positive Kontrolle, die h-tTG IgA ELISA High Positive Kontrolle und die ELISA Negative Kontrolle sollten bei jedem Testansatz mitgeführt werden, um sicherzustellen, daß alle Reagenzien und der Testansatz insgesamt ordnungsgemäß funktionieren.
2. Da die h-tTG IgA ELISA Low Positive Kontrolle, die h-tTG IgA ELISA High Positive Kontrolle und die ELISA Negative Kontrolle vorverdünnt sind, entfällt der Verdünnungsschritt der Patientenproben für

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die Kontrollen.

3. Zusätzliche Kontrollen zur Qualitätssicherung können gemäß den Richtlinien nationaler oder internationaler Regulierungs- oder Akkreditierungsbehörden eingesetzt werden. Geeignete Kontrollen können aus Humanserum gewonnen und bei $\leq$-20° C gelagert werden.
4. Um sicher zu sein, daß alle Patientenwerte korrekt sind, müssen die nachfolgenden Kriterien erfüllt werden (Wurden ein oder mehrere Kriterien nicht erfüllt, so ist das Ergebnis als ungültig zu betrachten und der Testansatz ist zu wiederholen).
   a. Die Absorption der vorverdünnten h-tTG IgA ELISA High Positive Kontrolle muß größer sein als die Absorption der vorverdünnten h-tTG IgA ELISA Low Positive Kontrolle. Die Absorption der Low Positive Kontrolle wiederum muß größer sein als die der vorverdünnten ELISA Negativkontrolle.
   b. Die Absorption der vorverdünnten h-tTG IgA ELISA High Positive Kontrolle muß größer als 1,0 sein, die Absorption der vorverdünnten Negative Kontrolle darf nicht größer als 0,2 sein.
   c. Die Absorption der h-tTG IgA ELISA Low Positive Kontrolle muß mehr als doppelt so hoch sein wie die der ELISA Negative Kontrolle sein oder über 0,25 liegen.
   d. Die ELISA Negative Kontrolle und die h-tTG IgA ELISA High Positive Kontrolle dienen der Sicherstellung der ordnungsgemäßen Funktionsweise des Testansatzes. Die h-tTG IgA ELISA High Positive Kontrolle stellt die Präzision am Cut-off des Tests nicht sicher.
   e. Der Anwender sollte unter anderem das NCCLS Dokument Nr. C24-A für zusätzliche Hinweise zur zeitgemäßen Qualitätskontrolle beachten.

Berechnung der Ergebnisse

Zunächst sind die Mittelwerte der OD für die Doppelbestimmungen zu berechnen. Alle weiteren Berechnungen werden dann mit den entsprechenden Mittelwerten durchgeführt. Die Reaktivität jeder Patientenprobe wird bestimmt durch die Division des Mittelwertes der Proben-OD durch den Mittelwert der Low Positive Kontrolle und der Multiplikation dieses Ergebnisses mit dem chargenspezifischen Wert der h-tTG IgA ELISA Low Positive Kontrolle. Die chargenspezifischen Werte sind auf dem Fläschchenetikett aufgedruckt.

$$\text{Probenwert (Units)} = \frac{\text{OD der Probe}}{\text{h-tTG IgA ELISA Low Positive Kontrolle OD Wert}} \times \text{h-tTG IgA ELISA Low Positive Kontrollwert (Units)}$$

Die Reaktivität verhält sich zur Konzentration der vorhandenen Antikörper nicht linear. Zunahme und Abnahme der Antikörperkonzentrationen von Patienten werden durch einen entsprechenden Anstieg oder Abfall der Reaktivität angezeigt, diese Änderungen sind jedoch nicht proportional (d.h. eine Verdoppelung der Antikörperkonzentration führt nicht zu einer Verdoppelung der Reaktivität). Wird eine genauere Quantifizierung der Patientenantikörper gewünscht, sind serielle Verdünnungen der Probe durchzuführen und der Titer der zuletzt als positiv gemessen wurde, sollte als Patienten-Antikörpertiter bewertet werden.

Interpretation der Ergebnisse

Dieser ELISA-Test ist sehr sensitiv und in der Lage, sogar kleine Unterschiede in Patientenpopulationen zu messen. Die folgenden Werte dienen als Beispiel für die Interpretation der Testergebnisse. Es wird empfohlen, daß sich jedes Labor seine eigenen Normalwerte, basierend auf eigener Technik, Kontrollen, Ausrüstung und Patientenpopulation erarbeitet.

Die Patientenprobe kann anschließend als negativ, schwach, deutlich positiv bis stark positiv klassifiziert werden.

	Units
Negativ	<20
Schwach positiv	20 – 30
Deutlich positiv bis stark positiv	>30

1. Ein positives Ergebnis zeigt das Vorhandensein von h-tTG IgA Antikörpern an und legt die Möglichkeit des gluteninduzierten Enteropathien wie Zöliakie und Dermatitis herpetiformis.
2. Ein negatives Ergebnis deutet auf das Nichtvorhandensein von h-tTG IgA Antikörpern hin oder auf Konzentrationen unterhalb der Erfassungsgrenze des Testsystems.
3. Wir schlagen vor, die Laborergebnisse mit folgendem Hinweis zu versehen: "Die folgenden Ergebnisse wurden mit dem INOVA QUANTA Lite™ h-tTG IgA ELISA erzielt. h-tTG IgA

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Werte, die mit Testsystemen anderer Hersteller ermittelt wurden, können nicht untereinander ausgetauscht werden. Die Höhe des gefundenen IgA-Titers kann nicht mit einem Endpunkttiter in Korrelation gebracht werden."

Grenzen des Verfahrens

1. Immunkomplexe oder andere Immunoglobulin-Aggregate im Patientenserum können nicht-spezifische Bindungen und falsch-positive Ergebnisse hervorrufen.
2. Ein negatives h-tTG IgA Ergebnis bei einem unbehandelten Patienten schließt eine gluten-sensitive Enteropathie nicht aus. Dieses Ergebnis kann des öfteren durch eine selektive IgA-Defizienz erklärt werden, ein relativ häufiges Vorkommen bei der Zöliakie. INOVA bietet ein h-tTG IgG Testkit zur Untersuchung dieser Patienten (QUANTA Lite™ h-tTG IgG).
3. Ergebnisse dieses Testes müssen im Zusammenhang mit klinischen Ergebnissen und anderen serologischen Tests verwendet werden.
4. Die Leistungscharakteristika für andere Untersuchungsmaterialien als Serum wurden nicht bestimmt.

Erwartungswerte

Die Fähigkeit des QUANTA Lite™ h-tTG (humane Gewebetransglutaminase) IgA ELISA, IgA Gewebetransglutaminase-Antikörper nachzuweisen, wurde im Vergleich mit einem anderen Test und klinischer Diagnose in zwei externen klinischen Evaluierungen und einer internen Studie untersucht.

Normalbereich

Die Normalbereichsstudie wurde im Forschungslabor von INOVA Diagnostics durchgeführt. Insgesamt wurden 185 Proben getestet, davon 102 von Männern. Das Alter reichte von 6-60 Jahren, mit einem Durchschnitt von 34 Jahren. 83 Frauen im Alter von 7-63 Jahren, mit einem Durchschnitt von 31 Jahren.

Bei der Analyse der 185 Proben im QUANTA Lite™ h-tTG IgA Testkit war nur eine Probe (0,5%) positiv. Diese eine Probe lag gerade oberhalb des Cutoff bei 20 Units. Von dieser schwach positiven Probe abgesehen, hatte die nächste einen Wert von 11,5 Units. Der Durchschnitt für diese Gruppe der Normalseren lag bei 4,4 Units.

Relative Sensitivität und Spezifität

Zur Bestimmung von Sensitivität und Spezifität dieses Testsystems wurden insgesamt 257 Patientenproben sowohl im QUANTA Lite™ h-tTG IgA als auch einem anderen, kommerziell verfügbaren tTG-Antikörpertest untersucht. Die Ergebnisse sind nachfolgend zusammengefaßt:

		h-tTG +	h-tTG -		
Meerschweinchen-leber tTG	+	51	4*	Relative Sensitivität	92,7%
				Relative Spezifität	91,6%
	-	17**	185	Relative Übereinstimmung	91,8%

* 2 dieser Proben waren Endomysium und Gliadin IgA negativ.

** Die meisten dieser Proben stammten von bekannten Zöliakiepatienten, die bei früheren Untersuchungen schwache oder negative serologische Ergebnisse hatten.

Klinische Sensitivität und Spezifität

Der QUANTA Lite™ h-tTG IgA (Gewebetransglutaminase) Antikörper Testkit wurde in einer externen klinischen und einer internen klinischen Studie evaluiert. Die nachfolgende Tabelle faßt die Ergebnisse dieser Studie zusammen.

Patientengruppe	Anzahl	Anzahl pos.	(%)
Normalseren	185	1*	(0,5)
Zöliakie	24	24	(100%)
Zöliakie Pädiatrische Proben	11	10	(91%)
Zöliakie IgA Defizienz	16	0	
Colitis ulcerosa	14	0	
Morbus Crohn	6	1**	(17%)
Perniziöse Anämia	6	0	

175

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Chronisch aktive Hepatitis	9	0
Verschied. Bindegewebe-Autoantikörper positive	55	0

* Diese Probe war schwach positiv mit 20 Units.

** Diese Probe war schwach positiv mit 23 Units und war auch schwach positiv mit 22 Units mit dem QUANTA Lite™ tTG Testkit, bei dem Meerschweinchenleber-Antigen verwendet wird.

Kreuzreaktivität

Zur Ermittlung der Spezifität des QUANTA Lite™ h-tTG IgA Testkits wurden insgesamt 90 Seren von Patienten mit anderen gastrointestinalen Problemen sowie von Patienten mit einer Reihe von hochtitrigen Bindegewebeerkrankungens getestet. 14 Seren stammten von Patienten mit Colitis ulcerosa, 6 mit Morbus Crohn, 6 mit perniziöser Anämia und 9 mit chronisch aktiver Hepatitis. Insgesamt 55 Seren wurden getestet, darunter 8 SS-A positive, 6 SS-B positive, 9 Sm-positive, 11 RNP-positive, 13 positiv für Scl-70 und 8 positiv für Jo-1.

Von diesen 90 Proben war nur eine positiv, sie stammte von einem Morbus Crohn-Patienten. Diese Probe war nur schwach positiv mit 23 Units IgA h-tTG. Die gleiche Probe hatte im Meerschweinchen-tTG Testkit einen positiven Wert mit 22 units. Abgesehen von dieser einen schwach positiven Proben hatten alle anderen keinen Wert oberhalb von 8 Units.

Präzision und Reproduzierbarkeit

Die Präzision und Reproduzierbarkeit des Tests wurde durch Messen einer negativ, einer schwach positiv und einer stark positiv Probe in sechs Wiederholungen in sechs verschiedenen Testansätzen ermittelt. Die mittlere Absorption der stark positiven Probe betrug 132,6, die der schwach positiven 25 und die der negativen 13,4. Die Standardabweichung (SD) und der Variationskoeffizient (VK) jeder Probe sind nachfolgend aufgeführt.

	Negativ		Stark positiv		Schwach positiv	
	SD	VK	SD	VK	SD	VK
Gesamt	1,05	7,8%	3,76	2,8%	0,66	2,6%
Intraassay	0,65	4,8%	3,01	2,3%	0,69	2,8%
Interassay	0,78	5,8%	3,21	2,4%	0,44	1,8%

Zusammenfassung der Testdurchführung

Testvorbereitung:

1. Alle Reagenzien auf Raumtemperatur (20-26°C) bringen und gut mischen.
2. Benötigte Menge Waschpufferkonzentrat mit Aqua dest. 1:40 verdünnen.
3. Die Patientenseren 1:101 mit HRP Probenverdünner verdünnen, z.B. 5 µL Serum mit 0,5 mL Probenverdünner. Die Kitkontrollen sind gebrauchsfertig.

Testdurchführung:

1. Pipettieren: Je 100 µL der **gebrauchsfertigen** ELISA High Positive Kontrolle, der ELISA Low Positive Kontrolle, der Negative Kontrolle und der verdünnten Patientenproben in die entsprechenden Kavitäten pipettieren.
2. Inkubation: 30 Minuten bei Raumtemperatur.
3. Waschen: Den Inhalt der Kavitäten absaugen. 200-300 µL der verdünnten Waschlösung in die Kavitäten füllen und danach absaugen. Insgesamt dreimal waschen.
4. Pipettieren: 100 µL Konjugat in jede Kavität.
5. Inkubation: 30 Minuten bei Raumtemperatur.
6. Waschen: Den Inhalt der Kavitäten absaugen. 200-300 µL der verdünnten Waschlösung in die Kavitäten füllen und danach absaugen. Insgesamt dreimal waschen.
7. Pipettieren: 100 µL TMB in jede Kavität.
8. Inkubation: 30 Minuten bei Raumtemperatur.
9. Pipettieren: 100 µL Stopplösung in jede Kavität.
10. Absorption bei einer Wellenlänge von 450 / 620 nm messen.

176

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QUANTA Lite™ h-tTG IgA (HumanTissue Transglutaminase) 708760

Per uso diagnostico *In Vitro*

Finalità d'uso

QUANTA-Lite h-tTG IgA es un test immunoenzimatico per la ricerca semi-quantitativa di anticorpi della classe IgA diretti contro la transglutaminasi tissutale (endomisio) nel siero umano. La presenza di IgA anti-h-tTG può essere usata, insieme al quadro clinico del paziente ed al risultato degli altri test di laboratorio eseguiti, come aiuto nella diagnosi di certe enteropatie sensibili al glutine quali la malattia celiaca e la dermatite erpetiforme.

Riassunto e Spiegazione del test

La malattia celiaca e la dermatite erpetiforme, due forme riconosciute di enteropatia sensibile al glutine (GSE) sono caratterizzate da infiammazione cronica della mucosa intestinale e da appiattimento dell'epitelio o "atrofia villosa" positiva.[1,2] L'intolleranza al glutine, la proteina del grano, della segale e dell'orzo, causa la GSE. I pazienti con malattia celiaca possono presentare diarrea, altri problemi gastrointestinali, anemia, affaticamento, problemi psichiatrici ed altri differenti effetti collaterali oppure possono essere asintomatici.[2] La dermatite erpetiforme è una patologia cutanea associata a GSE. Tutti i pazienti affetti da GSE presentano un elevato rischio di linfoma.[3] La dieta priva di glutine controlla la GSE ed i rischi correlati.

L'osservazione, fatta negli anni '50, che il glutine causava la malattia celiaca rese possibile il trattamento ponendo i pazienti in dieta senza glutine. In seguito alla messa a punto di un metodo per il prelievo per via orale di biopsie del piccolo intestino, i medici furono in grado di individuare l'atrofia villosa. Mentre atrofia villosa positiva può essere osservata in altri disordini, la GSE può essere provata mediante i seguenti criteri originali della European Society for Pediatric Gastroenterology and Nutrition (ESPGAN). Questi criteri richiedono circa un anno di studi difficili che comprendono: a) una iniziale biopsia intestinale positiva, b) 6 mesi di dieta priva di glutine, c) una secondas biopsia intestinale negativa, d) un test con glutine per 6 mesi, e) una terza biopsia intestinale positiva.

La messa a punto di test sierologici per la ricerca di tre differenti anticorpi dell'isotipo IgA[3] ha reso possibile la formulazione di criteri ESPGAN più rapidi e riveduti per la malattia celiaca come segnalato nel 1990.[2] Questi test includono la ricerca di anticorpi della classe IgA anti-endomisio (EMA),[4] IgA anti-gliadina (AGA) ed anti-reticolina R1 (ARA). I criteri ESPGAN riveduti comprendono: a) una singola biopsia intestinale positiva e b) la dimostrazione di almeno due dei tre anticorpi della classe IgA precedentemente citati. Da allora, numerosi studi hanno dimostrato che il test per le IgA EMA è caratterizzato dal 99% di specificità per la GSE e che ha una specificità maggiore rispetto ai test per ARA o AGA.[5-13] Poichè le IgA per EMA scompaiono quando il paziente con malattia celiaca o con dermatite erpetiforme osserva scrupolosamente la dieta priva di glutine, i test per la ricerca di questi anticorpi rappresentano anche un aiuto per individuare quei pazienti che si attengono scrupolosamente alla loro dieta.[4,5,12]

Recentemente, l'antigene di endomisio è stato identificato come l'enzima chiamato transglutaminasi tissutale (tTG).[14,15] I test ELISA specifici che incorporano la tTG forniscono una valida, oggettiva alternativa ai test tradizionali in immunofluorescenza assays su sezioni sottili di esofago di scimmia come substrato. I test ELISA possono essere usati per testare sia grandi sia piccoli numeri di campioni. Nel corso degli ultimi due anni è stato prodotto un'antigene tTG ricombinante. Gli antigeni umani ricombinanti possono presentare vantaggi nei confronti degli antigeni tradizionali estratti da fegato di cavia.[16,17,20]

Due ulteriori aggiornamenti della diagnosi sierologica della malattia celiaca sono rappresentati dall'uso di transglutaminasi tissutale umana nativa, estratta dagli eritrociti, e dall'uso di test in grado di individuare anticorpi della classe IgG diretti contro la transglutaminasi tissutale. L'utilizzo della tTG umana nativa estratta dagli eritrociti al posto di quella estratta dal fegato di cavia o di quella ricombinante consente una maggiore facilità di purificazione e di ottenere preparazioni senza contaminazioni da parte di proteine estranee.[21, 22, 23, 24] I lavori bibliografici presenti nella letteratura indicano che i test ELISA che utilizzano l'antigene tTG umano sembrano dare risultati migliori rispetto a quelli basati sull'antigene tradizionale estratto dalle cavie.[16, 17, 20-23]

I test per la ricerca delle IgG anti-gliadina sono ormai in uso da alcuni anni soprattutto per controllare i soggetti che non producono IgA. Numerosi ricercatori hanno dimostrato l'utilità clinica della ricerca delle IgG anti-tTG.[17,20,21] Nel corso di uno di questi studi la sensibilità del test è stata aumentata dal 91,5% per la sola ricerca di IgA al 98,5% quando venivano considerati i risultati dei test per la ricerca sia di IgA che di IgG.[17]

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Principio della Metodica

L'antigene tTG umana estratto da eritrociti freschi è adsorbito sulla parete dei pozzetti di una piastra microtiter in polistirene in condizioni adatte a preservare l'antigene nel suo stato nativo.
I controlli pre-diluiti ed i sieri diluiti dei pazienti vengono distribuiti nei pozzetti corrispondenti, consentendo alle IgA anti-h-tTG eventualmente presenti di legarsi all'antigene adsorbito. L'eccesso di campione non legato viene allontanato mediante il lavaggio e successivamente si aggiunge a ciascun pozzetto un anticorpo anti-IgA umane marcato con un enzima. Una seconda incubazione consente agli anticorpi anti-IgA umane marcati con l'enzima di legarsi agli anticorpi del paziente che si sono eventualmente legati alla parete dei pozzetti. Dopo ulteriore lavaggio per allontanare l'eccesso di anticorpi anti-IgA umane marcati con l'enzima, l'attività dell'enzima rimasto viene misurata aggiungendo un substrato cromogenico e misurando l'intensità del colore che si sviluppa. Il test può essere letto mediante uno spettrofotometro ed interpretato confrontando l'intensità del colore che si sviluppa nei pozzetti dei campioni con quella dei pozzetti dei controlli.

Reagenti

1. Piastra microtiter ELISA in polistirene adsorbita con antigene h-tTG purificato, (12-1 x 8 pozzetti), con supporto, in busta chiusa contenente materiale essicante
2. Controllo ELISA Negativo, 1 flacone di tampone contenente conservante e siero umano senza anticorpi umani della classe IgA anti-h-tTG, prediluito, 1,2mL
3. Controllo ELISA fortemente positivo per IgA anti-h-tTG, 1 flacone di tampone contenente conservante ed anticorpi umani della classe IgA anti-h-tTG, prediluito, 1,2mL
4. Controllo ELISA debolmente positivo per IgA anti-h-tTG, 1 flacone di tampone contenente conservante ed anticorpi umani della classe IgA anti-h-tTG, prediluito, 1,2mL
5. Diluente per campioni HRP, 1 flacone – di colore rosa contenente soluzione fisiologica tamponata con Tris, Tween 20, stabilizzatori delle proteine e conservante, 50mL
6. Soluzione di lavaggio HRP Concentrata, 1 flacone di soluzione concentrata 40x – di colore rosso contenente soluzione fisiologica tamponata con Tris e Tween 20, 25mL. Vedi la Sezione Metodi per le istruzioni sulla diluizione.
7. Coniugato HRP IgA, (montone), anti-IgA umane, 1 flacone – di colore giallo contenente tampone, stabilizzatori delle proteine e conservante, 10mL
8. Cromogeno TMB, 1 flacone contenente stabilizzatori, 10mL
9. Soluzione di arresto HRP, Acido solforico 0,344M, 1 flacone – incolore, 10mL

Avvertenze

1. ATTENZIONE: Questo prodotto contiene un composto chimico (cloramfenicolo allo 0,02%) nel diluente per i campioni, nei controlli e nel coniugato, noto allo Stato della California come causa di tumori.
2. Tutte le fonti umane di materiali usati nella preparazione dei controlli per questo prodotto sono state testate e sono risultate negative per la presenza di anticorpi anti-HIV, per HbsAg e per anticorpi anti-HCV mediante metodi approvati dall'FDA. Tuttavia nessun test offre la certezza completa dell'assenza di HIV, HBV, HCV o di altri agenti infettivi. Pertanto, i Controlli ELISA fortemente positivo per IgA anti h-tTG, ELISA debolmente positivo per IgA anti h-tTG ed ELISA Negativo devono essere maneggiati come materiali potenzialmente infettivi.[25]
3. La sodio azide è usata come conservante. La sodio azide è un veleno e può essere tossica se ingerita o assorbita attraverso la cute o gli occhi. La sodio azide può reagire con le tubature di piombo o rame formando azidi metalliche potenzialmente esplosive. Lasciar scorrere grandi quantità di acqua, se si usa un lavandino per eliminare i reagenti, per prevenire la formazione di azidi.
4. Il Coniugato HRP contiene un composto chimico velenoso/corrosivo, che può essere tossico se ingerito. Per prevenire possibili ustioni chimiche, evitare il contatto con la cute e con gli occhi.
5. Il Cromogeno TMB contiene un irritante, che può essere dannoso se inalato, ingerito o assorbito attraverso la cute. Per prevenire lesioni, evitare l'inalazione, l'ingestione o il contatto con la cute e con gli occhi.
6. La Soluzione di arresto HRP è costituita da una soluzione di acido solforico diluito. Evitare l'esposizione a basi, metalli o altri composti che possono reagire con gli acidi. L'acido solforico è velenoso e corrosivo e può essere tossico se ingerito. Per prevenire possibili ustioni chimiche, evitare il contatto con la cute e con gli occhi.
7. Usare appropriati indumenti personali protettivi mentre si lavora con i reagenti forniti.
8. I reagenti eventualmente rovesciati devono essere rimossi immediatamente. Seguire tutte le

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normative vigenti in materia di eliminazione dei residui di natura chimica.

Precauzioni

1. Questo prodotto è stato messo a punto per l'uso diagnostico *In Vitro*.
2. La sostituzione di componenti diversi da quelli forniti nel kit può causare risultati non attendibili.
3. Un lavaggio incompleto o non accurato e l'insufficiente aspirazione del liquido dai pozzetti ELISA può causare una scarsa precisione e/o un'elevato background.
4. L'adattamento di questo test all'uso con apparecchiature automatiche e altri dispositivi per trattamento dei campioni liquidi, in toto o in parte, può causare differenze nei risultati rispetto a quelli ottenuti mediante la procedura manuale. E' responsabilità di ciascun Laboratorio confermare che le procedure automatizzate utilizzate diano risultati entro limiti di accettabilità.
5. Una serie di fattori influenza le prestazioni del test. Essi comprendono l'iniziale temperatura dei reagenti, la temperatura dell'ambiente, l'accuratezza e la riproducibilità della tecnica di pipettamento, la scrupolosità delle operazioni di lavaggio e di aspirazione del liquido dai pozzetti delle piastre ELISA, il tipo di spettrofotometro usato per leggere i risultati e la lunghezza dei tempi di incubazione durante l'esecuzione del test. Bisogna prestare molta attenzione alla costanza per ottenere risultati accurati e riproducibili.
6. Si raccomanda di attenersi scrupolosamente alle istruzioni fornite.
7. Una chiusura incompleta della busta richiudibile contenente le strisce di pozzetti ed il materiale essicante causa la degradazione dell'antigene ed una scarsa precisione nei risultati.
8. Valori di assorbanza inaccettabilmente bassi possono essere osservati dopo aver usato **due** o più volte lo stesso flacone di coniugato HRP per un certo periodo di tempo. E' importante seguire attentamente le istruzioni fornite per il trattamento del coniugato HRP per evitare tale inconveniente.
9. Un'eventuale contaminazione chimica del coniugato HRP può essere conseguenza di una impropria pulizia o risciacquo di apparecchi o di strumenti. Residui di comuni reagenti chimici di laboratorio quali formalina, candeggina, etanolo o detergenti causano la degradazione nel tempo del coniugato HRP. Risciacquare molto accuratamente tutti gli apparecchi e gli strumenti dopo l'uso di detergenti chimici.

Condizioni di conservazione

1. Conservare tutti i reagenti del kit a 2-8°C. Non congelare. I reagenti sono stabili fino alla data di scadenza se conservati e trattati seguendo le istruzioni fornite.
2. Le strisce di pozzetti non utilizzate devono essere rimesse immediatamente nella busta richiudibile contenente il materiale essicante e conservate nella busta ben chiusa a 2-8°C.
3. Il tampone di lavaggio diluito è stabile per 1 settimana a 2-8°C.

Raccolta dei campioni

Questa tecnica deve essere usata con un campione di siero. L'aggiunta al campione di sodio azide o di altri conservanti può influenzare in modo negativo i risultati. Campioni con segni di contaminazione microbica, trattati con calore o contenenti particelle visibili non dovrebbero essere usati. Si dovrebbe evitare anche l'uso di sieri fortemente emolizzati o lipemici.

Dopo il prelievo, il siero dovrebbe essere separato dal coagulo. Il Documento H18-A dell'NCCLS raccomanda le seguenti condizioni di conservazione per i campioni: 1) Conservare i campioni a temperatura ambiente per non più di 8 ore. 2) Se il test non può essere eseguito entro 8 ore, conservare i campioni in frigorifero a 2-8°C. 3) Se il test non può essere eseguito entro 48 ore, oppure per la spedizione dei campioni, congelare a ≤-20°C. I campioni congelati devono essere mescolati bene dopo lo scongelamento e prima di essere testati.

Procedura

Materiali forniti

1 Piastra microtiter ELISA h-tTG IgA, (12-1 x 8 pozzetti), con supporto
1 1,2mL Controllo ELISA Negativo prediluito
1 1,2mL Controllo ELISA debolmente positivo per IgA anti h-tTG prediluito
1 1,2mL Controllo ELISA fortemente positivo per IgA anti h-tTG prediluito
1 50mL Diluente per campioni HRP
1 25mL Soluzione di lavaggio HRP concentrata, 40x concentrata
1 10mL Coniugato HRP IgA, (montone), anti-IgA umane

1 10mL Cromogeno TMB
1 10mL Soluzione di arresto HRP, 0,344M Acido solforico

Materiali richiesti ma non forniti

Micropipette in grado di erogare volume di 5, 100, 200-300, e 500µL
Puntali monouso per micropipette
Provette per la diluizione dei sieri, volume 4mL
Acqua distillata o deionizzata
Beuta da 1L per diluite la Soluzione di lavaggio HRP concentrata
Lettore per piastre ELISA in grado di leggere a 450nm (e 620nm per le letture a doppia lunghezza d'onda)

Metodica

Prima di incominciare

1. Portare tutti i reagenti ed i campioni a temperatura ambiente (20-26°C) e mescolarli bene.
2. Diluire la Soluzione di lavaggio HRP concentrata 1:40 aggiungendo il contenuto del flacone fornito con il kit a 975mL di acqua distillata o deionizzata. Se non si usa l'intera piastra in una sola volta, si può preparare una minore quantità di soluzione di lavaggio aggiungendo 2,0mL di concentrato a 78mL di acqua distillata o deionizzata ogni 16 pozzetti utilizzati. La soluzione di lavaggio diluita è stabile per 1 settimana a 2-8°C.
3. Preparare una diluizione 1:101 di ciascun campione da testare aggiungendo 5µL di siero a 500µL di Diluente per campioni HRP. I campioni diluiti devono essere testati entro 8 ore dalla preparazione. **NON DILUIRE** i Controlli ELISA debolmente positivo per IgA anti h-tTG, ELISA fortemente positivo per IgA anti h-tTG ed ELISA Negativo.
4. La determinazione della presenza o dell'assenza di IgA anti-h-tTG usando unità arbitrarie richiede due pozzetti per ciascuno dei tre controlli ed uno o due pozzetti per ciascun campione clinico. Si raccomanda di testare i campioni in duplicato.

Esecuzione del test

1. **TUTTI I REAGENTI DEVONO ESSERE PORTATI A TEMPERATURA AMBIENTE (20-26°C) PRIMA DI INIZIARE IL TEST.** Posizionare i pozzetti/le strisce necessarie nel supporto. **Rimettere immediatamente la strisce inutilizzate nella busta contenente il materiale essicante e sigillarla bene per minimizzare l'esposizione all'umidità ambientale.**
2. Distribuire 100µL dei Controlli ELISA debolmente positivo per IgA anti h-tTG, ELISA fortemente positivo per IgA anti h-tTG ed ELISA Negativo **prediluiti** e dei sieri diluiti nei rispettivi pozzetti. Coprire i pozzetti ed incubare per 30 minuti a temperatura ambiente su una superficie piana. Il tempo di incubazione inizia dopo l'aggiunta dell'ultimo campione.
3. Lavaggio: Aspirare completamente il contenuto di ciascun pozzetto. Distribuire 200-300µL di soluzione di lavaggio HRP **diluita** in tutti i pozzetti e quindi aspirarla. Ripetere questa operazione per altre due volte, per un totale di tre lavaggi. Dopo l'ultimo lavaggio capovolgere la piastra e scuoterla fermamente su tovaglioli di carta assorbente per rimuovere eventuali residui di liquido. E' importante vuotare completamente ciascun pozzetto dopo ciascun lavaggio. Per l'aspirazione mantenere la stessa sequenza usata per l'aggiunta dei campioni.
4. Distribuire 100µL di Coniugato HRP IgA in ciascun pozzetto. Il coniugato dovrebbe essere prelevato dal flacone mediante tecniche sterili. Prelevare dal flacone solo la quantità di coniugato necessaria per l'esecuzione del test. **PER EVITARE POSSIBILI CONTAMINAZIONI MICROBICHE E/O CHIMICHE, NON RIMETTERE MAI IL CONIUGATO INUTILIZZATO NEL FLACONE.** Incubare i pozzetti per 30 minuti come descritto al punto 2.
5. Lavaggio: Ripetere la procedura descritta al punto 3.
6. Distribuire 100µL di Cromogeno TMB in ciascun pozzetto ed incubare **al buio** per 30 minuti a temperatura ambiente.
7. Distribuire 100µL di Soluzione di arresto HRP in ogni pozzetto. Per l'aggiunta della Soluzione di arresto HRP mantenere la stessa sequenza e gli stessi tempi utilizzati per l'aggiunta del Cromogeno TMB. Agitare gentilmente la piastra con le dita per mescolare completamente i reagenti nei pozzetti.
8. Leggere l'assorbanza (OD) di ciascun pozzetto a 450nm entro 1 ora dall'aggiunta della soluzione di arresto. Se si preferisce una lettura a doppia lunghezza d'onda, si può usare la lunghezza d'onda di 620nm come riferimento.

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Records processed under FOIA Request 2014-6998; Released 10/30/14

Controllo di qualità

1. I Controlli ELISA debolmente positivo per IgA anti h-tTG, ELISA fortemente positivo per IgA anti h-tTG ed ELISA Negativo devono essere inclusi ogni volta che si esegue il test per assicurare che tutti i reagenti ed il test funzionino in modo corretto.
2. Si deve notare che, poichè i Controlli ELISA debolmente positivo per IgA anti h-tTG, ELISA fortemente positivo per IgA anti h-tTG ed ELISA Negativo sono prediluiti, essi non rappresentano un controllo procedurale per le tecniche di diluizione utilizzate per i campioni.
3. Ulteriori controlli possono essere inclusi in base alle indicazioni o alle richieste delle vigenti regolamentazioni o delle organizzazioni di accreditamento. Ulteriori sieri di controllo possono essere preparati raccogliendo un pool dei sieri umani, aliquotandolo e conservandolo a $\leq -20^{o}$ C.
4. Perchè i risultati del test siano considerati validi, tutti i seguenti criteri devono essere soddisfatti. Se anche uno solo non rientra nei valori specificati, i risultati non dovrebbero essere considerati validi ed il test dovrebbe essere ripetuto.
   a. L'assorbanza del Controllo ELISA fortemente positivo per IgA anti h-tTG prediluito deve essere maggiore di quella del Controllo ELISA debolmente positivo per IgA anti h-tTG prediluito che a sua volta deve essere maggiore di quella del Controllo ELISA Negativo prediluito.
   b. Il Controllo ELISA fortemente positivo per IgA anti h-tTG prediluito deve avere un'assorbanza maggiore di 1,0 mentre l'assorbanza del Controllo ELISA Negativo prediluito non deve essere maggiore di 0,2.
   c. L'assorbanza del Controllo ELISA debolmente positivo per IgA anti h-tTG deve essere maggiore di due volte rispetto a quella del Controllo ELISA Negativo oppure >0,25.
   d. Il Controllo ELISA Negativo e quello ELISA fortemente positivo per IgA anti h-tTG servono per controllare un'eventuale malfunzionamento dei reagenti. Il Controllo ELISA fortemente positivo per IgA anti h-tTG non assicura la precisione in corrispondenza del valore soglia del test.
   e. L'utente del test dovrebbe fare riferimento al Documento C24-A dell'NCCLS per ulteriori informazioni su appropriate tecniche di Controllo di Qualità.

Calcolo dei risultati

Si deve determinare innanzitutto il valore medio di assorbanza per ciascun set di duplicati. La reattività di ciascun campione viene quindi calcolata dividendo il valore medio di assorbanza del campione per il valore medio di assorbanza del Controllo ELISA debolmente positivo per IgA anti h-tTG e moltiplicando il risultato per il numero di unità assegnate al Controllo ELISA debolmente positivo per IgA anti h-tTG, che è stampato sull'etichetta del flacone.

$$\text{Valore del campione (unità)} = \frac{\text{Assorbanza campione}}{\text{Assorbanza Controllo ELISA debolmente positivo per IgA anti h-tTG}} \times \text{Valore Controllo ELISA debolmente positivo per IgA anti h-tTG (unità)}$$

La reattività è collegata in modo non lineare alla quantità di anticorpi presente. Mentre gli aumenti e le diminuzioni delle concentrazioni degli anticorpi vengono evidenziate da un corrispondente aumento o diminuzione della reattività, il cambiamento non è proporzionale (ad es., un raddoppio della concentrazione di anticorpi non raddoppia necessariamente la reattività). Se si desidera una più accurata quantificazione del contenuto di anticorpi, si possono allestire e testare diluizioni seriate del campione. L'ultima diluizione che risulta positiva nel test dovrebbe essere refertata come il titolo anticorpale del paziente.

Interpretazione dei risultati

Il metodo ELISA è molto sensibile alla procedura ed è in grado di individuare anche piccole differenze nella popolazione dei pazienti. I valori riportati qui di seguito sono solo valori suggeriti. Ciascun Laboratorio dovrebbe stabilire il proprio range di normalità basato sulla procedura da esso utilizzata, sui controlli, sull'apparecchiatura e sulla popolazione di pazienti secondo le proprie metodiche standard.

Il campione può essere quindi classificato come negativo, debolmente positivo, moderatamente o fortemente positivo in base alla seguente tabella.

	Unità
Negativo	<20

181

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Debolmente Positivo	20 – 30
Moderatamente a Fortemente Positivo	>30

1. Un risultato positivo indica la presenza di IgA dirette contro h-tTG e suggerisce la possibilità della presenza di certe enteropatie sensibili al glutine, quali la malattia celiaca e la dermatite erpetiforme.
2. Un risultato negativo indica l'assenza di IgA dirette contro h-tTG oppure la loro presenza a livelli inferiori al limite di sensibilità del metodo.
3. Sul referto si dovrebbe riportare la seguente frase: "I seguenti risultati sono stati ottenuti mediante il test QUANTA Lite™ h-tTG IgA ELISA della INOVA. I valori di IgA anti-h-tTG ottenuti con test prodotti da altre Ditte possono non essere utilizzabili in modo intercambiabile. Il livello di IgA determinato con questo metodo non può essere correlato ad un titolo endpoint."

Limitazioni del test

1. La presenza nel campione di complessi immuni o di altri aggregati di immunoglobuline può causare un aumento del livello di reazioni a specifiche con conseguenti risultati falsi positivi con questo test.
2. Un risultato negativo per IgA anti-h-tTG in un paziente non trattato non esclude una enteropatia sensibile al glutine. Questo risultato può essere spesso spiegato da una deficienza selettiva di IgA, un dato relativamente frequente nel corso della malattia celiaca.
3. I risultati di questo test devono essere valutati dal medico curante alla luce del quadro clinico del paziente e del risultato degli altri test sierologici.
4. Le prestazioni caratteristiche del test non sono state valutate per campioni diversi dal siero.

Valori attesi

La capacità del test QUANTA Lite™ h-tTG (transglutaminasi tissutale umana) IgA ELISA di individuare la presenza di IgA dirette contro la transglutaminasi tissutale è stata valutata confrontando le prestazioni del kit con quelle di un altro test per la ricerca di IgA anti-tTG e correlandole alla diagnosi clinica nel corso di due studi clinici esterni e di uno interno.

Range normale

Lo studio per valutare il range di normalità è stato eseguito presso i Laboratori di ricerca alla INOVA Diagnostics. In totale sono stati testati 185 sieri, appartenenti a 102 maschi, di età compresa tra 6 e 60 anni (media = 34), ed a 83 frmmine, di età compresa tra 7 e 63 anni (media = 31).

Solo 1 campione su 185 (0.5%) è risultato positivo con il test QUANTA Lite™ h-tTG (transglutaminasi tissutale umana) IgA ELISA. Questo siero presentava un risultato appena superiore al valore soglia di 20 unità. Altri campioni sono risultati debolmente positivi, anche se quello maggiore reattività presentava un valore pari a 11.5 unità. Il valore medio di questo gruppo di sieri normali era pari a 4.4 unità.

Sensibilità e specificità relative

La sensibilità e la specificità relativa del kit QUANTA Lite™ h-tTG IgA sono state valutate testando 257 campioni e paragonando i risultati a quelli ottenuti mediante un altro kit reperibile in commercio. La tabella seguente riassume i risultati:

		h-tTG +	h-tTG -		
tTG estratta da fegato di cavia	+	51	4*	Sensibilità relativa	92,7%
				Specificità relativa	91,6%
	-	17**	185	Concordanza relativa	91,8%

* 2 di questi campioni erano negativi per la ricerca di IgA anti-endomisio ed anti-gliadina.

** Per la maggior parte di questi pazienti era già stata posta o sospettata una diagnosi di malattia celiaca e nel corso di precedenti test avevano risultati sierologici debolmente positiv o negativi.

Sensibilità e Specificità Clínica

Il kit QUANTA Lite™ tTG (transglutaminasi tissutale) è stato valutato nel corso di uno studi clinici esterni e di uno studio interno. La tabella successiva riassume i risultati degli studi clinici sia esterni che interni.

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Gruppo di pazienti	N.	N. Pos	(%)
Normali	185	1*	(0,5)
Malattia celiaca	24	24	(100)
Malattia celiaca Pediatrici	11	10	(91)
Pazienti celiaci senza IgA	16	0	
Colite ulcerativa	14	0	
Morbo di Crohn	6	1**	(17%)
Anemia perniciosa	6	0	
Epatite cronica attiva	9	0	
Pz. pos. per autoanticopi anti-tess. conn. vari	55	0	

* Questo campione risultava debolmente positivo positive (20 unità).

** Il campione debolmente positivo (23 unità) è risultato debolmente positivo (22 unità) utilizzando il kit QUANTA Lite™ tTG basato sull'antigene estratto dal fegato di cavia.

Reazioni crociate

Per valutare appieno la sensibilità del test QUANTA Lite™ h-tTG IgA sono stati testati 90 pazienti con problemi gastrointestinali di varia natura nonchè pazienti con alti titoli di autoanticopri associati a varie malattie del tessuto connettivo. Sono stati inclusi nel gruppo 14 pazienti con colite ulcerativa, 6 con Morbo di Crohn, 6 con anemia perniciosa e 9 con epatite cronica attiva. Inoltre è stato testato un gruppo di sieri scelti dalla collezione di campioni utilizzati per la messa a punto di altri kit ELISA della INOVA. Questo gruppo era costituito da 55 sieri che comprendevano anche 8 campioni positivi per SS-A, 6 per SS-B, 9 per Sm, 11 per RNP, 13 per Scl-70 e 8 per Jo-1. Solo 1 di questi 90 campioni, appartenente ad un paziente affetto da Morbo di Crohn, è risultato positivo al test per la ricerca di IgA anti-tTG, con un valore pari a 23 unità. Lo stesso campione, sottoposto ad ulteriore test con il kit basato sull'antigene estratto dal fegato di cavia, si confermava debolmente positivo (22 unità). A parte questo siero debolmente positivo, nessuno degli altri mostrava valori superiori a 8 unità.

Precisione e riproducibilità

La precisione e la riproducibilità del metodo sono state valutate testando sei repliche di ciascun campione negativo, debolmente positivo e fortemente positivo in sei esperimenti diversi. Il rapporto medio del campione fortemente positivo era pari a 132,6, quello del campione debolmente positivo era 25 e quello del campione negativo 13,4. La deviazione standard ed il coefficiente di variazione per ciascun campione sono riportati nella seguente tabella.

	Negativo		Fortemente Positivo		Debolmente Positivo	
	DS	CV	DS	CV	DS	CV
Generale	1,05	7,8%	3,76	2,8%	0,66	2,6%
Intra-test	0,65	4,8%	3,01	2,3%	0,69	2,8%
Inter-test	0,78	5,8%	3,21	2,4%	0,44	1,8%

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QUANTA Lite™ h-tTG IgA (HumanTissue Transglutaminase) 708760

Para Diagnóstico *In Vitro*

Aplicación

QUANTA-Lite h-tTG IgA es un ensayo basado en la técnica ELISA (Enzyme-Linked Immunosorbent Assay) para la detección semi cuantitativa de anticuerpos IgA antitransglutaminasa del endomisio en suero humano. La presencia de anticuerpos anti-h-tTG IgA puede ser utilizada en conjunción con hallazgos clínicos y otras pruebas de laboratorio como ayuda en el diagnóstico de ciertas enteropatías sensibles al gluten, como la enfermedad celíaca y la dermatitis herpetiforme.

Sumario y Explicación de la prueba

La enfermedad celíaca y la dermatitis herpetiforme, las dos formas identificadas de enteropatías sensibles al gluten (ESG), se caracterizan por inflamaciones crónicas de la mucosa intestinal y por un aplanamiento del epitelio o atrofia de las vellosidades.[1,2] La intolerancia al gluten, proteína que se encuentra en el trigo, el centeno y en la cebada, causa ESG. Los pacientes con enfermedad celíaca pueden sufrir diarrea, problemas gastrointestinales, anemia, fatiga, problemas psiquiátricos y otros efectos secundarios, o por el contrario, ser asintomáticos[2]. La dermatitis herpetiforme es una enfermedad cutánea asociada a la ESG. Todos los pacientes con ESG presentan un mayor riesgo de linfoma[3]. Las ESG y los riesgos derivados pueden controlarse mediante una dieta libre de gluten.

El descubrimiento en los años 1950 de que el gluten causaba la enfermedad celíaca hizo posible el tratamiento sometiendo a los pacientes a una dieta libre de este compuesto. Tras el desarrollo de un método para obtener pequeñas biopsias del intestino por vía oral, los médicos fueron capaces de comprobar la existencia de atrofia vellositaria. Mientras que la atrofia vellositaria positiva puede presentarse en otros trastornos, las ESG pueden constatarse siguiendo los criterios originales de la Sociedad Europea de Gastroenterologia Pediátrica y Nutrición (ESPGAN). Estos criterios exigen casi un año de arduo estudio con: a) una biopsia inicial positiva del intestino, b) 6 meses de dieta libre de gluten, c) una segunda biopsia del intestino negativa, d) una dieta con gluten durante 6 meses, y e) una tercera biopsia del intestino positiva.

El desarrollo de tests serológicos para tres anticuerpos diferentes del isotipo IgA [3] ha permitido la creación de unos criterios más rápidos y revisados de la ESPGAN en lo que se refiere a la enfermedad celíaca, tal y como se concluyó en 1990 [2]. Estos tests incluyen Anticuerpos IgA antiendomisio (EMA),[4] anticuerpos IgA antigliadina (AGA) y anticuerpos R1 antireticulina (ARA). Los criterios revisados de la ESPGAN requieren: a) una sola biopsia positiva del intestino y b) la demostración de la existencia de al menos dos de los tres tipos de anticuerpos IgA mencionados anteriormente. Desde entonces, muchos estudios han demostrado que los tests de IgA EMA tienen una especificidad por encima del 99% para ESG y una mayor sensibilidad para los tests de ARA o AGA.[5-13] Puesto que la IgA EMA desaparece cuando los pacientes con enfermedad celíaca o dermatitis herpetiforme siguen una dieta libre de gluten, los tests de anticuerpos también ayudan a comprobar el estricto cumplimiento de dichas dietas por los pacientes.[4,5,12]

Recientemente, se ha identificado el antígeno endomisial con el enzima conocido como transglutaminasa tisular (tTG).[14,15] Los procedimientos ELISA específicos para antígenos que incorporan tTG constituyen una alternativa fiable y objetiva a los ensayos tradicionales basados en la inmunofluorescencia que incorporan finas secciones de esófago de primate como sustrato.[14-19] El procedimiento ELISA puede adaptarse tanto a grandes como a pequeñas cantidades de muestras de pacientes. En los últimos dos años, el antígeno tTG humano se ha venido produciendo mediante tecnología recombinante. El antígeno humano recombinante puede tener ciertas ventajas comparado con el antígeno tradicional obtenido a partir del hígado de cobaya.[16,17,20]

Dos nuevos descubrimientos en el campo de la serología de la enfermedad celíaca son el uso de transglutaminasa tisular nativa humana aislada de los glóbulos rojos y el uso de tests capaces de detectar transglutaminasa tisular de clase IgG. El uso de tTG nativa de glóbulos rojos humanos en lugar de los de cobaya o los antígenos recombinantes humanos ofrece la ventaja de que facilita la purificación y la obtención de resultados en las preparaciones libres de bacterias, insectos u otras proteínas contaminantes.[21,22,23,24] Los ensayos ELISA de antígeno tTG humano presentes en la bibliografía disponible parecen funcionar bien en comparación con los tests más tradicionales basados en antígenos de cobaya.[16,17,20-23]

Los kits para anticuerpos antigliadina de clase IgG se han venido utilizando ya hace algún tiempo

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principalmente para ayudar en la evaluación de personas con carencia de IgA.Varios investigadores han demostrado la utilidad clínica de la detección de anticuerpos IgG anti tTG.[17,20,21] En un estudio, la sensibilidad del test sólo para el anticuerpo IgA aumentó de 91.5% a 98.5% cuando se tomaban en cuenta tanto los resultados de IgA como los de IgG.[17]

Procedimiento de trabajo

Los pocillos de la microplaca contienen antígeno nativo humano de la transglutaminasa tisular (h-tTG) aislado de los eritrocitos de sangre fresca que se ha unido en condiciones que mantienen su estado nativo. Se añaden controles y muestras convenientemente diluidas en pocillos separados, uniéndose durante la incubación los anticuerpos anti h-tTG IgA al antígeno que los recubre. El resto de componentes no unidos se elimina mediante lavado y se añade conjugado anti IgA humana a cada pocillo. Un segundo paso de incubación permite que el conjugado se una a los anticuerpos presentes. Tras un lavado que elimina el conjugado sobrante, se añade un sustrato cromogénico y tras incubación la actividad enzimática presente en el pocillo es proporcional a la intensidad de color desarrollado. El ensayo puede ser evaluado fotométricamente comparando la intensidad de color en las muestras con la de los controles.

Reactivos

1. Microplaca ELISA de poliestireno con pocillos recubiertos con antígeno h-tTG purificado, (12-1 x 8 pocillos), envasada en su soporte en una bolsa de aluminio con desecante.
2. Control negativo ELISA, 1 frasco de tampón conteniendo conservante y suero humano ausente de anticuerpos humanos anti h-tTG IgA, prediluido, 1.2 ml.
3. Control ELISA h-tTG IgA Positivo Débil, 1 frasco de tampón conteniendo conservante y suero humano con anticuerpos anti h-tTG IgA, prediluido, 1.2 ml.
4. Control ELISA h-tTG IgA Positivo Fuerte, 1 frasco de tampón conteniendo conservante y suero humano con anticuerpos anti h-tTG IgA, prediluido, 1.2 ml.
5. Diluyente de Muestra HRP, 1 frasco color rosado conteniendo tampón con Tris, Tween 20 y conservante, 50 ml.
6. Solución de Lavado Concentrada HRP, 1 frasco de concentrado 40x color rojo conteniendo tampón con Tris y Tween 20, 25ml. Referirse a la Sección "Metodología" para instrucciones de dilución.
7. Conjugado HRP IgA, con anti- IgA humana de cabra, 1 frasco - color amarillo conteniendo tampón, estabilizante de proteína y conservante, 10ml.
8. Cromógeno TMB, 1 frasco conteniendo estabilizantes, 10ml.
9. Solución de Parada HRP, Acido Sulfúrico 0.344 M, 1 frasco, incolora, 10 ml.

Advertencias

1. Aviso: Este producto contiene cloramfenicol al 0.02% en el Diluyente de muestras, controles y conjugado considerado en el estado de California como causante de cáncer.
2. Todo material de origen humano usado en la preparación de los controles para este producto se ha examinado resultando negativo para anticuerpos contra HIV, HbsAg, y HCV por métodos aprobados por la FDA. Ningún método puede sin embargo ofrecer garantía completa que HIV, HBV, HCV o otros agentes contagiosos estén ausentes. Por lo tanto, los controles h-tTG IgA ELISA positivo fuerte, h-tTG IgA ELISA positivo débil y ELISA negativo deben manejarse como si fueran material potencialmente contagioso.[25]
3. Dado que se utiliza azida sódica como conservante, este producto puede ser tóxico por ingestión o absorción a través de piel o mucosas. La azida sódica puede reaccionar con el plomo o cobre de las tuberías para formar azidas metálicas potencialmente explosivas. Si se utilizan desagües para la eliminación de reactivos se recomienda lavarlos con abundante agua para prevenir la formación de dichas azidas metálicas.
4. El Conjugado HRP contiene diluido un producto químico venenoso y corrosivo que puede ser tóxico si se ingiere. Para impedir quemaduras, evitar el contacto con piel o ojos.
5. El Cromógeno TMB contiene un irritante. Puede ser dañino si es inhalado, ingerido o absorbido por la piel. Evitar la inhalación, ingestión o contacto con piel y ojos.
6. La Solución de parada HRP consiste de una solución diluida de ácido sulfúrico. Evitar exposición a bases, metales, u otros compuestos que puedan reaccionar con ácidos. El ácido Sulfúrico es venenoso y corrosivo, puede ser tóxico si ingerido. Para impedir quemaduras, evitar contacto con piel y ojos.
7. Se recomienda utilizar el equipo protector apropiado al trabajar con este kit.
8. Los reactivos derramados deben limpiarse inmediatamente. Siga las normas aplicables en su

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laboratorio acerca de la eliminación de residuos.

Precauciones

1. Este producto es para ser usado en el Diagnóstico *In Vitro.*
2. La sustitución de componentes diferentes de los incluidos en el sistema puede generar resultados inconsistentes.
3. Un lavado incompleto o ineficiente y una eliminación insuficiente de líquido de los pocillos puede dar lugar a una pérdida de precisión y/o un fondo elevado.
4. La adaptación de este ensayo para su uso en procesadores automáticos u otros dispositivos, totalmente o en parte, puede producir diferencias en los resultados en comparación con el procedimiento manual. Es responsabilidad de cada laboratorio de validar sus procedimiento automatizado y comprobar que produce resultados dentro de los límites aceptables.
5. Existe una variedad de factores que influyen en la realización del ensayo. Esto incluye la temperatura inicial de los reactivos, la temperatura ambiente, la exactitud y reproducibilidad de técnica de pipeteo, la calidad de la técnica de lavando, el fotómetro que se utiliza para medir los resultados, y los tiempos de incubación durante el ensayo. Es necesario un exquisito cuidado y un trabajo consistente para obtener resultados exactos y reproducibles.
6. Se recomienda seguir estrictamente el protocolo.
7. El sellado incompleto de la bolsa "zip-lock" que contenga tiras sin usar y desecante provocará la degradación del antígeno que se apreciará en un empeoramiento de la precisión de resultados.
8. Pueden observarse absorbancias inaceptablemente bajas tras **dos** o más usos separados de un conjugado HRP. Es importante seguir todas las recomendaciones de manipulación específicas para este producto para evitar que esto ocurra.
9. La contaminación química del conjugado HRP puede ocurrir como resultado de una limpieza o secado inadecuados del equipo o instrumentos. Residuos de productos químicos comunes en el laboratorio como formalina, lejía, etanol, o detergente causan la degradación del conjugado HRP con el tiempo. Enjuague bien todo equipo o instrumentos después de usar dichos productos.

Condiciones de Almacenaje

1. Guardar todos los reactivos del kit en nevera a 2-8°C. No congelar. Los reactivos son estables hasta la fecha de caducidad si son almacenados y manipulados correctamente.
2. Las tiras sin utilizar deben volverse a guardar en la bolsa de aluminio que contiene desecantes cerrándola herméticamente y almacenándola a 2-8°C.
3. La solución de lavado diluida es estable 1 semana a 2-8°C.

Recolección de Muestras

Este kit requiere suero como muestra. La adición de azida u otros conservantes a las muestras puede afectar adversamente los resultados. No deben utilizarse muestras contaminadas, tratadas por calor o que contengan partículas visibles. Deben asimismo evitarse las muestras lipémicas o hemolizadas.

Tras la recolección de las muestras de sangre, el suero debe ser separado del coágulo. El documento NCCLS H18-A recomienda las siguientes condiciones de almacenaje para muestras: 1) Guardar las muestras a temperatura ambiente no más de 8 horas. 2) Si el ensayo no se va completar en 8 horas, refrigerar la muestra a 2-8°C. 3) Si el ensayo no se va completar entre 48 horas, o para enviar las muestras, congelar a -20°C o a temperatura inferior. Una vez descongeladas las muestras deben agitarse bien antes de utilizarse.

Procedimiento

Materiales Suministrados

1 Microplaca h-tTG IgA ELISA (12-1 x 8 pocillos) con soporte
1 1.2 ml control negativo ELISA prediluido
1 1.2ml control prediluido h-tTG IgA ELISA Positivo Débil
1 1.2ml control prediluido h-tTG IgA ELISA Positivo Fuerte
1 50ml Diluyente de muestra HRP
1 25ml Solución de lavado concentrada 40X
1 10ml Conjugado IgA (cabra) anti IgA humana
1 10ml Cromógeno TMB

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1 10ml Solución parada HRP (Ácido Sulfúrico 0.344M)

Material necesario no incluido

Micropipetas para 5, 100, 200-300, y 500µl
Puntas desechables para micropipeta
Tubos para dilución de muestras, 4ml
Agua destilada
Recipiente de 1L para la solución de lavado reconstituida
Lector de microplacas capaz de medir densidades ópticas a 450nm (y a 620nm para lecturas de doble longitud de onda

Metodología

Antes de empezar

1. Llevar todos reactivos y muestras a la temperatura ambiente (20-26°C) y agitar.
2. Diluir la solución de lavado HRP 1:40 añadiendo el contenido del envase con concentrado a 975 ml de agua destilada. Si no va a utilizar toda la placa, puede preparar una cantidad menor de solución de lavado añadiendo 2.0 ml del concentrado a 78ml de agua destilada para cada 16 pocillos que vayan a utilizarse. El tampón diluido es estable 1 semana a 2-8°C.
3. Preparar una dilución 1:101 de cada muestra a procesar añadiendo 5 µl de muestra a 500 µl de diluyente. Las muestras diluidas deben ser utilizadas dentro de las 8 horas de su preparación. **NO DILUIR** los controles h-tTG IgA ELISA positivo débil, positivo fuerte y ELISA negativo.
4. La determinación de la presencia o ausencia de anticuerpos anti h-tTG IgA usando unidades arbitrarias requiere dos pocillos para cada uno de los tres controles y uno o dos pocillos para cada muestra. Se recomienda que las muestras se hagan por duplicado.

Procedimiento de Ensayo

1. **TODOS REACTIVOS DEBEN ESTAR A TEMPERATURA AMBIENTE (20-26°C) ANTES DE EMPEZAR EL ENSAYO.** Ponga el número necesario de tiras en el soporte. **Devolver inmediatamente las tiras sin utilizar a la bolsa de aluminio y sellarla firmemente para minimizar la exposición a la humedad.**
2. Agregar 100µl de los controles prediluidos h-tTG IgA ELISA Positivo Débil, h-tTG IgA ELISA Positivo Fuerte, ELISA Negativo y las muestras prediluidas a los pocillos. Cubrir los pocillos e incubar 30 minutos a temperatura ambiente en una superficie plana. El tiempo de incubación empieza después de la adición de la última muestra.
3. Lavado: Aspirar el contenido de cada pocillo. Agregar 200-300µl de solución de lavado a todos pocillos y aspirar. Repetir esta secuencia dos veces más para un total de tres lavados. Invertir la placa y golpearla suavemente en material absorbente para eliminar cualquier fluido residual tras el último lavado. Es importante que cada pocillo esté completamente vacío después de cada paso de lavado. Mantener la misma secuencia para la aspiración que la usada para la adición de muestras.
4. Agregar 100µl de Conjugado IgA HRP a cada pocillo. El conjugado debe pipetearse en las condiciones más asépticas posibles y siguiendo buenas técnicas de laboratorio. Aspirar solamente la cantidad de conjugado necesaria para el ensayo. **PARA EVITAR CUALQUIER CONTAMINACIÓN POTENCIAL MICROBIANA Y/O QUÍMICA, NUNCA DEVOLVER EL CONJUGADO SIN USAR A SU RECIPIENTE ORIGINAL.** Incubar los pocillos 30 minutos como en el paso 2.
5. Lavado: Repetir el paso 3.
6. Agregar 100µl de Cromógeno TMB a cada pocillo e incubar 30 minutos en **oscuridad** a temperatura ambiente.
7. Agregar 100µl de Solución de parada a cada pocillo. Mantener la misma secuencia y temporalización que la efectuada en la adición de Cromógeno. Agitar suavemente la placa para mezclar bien los pocillos.
8. Leer la absorbancia (OD) de cada pocillo a 450nm en un plazo máximo de una hora. Si se desea seguir el método de lectura bicromática puede utilizarse 620 nm como longitud de onda de referencia.

Control de Calidad

1. Los controles h-tTG IgA ELISA Positivo Débil, h-tTG IgA ELISA Positivo Fuerte y Control Negativo ELISA deben procesarse con cada lote de muestras para asegurar que todos reactivos y procedimientos funcionan correctamente.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118 187

2. El usuario debe notar que debido a que los controles h-tTG IgA ELISA Positivo Débil, h-tTG IgA ELISA Positivo Fuerte y ELISA Negativo son prediluidos, no controlan procedimientos asociados con dilución de especímenes.
3. Pueden añadirse controles adicionales según las directivas o requisitos del laboratorio. Pueden prepararse controles válidos haciendo alícuotas de un "pool" de suero humano que debe almacenarse a ≤ -20°C.
4. Para considerar válidos los resultados deben considerarse satisfechos todos los criterios especificados a continuación, si alguno de ellos no se cumple la prueba debe considerarse inválida y repetirse.
   a. La absorbancia del control h-tTG IgA Positivo debe ser superior a la del control h-tTG IgA positivo débil y la de este debe ser superior a la del control negativo.
   b. El control h-tTG IgA positivo fuerte debe tener una absorbancia mayor que 1.0 mientras que la del control negativo no debe exceder de 0.2.
   c. La absorbancia del control h-tTG IgA positivo débil debe ser superior al doble del control negativo, u oscilar entre 0.25.
   d. Los controles ELISA negativo y h-tTG IgA positivo fuerte están pensados para monitorizar problemas de reactivo de magnitud substancial. El control h-tTG IgA positivo fuerte no puede asegurar precisión alguna en el punto de corte del ensayo.
   e. El usuario puede referirse al documento NCCLS (National Committee of Clinical Laboratory Standards) C24-A para una guía adicional acerca de buenas prácticas de laboratorio.

Cálculo de Resultados

Debe determinarse en primer lugar el promedio de densidades ópticas de cada juego de duplicados. La reactividad para cada muestra se calcula dividiendo la densidad óptica promedio de la muestra por la densidad óptica promedio del control h-tTG IgA positivo débil.

$$\text{Valor de la Muestra (unidades)} = \frac{\text{DO Muestra}}{\text{DO ELISA Positivo Débil}} \times \text{Valor ELISA Positivo Débil (unidades)}$$

La reactividad de la muestra está relacionada de manera no lineal con la cantidad de anticuerpo presente. Mientras que los aumentos y disminuciones en la concentración de anticuerpos del paciente se reflejarán en el aumento o disminución correspondiente de la reactividad, los cambios no son proporcionales (por ejemplo, al doblarse la concentración de anticuerpo no se dobla la reactividad). Si se deseara una cuantificación más precisa de la cantidad de anticuerpo, puede informarse como el título de anticuerpo de la muestra la última dilución positiva de una serie de diluciones seriadas de la muestra.

Interpretación de los Resultados

La técnica ELISA es muy sensible y es capaz de detectar pequeñas diferencias entre poblaciones de pacientes. Los valores presentados a continuación son sólo valores sugeridos. Cada laboratorio debe establecer su propio rango normal basado en su propia metodología, controles, equipo y población de pacientes.

La muestra puede clasificarse con un valor negativo, positivo débil, positivo moderado o positivo fuerte según la tabla siguiente.

	Unidades
Negativo	<20
Positivo Débil	20 – 30
Positivo Moderado a Positivo Fuerte	>30

1. Un resultado positivo indica la presencia de anticuerpos anti-h-tTG IgA y sugiere la posibilidad de ciertas enteropatías sensibles al gluten, como la enfermedad celíaca y la dermatitis herpetiforme.
2. Un resultado negativo indica la ausencia de anticuerpos anti-h-tTG IgA o niveles inferiores al punto de corte del ensayo.
3. Se sugiere que los resultados informados por el laboratorio incluyan la afirmación "Los siguientes resultados se obtuvieron con el kit INOVA Quanta Lite h-tTG IgA ELISA. Los valores obtenidos con kits de diferentes fabricantes pueden variar y no pueden intercambiarse entre ellos. La magnitud de los niveles informados de IgA no puede correlacionarse con un punto final".

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Limitaciones del Procedimiento

1. La presencia de inmunocomplejos u otros agregados de inmunoglobulinas en la muestra del paciente puede producir un aumento de uniones inespecíficas y causar falsos positivos en este ensayo.
2. Un resultado negativo de h-tTG IgA en un paciente no sometido a tratamiento no descarta una enteropatía sensible al gluten. Este hecho puede explicarse a menudo a partir de una deficiencia selectiva de IgA, cosa que suele ocurrir con relativa frecuencia en la enfermedad celíaca. INOVA ofrece un kit h-tTG IgG para estudiar estos pacientes (QUANTA Lite™ h-tTG IgG).
3. Los resultados de este ensayo deben utilizarse conjuntamente con hallazgos clínicos y otras pruebas serológicas.
4. La funcionalidad del ensayo no ha sido establecida para matrices diferentes del suero.

Valores Esperados

La capacidad del ensayo QUANTA Lite™ h-tTG (transglutaminasa tisular humana) IgA ELISA para detectar anticuerpos IgA antitransglutaminasa tisular fue evaluada comparándola con otro test para la detección de anticuerpos IgA anti-tTG, y comparando los resultados con diagnósticos clínicos reales realizados en dos evaluaciones clínicas externas y un estudio interno.

Rango Normal

El estudio de intervalo normal se realizó en el laboratorio de investigación de INOVA Diagnostics. Se analizó un total de 185 muestras. De estas muestras, 102 procedían de varones. Las edades estaban comprendidas entre los 6 y 60 años, con una media de 34.
Se analizó un total de 83 mujeres con edades comprendidas entre los 7 y 63 años, y una media de 31.

Cuando se testaron estas185 muestras normales con el kit QUANTA Lite™ h-tTG IgA, sólo una muestra dio positivo (0,5%). Esta única muestra estaba justo por encima del umbral de 20 unidades. Aparte de este positivo débil, la siguiente muestra con mayor reactividad obtuvo un valor de 11.5 unidades, y la mayoría de los valores restantes no pasaron de 10. El valor medio para este grupo de muestras normales fue de 4.4 unidades.

Sensibilidad y Especificidad Relativas

Para determinar la sensibilidad y especificidad del ensayo, un total de 257 muestras fueron analizadas con el kit QUANTA Lite™ h-tTG IgA y con otro kit comercial para el anticuerpo anti tTG. Los resultados se resumen a continuación:

		h-tTG +	h-tTG -		
	+	51	4*	Sensibilidad relativa	92.7%
cobaya transglutaminasa				Especificidad relativa	91.6%
de tejido hepático	-	17**	185	Eficiencia relativa	91.8%

* 2 de estas muestras dieron negativo para IgA en los tests de endomisio y gliadina.
** La mayoría de estas muestras pertenecían a pacientes celíacos, o que se sospechaba que podían serlo, y que habían dado unos resultados serológicos positivos débiles o negativos en ensayos anteriores.

Sensibilidad y Especificidad Clínicas

Se evaluó el kit QUANTA Lite™ h-tTG IgA (tissue transglutaminase) mediante un estudio clínico externo y otro interno. La tabla siguiente resume las conclusiones de ambos estudios.

Grupo de pacientes	Núm.	Núm. Pos	(%)
Normales	185	1***	(0.5)
Celíacos	24	24	(100)
Celíacos (pediátricos)	11	10	(91)
Celíacos con carencia de IgA	16	0	
Colitis ulcerativa	14	0	
Enfermedad de Crohn	6	1**	(17%)
Anemia perniciosa	6	0	
Hepatitis crónica activa	9	0	

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Varios positivos para anticuerpos del tejido conectivo	55	0

* Esta única muestra fue un positivo débil, con un valor de 20 unidades.

** Esta única muestra fue un positivo débil con 23 unidades y también dio positivo débil, esta vez con 22 unidades, con el kit QUANTA Lite™ tTG a base de antígeno de hígado de cobaya.

Reactividad Cruzada

Con el fin de evaluar mejor la especificidad del kit QUANTA Lite™ h-tTG IgA, se analizó un total de 90 pacientes con otros problemas gastrointestinales, así como pacientes con una serie de enfermedades con alta presencia de autoanticuerpos del tejido conectivo. También se analizó un total de 14 muestras de suero de pacientes con colitis ulcerativa, 6 con la enfermedad de Crohn, 6 con anemia perniciosa y 9 con hepatitis crónica activa. Además, se estudió un grupo de muestras de suero recogidas para elaborar otros kits ELISA de autoanticuerpos de INOVA. Se analizaron un total de 55 muestras de suero, incluyendo 8 positivos para SS-A, 6 positivos para SS-B, 9 positivos para Sm, 11 positivos para RNP, 13 positivos para Scl-70 y 8 positivos para Jo-1.

De estas 90 muestras, sólo dio positivo la de un paciente con la enfermedad de Crohn. El valor para este paciente fue tan sólo un positivo débil, con 23 unidades para IgA h-tTG. Cuando se analizó con el kit de tTG de cobaya, esta muestra también dio positivo con 22 unidades. Aparte de este positivo débil, ninguna otra muestra superó las 8 unidades.

Precisión y Reproducibilidad

La precisión y reproducibilidad del ensayo se midió mediante seis replicados de muestras fuertemente positivas, débilmente positivas y negativas en seis ensayos separados. El promedio de positivos fuertes fue de 132.6, de 25 para los positivos débiles y de 13.4 para los negativos. La desviación estándar y el coeficiente de variación para cada muestra se resume a continuación.

	Negativo		Positivo Fuerte		Positivo Débil	
	SD	CV	SD	CV	SD	CV
Global	1.05	7.8%	3.76	2.8%	0.66	2.6%
Intra ensayo	0.65	4.8%	3.01	2.3%	0.69	2.8%
Inter ensayo	0.78	5.8%	3.21	2.4%	0.44	1.8%

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QUANTA Lite™ h-tTG IgA (HumanTissue Transglutaminase) 708760

Uniquement pour "Diagnostics *In-Vitro*"

Application

QUANTA Lite™ h-tTG IgA est un test ELISA (Enzyme-linked Immunosorbent Assay) pour la détection semi-quantitative des anticorps anti-Transglutaminase de tissu (endomysium) d'isotype IgA dans le sérum humain. La recherche d'anticorps anti-h-tTG IgA peut être utilisée conjointement aux signes cliniques et à d'autres tests de laboratoire pour faciliter le diagnostic de certaines entéropathies sensibles au gluten comme la Maladie Cœliaque et la Dermatite Herpétiforme.

Informations concernant le test

La maladie cœliaque et la dermatite herpétiforme, les deux formes reconnues d'entéropathies sensibles au gluten (ESG) sont caractérisées par une inflammation de la muqueuse intestinale et un aplatissement de l'épithélium ou une « atrophie villositaire » positive[1, 2]. L'intolérance au gluten, qui est une protéine de blé, de seigle et d'orge, provoque une ESG. Les patients atteints de maladie cœliaque peuvent souffrir de diarrhée, de problèmes gastro-intestinaux, d'anémie, de fatigue, de problèmes psychiatriques et de divers maux qui y sont associés, ils peuvent aussi être cliniquement asymptomatique[2]. La dermatite herpétiforme est une maladie de peau associée aux ESG. Tous les patients ESG ont un risque augmenté de développer un lymphome[3]. Un régime sans gluten contrôle l'ESG et les risques associés.

La découverte en 1950 que le gluten est la cause de la maladie cœliaque a rendu le traitement de cette maladie possible en plaçant le patient sous un régime sans gluten. Grâce au développement de la fibroscopie pour effectuer une biopsie intestinale, les médecins ont pu confirmer leur diagnostic en surveillant l'atrophie villositaire. Comme l'atrophie villositaire peut être observée dans d'autres pathologies, l'association « European Society for Pediatric Gastroenterology and Nutrition » (ESPGAN) a établi les critères suivants pour confirmer le diagnostic d'une ESG, critères qui demandent un an d'étude laborieuse : a/ Biopsie initiale positive, b/ Biopsie négative après 6 mois de régime sans gluten c/ Biopsie positive après 6 mois de reprise d'une alimentation normale avec gluten.

Le développement de tests sérologiques pour la détection de 3 types d'anticorps d'isotype IgA[3] a rendu possible un diagnostic plus rapide de la maladie cœliaque et a d'ailleurs conduit les experts de l'ESPGAN à changer les critères de diagnostic en 1990[2]. Ces tests comprennent la détection des anticorps anti-IgA endomysium (EMA), des anticorps anti-IgA gliadine (AGA) et d'anticorps anti-IgA réticuline R1 (ARA). Ainsi les critères révisés de l'ESPGAN sont les suivants : a/ une unique biopsie positive, b/ la présence d'au moins 2 des 3 anticorps d'isotype IgA mentionnés plus haut. Depuis, différentes études ont démontré que les tests de détections des EMA IgA montrent plus de 99% de spécificité pour l'ESG et une plus grande sensibilité que les tests ARA ou AGA[5-13]. Comme les anticorps EMA IgA disparaissent quand les patients atteints de maladie cœliaque ou de dermatite herpétiforme suivent strictement un régime sans gluten, les tests de détection de ces anticorps sont aussi une aide précieuse pour le suivi de l'adhésion du patient à son régime sans gluten[4, 5,12].
Récemment, l'antigène endomysium a été identifié comme étant l'enzyme provoquant la réticulation (cross-linking) des protéines, la transglutaminase de tissu (tTG)[14,15]. L'antigène spécifique tTG incorporé dans un test ELISA permet de proposer une technique fiable, objective. C'est une alternative à la technique d'Immunofluorescence impliquant de fines coupes d'œsophage de primates comme substrat. La technique ELISA peut être adaptée à la fois à de petites ou de grandes séries. Au cours de ces deux dernières années, l'antigène humain tTG est produit par la technologie de recombinant. L'antigène humain recombinant offre certains avantages par rapport à l'antigène issu de foies de cobaye.[16,17,20]
Deux développements récents en sérologie de la maladie coeliaque sont l'utilisation de la transglutaminase de tissu humaine native, extraite des globules rouges et la mise au point des tests capable de détecter d'anti-transglutaminase d'isotype IgG. L'utilisation de tTG humaine native provenant de globules rouges plutôt que la tTG de cobaye ou tTG humaine recombinante bénéficie l'avantage d'une purification plus aisée permettant d'offrir une préparation exempte protéines contaminantes issues de bactéries ou d'insectes.[21, 22, 23, 24] Selon les publications, les tests ELISA utilisant l'antigène humain tTG fournissent de bons résultats, comparés aux tests traditionnels utilisant l'antigène extrait de foie de cobaye.[16, 17, 20, 23]

Les coffrets anti-IgG Gliadine sont utilisés depuis un certain temps pour aider au diagnostic des patients déficitaires en IgA. Plusieurs études ont montré qu'il est cliniquement utile de détecter les anti-tTG de classe

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118 191

IgG.[17, 20,21] Dans une des études, la sensibilité du test a augmenté de 91,5% pour l'anti-IgA seul à 98,5% lorsque l'on détecte à la fois l'anti-IgA et l'anti-IgG.[17]

Principe du test

L'antigène humain natif de transglutaminase de tissu (h-tTG), extrait des globules rouges fraîches, est fixé dans les puits d'une plaque de microtitration en polystyrène, selon les conditions qui préservent son état natif. Les contrôles pré-dilués et les sérums de patients dilués sont ajoutés dans différents puits. Une étape d´incubation permet la liaison entre les anticorps anti-h-tTG IgA présents dans le sérum et l´antigène immobilisé dans le puits. Les molécules non liées aux antigènes sont éliminées par lavage. Un conjugué enzymatique anti-IgA humaine est alors ajouté dans chaque puits pour révéler les auto-anticorps du patient. Après une étape d´incubation, le conjugué non fixé est éliminé par lavage. L´activité enzymatique résiduelle est quantifiée grâce à l´addition d´un substrat chromogène suivie d'une étape de mesure de l´intensité de la coloration ainsi développée. Les résultats peuvent être évalués par comparaison entre la couleur du puits échantillon et celle des puits de contrôle.

Contenu du coffret

1. Plaque de microtitration ELISA revêtue d´antigène h-tTG, portoir de 12 barrettes de 8 puits en polystyrène sécables, dans un sachet d'aluminium avec un dessiccant.
2. Contrôle Négatif pré-dilué, 1 flacon de 1,2 ml de sérum humain sans anticorps humains anti-h-tTG IgA, dans du tampon avec du stabilisateur.
3. Contrôle ELISA h-tTG IgA faiblement positif pré-dilué, 1 flacon de 1,2 ml de sérum humain avec des anticorps humains anti- h-tTG IgA, dans du tampon avec du stabilisateur.
4. Contrôle ELISA h-tTG IgA fortement positif pré-dilué, 1 flacon de 1,2 ml de sérum humain avec des anticorps humains anti-h-tTG IgA, dans du tampon avec du stabilisateur.
5. Diluant d´échantillons HRP, teinté en rose, 1 flacon de 50 ml contenant du tampon Tris salin, du Tween 20, des stabilisateurs de protéines et des conservateurs.
6. Tampon de lavage HRP concentré 40X – tampon Tris salin avec du Tween 20, teinté de rouge, 1 flacon de 25 ml. Consulter le paragraphe "Méthode" pour la dilution.
7. Conjugué IgA-HRP, anticorps de chèvre anti-IgA humaines dans du tampon avec des stabilisateurs de protéines et des conservateurs, teinté en jaune, 1 flacon de 10 ml.
8. TMB Chromogène, avec stabilisateurs, 1 flacon de 10 ml.
9. Solution d´arrêt HRP, acide sulfurique 0,344M, non teinté, 1 flacon de 10 ml.

Avertissements

1. ATTENTION : Le diluant des échantillons, les contrôles et le conjugué contiennent 0,02% de chloramphénicol, considéré comme cancérigène dans l'état de Californie.
2. Tout le matériel d'origine humaine utilisé pour la préparation des contrôles a été testé et trouvé négatif lors de la recherche d'anticorps anti-VIH, anti-antigène de surface de l'Hépatite B et anti-Virus de l'Hépatite C à l'aide de tests approuvés par la FDA. Cependant, aucune méthode ne peut donner une garantie complète quant à l'absence de VIH, VHB, du VHC ou d'autres agents infectieux. En conséquence, tous les sérums humains de contrôle doivent être manipulés avec les mêmes précautions que celles utilisées pour un matériel potentiellement infectieux.[25]
3. L´azide de sodium (NaN_3) est utilisé comme conservateur. NaN_3 est un poison et il est toxique en cas d´ingestion et de contact avec les yeux et la peau. Eviter de l´exposer aux bases, métaux ou autres composants, réagissant avec les acides. Après l´évacuation des réactifs, laver à grande eau afin d´éviter des dépôts dans les canalisations.
4. Le conjugué Peroxydase HRP contient un composant chimique dilué qui est un poison corrosif, toxique en cas d'ingestion en grande quantité. Pour éviter des brûlures chimiques, il est recommandé d'éviter tout contact avec la peau ou les yeux.
5. Le chromogène TMB est irritant et peut être toxique en cas d'inhalation, d'ingestion et d'absorption en grande quantité. Eviter tout contact avec la peau ou les yeux et toute inhalation.
6. La solution d'arrêt HRP est une solution diluée d'acide sulfurique. Eviter de l'exposer aux bases, aux métaux, aux oxydants ou tout autre composé susceptible de réagir avec les acides. L´acide sulfurique est toxique et corrosif en cas d´absorption. Eviter le contact avec la peau et les yeux pour éviter toute brûlure chimique.
7. Lors de la manipulation de ces produits, utiliser les équipements de protection personnelle appropriés.
8. Les éclaboussures de réactifs doivent être nettoyées immédiatement. Respecter les règlements en

vigueur concernant l´élimination des déchets.

Précautions

1. Ce test est pour un usage diagnostic in vitro.
2. Toute substitution par d´autres réactifs que ceux fournis dans le coffret entraîne des variations de résultats.
3. Un lavage incomplet ou insuffisant et une aspiration insuffisante du liquide dans les puits d´ELISA entraînent des imprécisions et un bruit de fond élevé.
4. L'automatisation, complète ou partielle, du traitement des échantillons dans ce test peut produire des différences de résultats par rapport au traitement manuel. Chaque laboratoire est responsable de la validation des procédures automatiques afin que les résultats des tests restent dans les limites acceptables.
5. Un certain nombre de facteurs influencent la performance du test: la température initiale des réactifs, la température ambiante, l´exactitude et la reproductibilité de la technique de pipetage, la qualité du lavage et de vidange des puits d´ELISA, le photomètre utilisé pour mesurer les résultats et la durée des temps d´incubation pendant le test. Il est donc recommandé de prêter attention à tous ces facteurs pour obtenir des résultats reproductibles et rigoureux.
6. Il est recommandé de suivre strictement le protocole. Chaque modification est au risque de l´utilisateur.
7. La fermeture incomplète de la pochette contenant les puits de microtitration et le dessiccant entraîne la dégradation de l´antigène et en conséquence une mauvaise précision des résultats.
8. Des valeurs faibles et inacceptables d´absorption peuvent être progressivement observées dès le **deuxième** prélèvement dans le même flacon de conjugué HRP. Il est donc important de suivre exactement les instructions fournies pour éviter ce risque.
9. Un nettoyage ou rinçage inapproprié des appareils et instruments pourrait provoquer une contamination chimique du conjugué HRP. Des résidus de produits chimiques courants de laboratoire comme le formol, l´eau de javel, l´éthanol ou les détergents, entraînent la dégradation progressive du conjugué HRP. Il est absolument recommandé de rincer abondamment tous les instruments et appareils après l´utilisation des détergents chimiques.

Conditions de conservation

1. Conserver tous les réactifs du coffret entre 2-8°C.Ne pas congeler. Les réactifs sont stables jusqu´à la date limite d'utilisation dans des conditions de stockage et d'utilisation conformes.
2. Les puits non utilisés devront être remis dans l'emballage en aluminium avec le dessiccant, bien refermé et stocké à 2-8°C.
3. La solution de tampon diluée reste stable une semaine à 2-8°C.

Echantillons

Ce test doit être réalisé avec des sérums comme échantillons. L´addition d´azide ou d´autres conservateurs aux échantillons peut affecter le bon déroulement de la procédure. Les sérums contaminés par des agents microbiens, prétraités par la chaleur ou contenant des particules visibles ne doivent pas être utilisés. Les sérums ou échantillons hémolysés ou lipémiques sont à éviter.

Après prélèvement, le sérum doit être séparé du culot. Le document H18-A du NCCLS recommande les conditions suivantes de conservation: 1) Conserver les échantillons à température ambiante pas plus de 8 heures. 2) Si le test ne peut pas être fait dans les 8 heures, conserver les échantillons à 2-8°C. 3) Si le test ne s'effectue pas dans les 48 heures ou si on doit envoyer les échantillons, les congeler à –20°C ou à plus basse température. Bien agiter les échantillons décongelés.

Procédure

Matériel fourni

1 Plaque de microtitration ELISA h-tTG IgA, (12-1 x 8 puits), avec support
1 1,2 ml Contrôle ELISA Négatif pré-dilué
1 1,2 ml Contrôle ELISA faiblement positif h-tTG IgA pré-dilué
1 1,2 ml Contrôle ELISA fortement positif h-tTG IgA pré-dilué
1 50 ml diluant HRP pour échantillons
1 25 ml tampon de lavage HRP concentré 40X
1 10 ml conjugué anti-IgA humaines de chèvre

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1 10 ml substrat TMB
1 10 ml solution d´arrêt HRP (acide sulfurique 0,344M)

Autre matériel nécessaire non fourni

Pipettes 5, 100, 200-300 et 500 µl
Cônes jetables
Tubes de 4ml pour la dilution de sérum
Eau distillée ou déionisée
Récipient de 1 litre pour le tampon de lavage HRP dilué
Lecteur ELISA avec filtre de 450nm (et 620nm pour les lectures à double longueur d´ondes)

Méthode

Préparation du test

1. Porter tous les réactifs et échantillons à la température ambiante (20-26°C) et bien les mélanger avant de les utiliser.
2. Diluer la totalité de la solution de lavage (25ml) avec 975 ml d´eau distillée (1/40). La solution de tampon diluée reste stable une semaine à 2-8°C. Si la totalité de la plaque de microtitration n´est pas utilisée en une seule fois, un plus petit volume de tampon sera suffisant, par exemple pour traiter 16 puits, diluer 2ml de tampon concentré dans 78ml d´eau distillée.
3. Préparer les sérums des patients en les diluant au 1/101 dans le diluant d'échantillon (5µl dans 500µl). Les sérums doivent être utilisés dans les 8 heures qui suivent leur dilution. **Ne pas diluer** les contrôles ELISA fortement positif, faiblement positif en h-tTG IgA et négatif.
4. La détermination de la présence ou de l'absence de la h-tTG IgA en unités arbitraires nécessitent deux puits pour chacun des trois contrôles et un ou deux puits pour chacun des sérums de patients. Il est recommandé de tester les échantillons en double.

Exécution du test

1. PORTER TOUS LES REACTIFS ET LES ECHANTILLONS À TEMPERATURE AMBIANTE (20-26°C) AVANT DE LES UTILISER. Placer le nombre nécessaire de micro puits ou de barrettes sur le portoir. Remettre immédiatement les barrettes non utilisées dans la pochette en aluminium avec le dessiccant, fermer hermétiquement pour éviter toute condensation de vapeur d'eau.
2. Distribuer 100 µl de chacun des contrôles ELISA fortement, faiblement positifs en h-tTG IgA et négatif pré-dilués et de sérums des dilués patients dans les puits. Recouvrir les barrettes et laisser incuber pendant 30 minutes à température ambiante. Le temps d´incubation commence après l´ajout du dernier échantillon.
3. Lavage: aspirer le contenu de tous les puits. Ajouter 200-300µl de tampon dilué dans tous les puits et l'aspirer ensuite complètement. Répéter cette opération deux fois supplémentaires pour un total de trois lavages. Après le dernier lavage, retourner la plaque en la tapotant sur du papier absorbant, pour enlever tout liquide de lavage résiduel. Pour l'aspiration, maintenir la même séquence que celle utilisée lors de la distribution des échantillons.
4. Distribuer 100 µl de conjugué HRP IgA dans chaque puits. Le conjugué doit être prélevé du flacon dans des conditions aseptiques. Prélever en une seule fois la quantité de conjugué nécessaire à la réalisation de la série. POUR EVITER LE RISQUE DE CONTAMINATION, NE JAMAIS REMETTRE LE RESTE DU CONJUGUE NON UTILISE DANS LE FLACON. Recouvrir les barrettes et incuber pendant 30 minutes à température ambiante, comme décrit à l'étape 2.
5. Lavage: Répéter la procédure décrite à l'étape 3.
6. Distribuer 100 µl de chromogène TMB dans chaque puits et laisser incuber à l´obscurité 30 minutes à température ambiante.
7. Ajouter 100 µl de solution d´arrêt HRP dans chaque puits. Maintenir la même séquence et le même timing lors de l´addition de la solution d´arrêt que ceux utilisés lors de la distribution du chromogène TMB. Tapoter délicatement les plaques pour bien mélanger le contenu des puits.
8. Lire la densité optique (D.O.) de chaque puits à 450nm dans l´heure qui suit l´arrêt de la réaction. Pour une lecture bichromatique, la longueur d´onde de référence peut être 620nm.

Contrôle de qualité

1. Les contrôles ELISA fortement positif h-tTG IgA, faiblement positif en h-tTG IgA et négatif doivent être inclus à chaque série de tests afin de s´assurer du bon fonctionnement des réactifs et du bon

déroulement de la procédure.

2. Etant donné que les contrôles ELISA fortement positifs h-tTG IgA, ELISA faiblement positifs en h-tTG IgA et négatifs sont prédilués, il n'est pas possible de valider le procédé de dilution des échantillons.
3. Des contrôles de qualité supplémentaires peuvent être réalisés selon les directives nationales, les réglementations internationales ou celles des organismes d´accréditation. Des contrôles appropriés peuvent être obtenus en aliquotant un pool de sérums humains conservé à une température inférieure ou égale à -20°C.
4. Pour valider les résultats obtenus, tous les critères décrits ci-dessous devront être vérifiés. Si un ou plusieurs de ces critères ne sont pas réalisés, le test devra être considéré comme non valide et à refaire.
   a. La densité optique du Contrôle ELISA fortement positif en h-tTG IgA pré-dilué doit être supérieure à celle du contrôle ELISA faiblement positif en h-tTG IgA pré-dilué. D´autre part, la densité optique du contrôle ELISA faiblement positif en h-tTG IgA doit être supérieure à celle du contrôle ELISA négatif pré-dilué.
   b. La densité optique du contrôle ELISA fortement positif en h-tTG IgA pré-dilué doit être supérieure à 1,0. Celle du contrôle négatif pré-dilué doit être inférieure à 0,2.
   c. L´absorbance du Contrôle ELISA faiblement positif en h-tTG IgA doit être deux fois supérieure à celle du contrôle négatif d´ELISA ou supérieure à 0,25.
   d. Les contrôles ELISA négatif et ELISA fortement positif en h-tTG IgA permettent de contrôler le bon fonctionnement des tests. Le Contrôle ELISA fortement positif en h-tTG IgA n´assure pas la précision de la valeur seuil du test.
   e. L´utilisateur du test peut se référer au document C24-A du NCCLS pour des informations supplémentaires concernant le contrôle qualité.

Calcul des résultats

Déterminer d´abord la valeur moyenne des duplicates. La réactivité de chaque échantillon est calculée en divisant la DO moyenne de l'échantillon par la DO moyenne du contrôle faible et multiplier le résultat obtenu par la valeur (en unités) affectée au contrôle faible.
La valeur en unités est indiquée sur l'étiquette du flacon de contrôle ELISA positif faible en h-tTG IgA.

$$\text{Valeur de l´échantillon (unités)} = \frac{\text{DO de l´échantillon}}{\text{DO du contrôle faible}} \times \text{valeur du contrôle faible (unités)}$$

La réaction n´évolue pas de manière linéaire en fonction de la quantité d´anticorps présents dans le sérum. L'augmentation et la diminution de la concentration d´anticorps se traduisent par une augmentation ou une diminution de réactivité correspondante mais ces modifications du signal ne sont pas proportionnelles (par exemple un doublement de la quantité d´anticorps ne fera pas doubler le signal). Pour une évaluation quantitative rigoureuse des anticorps du patient, il faudra réaliser des dilutions sériées du sérum. Dans ce cas, la dernière dilution du sérum qui donne encore un signal positif sera considéré comme étant le titre en anticorps du patient.

Interprétation des résultats

Le test ELISA est une technique très sensible et peut en conséquence détecter de très faibles différences au sein d´un groupe de patients. Les valeurs limites détaillées dans le paragraphe "calcul des résultats" sont indicatives. Il est recommandé à chaque laboratoire d´établir ses propres valeurs seuils d´interprétation en fonction de ses techniques, contrôles, équipements et populations de patients.

Les échantillons peuvent être interprétés comme négatifs, faiblement, modérément ou fortement positif selon les valeurs en unités détaillées ci-dessous:

	Unités
Négatif	<20
Faiblement positif	20 – 30
Modérément á Fortement positif	>30

1. Un résultat positif indique la présence d´anticorps h-tTG IgA et la possibilité du certaines entéropathies sensibles au gluten comme la Maladie Coeliaque et la Dermatite Herpétiforme.
2. Un résultat négatif indique l'absence d´anticorps anti-h-tTG IgA ou bien un taux d'anticorps

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en dessous de la valeur seuil.

3. Nous suggérons de rendre les résultats avec la remarque suivante: "Les résultats suivants ont été obtenus avec le test ELISA INOVA QUANTA Lite™ h-tTG IgA. Les valeurs d'anti-h-tTG IgA obtenues avec des tests de différents fabricants ne sont pas interchangeables. Le taux d'anti-IgA trouvé n'est pas corrélé à une titration en point final."

Limites du test

1. La présence de Complexes Immuns ou d´autres agrégats d´Immunoglobulines dans le sérum humain peut entraîner l'augmentation de liaisons non spécifiques et des résultats faussement positifs.
2. Un résultat négatif en h-tTG IgA chez un patient non traité n'exclue pas une entéropathie sensible au gluten. Ce cas de figure est souvent expliqué par une déficience sélective en IgA, assez courant en maladie cœliaque. INOVA présente le coffret h-tTG IgG pour tester ces patients (QUANTA Lite™ h-tTG IgG).
3. Les résultats obtenus à l´aide de ce test devront être corrélés avec les signes cliniques et d´autres tests sérologiques.
4. Les performances du coffret pour les échantillons autres que les sérums n'ont pas été établies.

Performances

La capacité du coffret ELISA QUANTA Lite™ h-tTG (transglutaminase de tissu humaine) IgA de détecter les anti-htTG IgA est évaluée par comparaison avec un autre test de détection des anti-IgA tTG et avec les données cliniques issues de 2 études cliniques externes et d' une étude interne.

Valeur normale

L'étude de la valeur normale a été réalisée au sein du laboratoire de recherche de INOVA Diagnostics. Un total de 185 échantillons ont été testés : 102 personnes de sexe masculin, âgées de 6 à 60 ans, (âge moyen de 34 ans) et 83 personnes de sexe féminin, âgées de 7 à 63 ans (âge moyen de 31 ans).
Lorsque l'on utilise le coffret QUANTA Lite™ h-tTG IgA, un seul échantillon est positif (0,5%). Cet échantillon est juste au dessus du seuil de 20 unités. En dehors de cet échantillon faiblement positif, l'échantillon le plus réactif suivant a 11,5 unités et la plupart des autres échantillons ont des valeurs à un seul chiffre. La valeur moyenne de ce groupe d'échantillons normaux est de 4,4 unités.

Sensibilité et spécificité relatives

Pour déterminer la sensibilité et la spécificité du coffret, 257 échantillons ont été testés à la fois avec le coffret QUANTA Lite™ h-tTG IgA et un autre coffret ELISA anti-tTG déjà commercialisé. Les résultats sont résumés ci-dessous :

		h-tTG +	h-tTG -		
tTG de foie de cobaye	+	51	4*	Sensibilité Relative	92,7%
	-	17**	185	Spécificité Relative	91,6%
				Efficacité Relative	91,8%

* 2 de ces sérums sont négatifs en endomysium et en gliadine IgA
** La plupart de ces serums proviennent de patients confirmés ou suspectés d'avoir la maladie coeliaque. Ils ont été rendus faiblement positifs ou négatifs lors du test précédent.

Sensibilité et spécificité clinique

Le coffret QUANTA Lite™ h-tTG IgA a été évalué dans une études cliniques externes et une interne. Le tableau ci-dessous résume les résultats de l'ensemble de ces études :

Groupe de patients	Nombre	h-tTG IgA pos.	(%)
Sains	185	1*	(0,5)
Maladie Cœliaque	24	24	(100)
Maladie Cœliaque Pédiatrique	11	10	(91)
Maladie Cœliaque déficient en IgA	16	0	
Colite ulcéreuse	14	0	
Maladie de Crohn	6	1**	(17%)
Anémie pernicieuse	6	0	

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Hépatite chronique active	9	0
Connectivite mixte avec autoanticorps	55	0

* Cet échantillon est faiblement positif à 20 unités.

** Cet échantillon est faiblement positif à 23 unités et est aussi faiblement positif à 22 unités avec le coffret QUANTA Lite™ tTG dont l'antigène est issu du foie de cobaye.

Réactions croisées

Pour vérifier la spécificité du coffret QUANTA Lite™ h-tTG IgA, 90 patients atteints d'autres problèmes gastro-intestinaux ou de connectivite avec des taux élevés en auto-anticorps ont étés testés. 14 patients sont atteints de colite ulcéreuse, 6 de maladie de Crohn, 6 d'anémie pernicieuse, 9 d'hépatite chronique active. A été aussi testé un ensemble de sérums, servant à la fabrication d'autres coffret ELISA d'auto-anticorps de INOVA. Il y a en tout 55 sérums dont 8 SS-A positifs, 6 en SS-B, 9 en Sm, 11 en RNP, 13 en Scl-70 et 8 en Jo-1.

Parmi ces 90 sérums, il n'y a qu'un seul positif, provenant d'un patient de maladie de Crohn. Il est faiblement positif à 23 unités en IgA h-tTG. Lorsque cet échantillon a été testé avec le coffret tTG dont l'antigène provient du cobaye, il est aussi positif à 22 unités. A part cet échantillon faiblement positif, aucun ne dépasse les 8 unités.

Précision

Un sérum négatif , un sérum faiblement positif et un sérum fortement positif ont été testés chacun 6 fois par essai dans 6 essais séparés. La valeur moyenne du sérum fortement positif est de 132,6 unités, celle du faiblement positif est de 25 unités et celle du négatif est de 13,4 unités. Les écarts type (ET) et les coefficients de variation (CV) de chaque échantillon sont résumés dans le tableau ci-dessous.

	Négatif		Fortement Positif		Faiblement Positif	
	ET	CV	ET	CV	ET	CV
Global	1,05	7,8%	3,76	2,8%	0,66	2,6%
Intra-essais	0,65	4,8%	3,01	2,3%	0,69	2,8%
Inter-essais	0,78	5,8%	3,21	2,4%	0,44	1,8%

References, Referenzen, Bibliografia, Referencias, Références

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Appendix D

ImmuGlo EMA Product Insert

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IMMCO® DIAGNOSTICS

ImmuGlo™

Anti-Endomysial Antibody (EMA) Test System

For *in vitro* Diagnostic Use
CLIA Complexity: High
CDC Analyte Identification Code: 0497
CDC Test System Identification Code: 28281

PRODUCT INSERT

Catalog No. 1114 *48 Determinations*

INTENDED USE

An indirect immunofluorescence antibody test for the qualitative and semi-quantitative detection of endomysial antibodies (EMA) in human serum as an aid in the diagnosis of celiac disease and dermatitis herpetiformis.

SUMMARY AND EXPLANATION

Endomysial antibodies (EMA), as reported in the literature, are detected primarily on the smooth muscle of monkey esophagus by indirect immunofluorescence. The detection of EMA aids in the diagnosis of *gluten sensitive enteropathy*, i.e. *celiac disease* (CD) and *dermatitis herpetiformis* (DH). Patients with CD and DH are reported to have antibodies to endomysium, reticulin and gliadin[1-12]. These serological markers have recently been incorporated into the revised criteria for the diagnosis of CD by the European Society of Pediatric Gastroenterology and Nutrition[13]. Of the various antibody markers of CD and DH, EMA of the IgA class seem to be the most sensitive and specific marker. EMA of the IgG class also occur when IgA class EMA are in high titer or in individuals who are IgA deficient. A rapid decrease in EMA levels results with adherence to a gluten free diet. A gluten challenge or a failure to maintain a gluten free diet leads to the appearance or an increase in endomysial antibody titers. Patients on a gluten free diet >9 months have reduced or negative EMA titers if they adhere to their diet restrictions[1,6-8,10].

PRINCIPLES OF PROCEDURE

In the indirect immunofluorescence method, patient serum is incubated on tissue sections to allow binding of antibodies to the substrate. Any antibodies not bound are removed by rinsing. Bound antibodies of the IgA and IgG class are detected by incubation of the substrate with fluorescein-labeled, anti-human immunoglobulin conjugate. Reactions are observed under a fluorescence microscope equipped with appropriate filters. The presence of EMA is demonstrated by an apple green fluorescence of the endomysial lining of smooth muscle bundles. The titer (the reciprocal of the highest dilution giving a positive reaction) of the antibody is then determined by testing serial dilutions[14]

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REAGENTS

Storage and Preparation

Store all reagents at 2°-8°C. Ready for use after equilibration to room temperature.

Materials Provided

Anti-Endomysial Antibody (EMA) Test System. *Catalog No. 1114*

Kit contains sufficient reagents to perform 48 determinations

8 x	6-well - Primate Smooth Muscle Tissue Substrate Slides.
1 x 0.5 ml	EMA Positive Control*.
1 x 0.5 ml	Negative Control*.
1 x 5.0 ml	Goat anti-human polyvalent FITC Conjugate. Ready to use. **Protect from light***.
1 x 60 ml	Buffered Diluent. Ready to use*.
2 vials	Phosphate Buffered Saline. **Dissolve each vial to 1 liter.**
1 x 5.0 ml	Mounting Medium. **Do not freeze***.
1 x 1.0 ml	Evans Blue Counterstain
1 x 12	Cover Slips

*CAUTION - Contains <0.1% NaN_3

Precautions

For *in vitro* Diagnostic Use. All human derived components used have been tested for HBsAg, HCV, HIV-1 and 2 and HTLV-I and found negative by FDA required tests. All human serum specimens and human derived products should be treated as potentially hazardous, regardless of their origin. Follow good laboratory practices in storing, dispensing and disposing of these materials[15].

WARNING - Sodium azide (NaN_3) may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal of liquids, flush with large volumes of water to prevent azide buildup. Sodium azide may be toxic if ingested. If ingested, report incident immediately to laboratory director or poison control center.

Instructions should be followed exactly as they appear in this insert to ensure valid results. Do not interchange kit components with those from sources other than the same catalog number from IMMCO Diagnostics. Do not use beyond expiration date.

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Materials Required but not Provided

- Fluorescence microscope
- Micropipette or Pasteur pipette
- Serological pipettes
- Staining dish (e.g. Coplin jar)
- Small test tubes (e.g. 13 x 75 mm) and test tube rack
- Distilled or deionized water
- 1 liter container
- Wash bottle
- Paper towels
- Incubation chamber

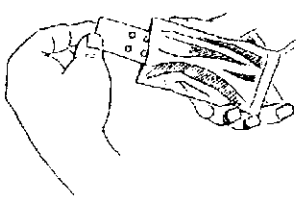
SPECIMEN COLLECTION AND PREPARATION

Only serum specimens should be used for this procedure. Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of this test and should not be used. Store specimens at 2°- 8°C for no longer than one week. For longer storage, serum should be frozen at -20°C. Avoid repeated freezing and thawing of samples.

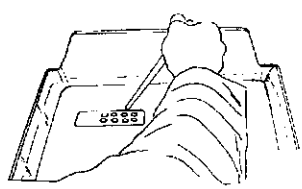
PROCEDURE

Test Method

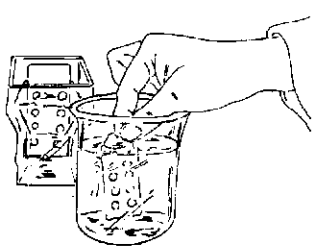
The indirect immunofluorescence staining procedure is illustrated in the following figures:



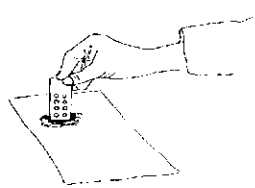
1. Let pouch equilibrate to room temperature, then remove slide(s) from pouch.



2. Place slide(s) into moisture chamber and add samples and controls. Cover and incubate 30 minutes.

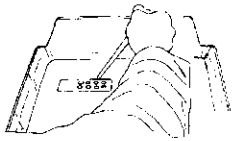


3. Rinse slide(s) by dipping into beaker with PBS. Transfer slide(s) into Coplin jar and wash 10 minutes.

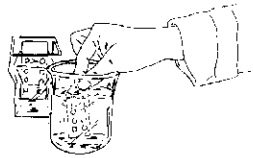


4. Blot edge of slide(s) on absorbent paper. Proceed immediately with Conjugate step.

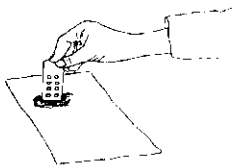
Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118



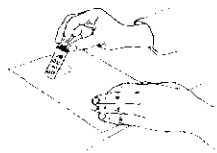
5. Apply Conjugate to each well. Cover and incubate 30 minutes.



6. Rinse slide(s) by dipping into beaker with PBS. Transfer slide(s) into Coplin jar and wash 10 minutes.



7. Blot edge of slide(s) on absorbent paper. Proceed immediately with next step.



8. Mount cover slip and read under fluorescent microscope.

A. Screening:

Step 1. Dilute each patient serum **1:2.5** with the Buffered Diluent provided (0.2 ml serum + 0.3 ml Diluent). **Do not dilute the Positive or Negative controls**. Save the undiluted sera to determine antibody titers if screening tests are found to be positive.

Step 2 Allow pouches containing substrate slides to equilibrate to room temperature for **10-15 minutes**. Carefully remove the slides without touching the substrate.

Step 3 Label the slides and place them in an incubation chamber lined with paper towels moistened with water to prevent drying.

Step 4 Invert dropper vial and gently squeeze to apply **1 drop** (approximately 50 µl) of the **Negative Control** to well #1. Similarly apply **1 drop** of **Positive Control** to well #2. Avoid overfilling the wells.

Step 5 Using a micropipette or Pasteur pipette, apply **1 drop** of patient's diluted serum (approximately 50 µl) to the other wells. Avoid overfilling the wells.

Step 6 Place the lid on the incubation chamber and incubate slides **30 minutes** at room temperature.

Step 7 Remove a slide from the incubation chamber. Hold slide at tab end and rinse gently with approximately **10 ml** of PBS using a pipette, or rinse slide in a beaker filled with PBS. Do not use a wash bottle. Transfer slide immediately into Coplin jar and wash **10 minutes**. Repeat process with all remaining slides.

Step 8 Remove slide(s) from Coplin jar. Blot the edge of the slide on a paper towel to remove excess PBS. Place the slide in the incubation chamber. Immediately invert the **Conjugate** dropper vial and gently squeeze to apply **1 drop** (approximately 50 µl) to each well.

Step 9. Repeat **Steps 7 and 8** for each slide.

Step 10 Replace the lid on the incubation chamber. Incubate **30 minutes** at room temperature.

Step 11 Remove a slide from incubator. Hold the slide at the tab end and dip the slide in a beaker containing PBS to remove excess conjugate. Place slide(s) in a staining dish filled with PBS for **10 minutes**. If desired, 2-3 drops of Evans blue counterstain may be added to the final wash. Repeat for the remaining slides. **NOTE:** Improper washing may lead to increased background fluorescence.

Step 12 Remove a slide from the staining dish. Blot the edge of the slide on a paper towel to remove excess PBS. **To prevent slide from drying, proceed immediately with Step 13 while the slide is still wet.**

Step 13 Mount the coverslip. Place **3 drops** of the Mounting Medium evenly spaced on a coverslip and invert the slide onto the coverslip. To remove any air bubbles gently apply pressure along the edge of the coverslip. Avoid any movement of the coverslip.

Step 14 Repeat **Steps 12 and 13** for each slide.

Step 15 Examine for specific fluorescence under a fluorescence microscope at a magnification of **200x** or greater.

Slides may be read as soon as prepared. However, because of the presence of antifading agent in the mounting medium, no significant loss of staining intensity occurs if reading is delayed. Slides should be stored in the dark at 2-8°C.

B: <u>End Point Determination</u> (Titration)

A serum positive in the screening test may be further tested following **Steps 5 through 13** to determine the titer. Each test run should include the Positive and Negative Controls. Make serial twofold dilutions starting at 1:5. Using one slide, a serum may be tested at dilutions ranging from 1:5 to 1:40. If positive at a 1:40 dilution, the titer is reported as greater or equal to 40, or additional slides may be used to obtain endpoints. The reciprocal of the highest dilution producing a positive reaction is the titer.

Preparation of Serial Dilutions

Number four tubes 1 through 4. Add 0.4 ml of Buffered Diluent to tube 1 and 0.2 ml to tubes 2 through 4. Pipette 0.1 ml of undiluted serum to tube 1 and mix thoroughly. Transfer 0.2 ml from tube 1 to tube 2 and mix thoroughly. Continue transferring 0.2 ml from one tube to the next after mixing to yield the dilutions depicted in the following table:

Tubes	1	2	3	4
Serum	0.1 ml			
+				
Buffered Diluent	0.4 ml	0.2 ml	0.2 ml	0.2 ml
Transfer		0.2 ml	0.2 ml	0.2 ml
Final dilution	1:5	1:10	1:20	1:40 etc.

Quality Control

Both the Positive and Negative Controls should be included with each test run. The Negative Control should show no specific fluorescence of the endomysium lining of the smooth muscle bundles, whereas the Positive Control should have 2+ or greater staining intensity of the tubules of these structures.

If expected results are not obtained, the run should be repeated. If inadequate results continue to occur with the controls, these may be due to:

- If signs of turbidity is seen, discard and use another control.
- Problems with the optical system of the fluorescence microscope. These may include: improper alignment, use of the bulb beyond the expected performance life, etc.
- Allowing the slide to dry during the procedure.

RESULTS

The results of the tests for endomysial antibodies should be reported as negative (<2.5), positive greater or equal to 40, or preferably, positive with titer.

Read for specific staining of the endomysium lining of the smooth muscle bundles. **See Figure 1a**. Endomysial antibodies react as a network of thin, irregular lines around the sarcolemma of the individual smooth muscle fibrils. This is in a sharp contrast to anti-smooth muscle antibodies which react with the sarcoplasm. **See Figure 1b**.

Other detectable antibodies besides anti-smooth muscle antibodies (ASMA) include antinuclear antibodies (ANA). The presence of ASMA is known to cause false negative results for endomysial antibodies. If ASMA are detected, then the sample should be tested at higher dilutions[1]. ANA reactions on smooth muscle tissue, when they occur, are usually weak and sparsely distributed and, therefore, unlikely to cause false negative results.

Figure 1a. EMA staining reaction on primate smooth muscle, 200X. Note staining of lining of the smooth muscle bundles.

Figure 1b. ASMA staining reaction on primate smooth muscle, 200X. Note staining of the smooth muscle sarcoplasm.

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LIMITATIONS OF THE PROCEDURE

The ImmuGlo™ EMA IFA Test System: CLIA Complexity: High; CDC Analyte Identification Code: 0497; CDC Test System Identification Code: 2828.

In some cases, sera positive for EMA may either be very weak or negative at the initial screening dilution (prozone phenomenon). In such doubtful cases the sera should be screened at higher dilutions and, if positive, antibody titer determined.

The presence of two or more antibodies in a serum which are reactive with the same tissue may cause an interference in their detection by immunofluorescence. This interference may cause either a failure to detect EMA or a suppression of its titer if the interfering antibody has a higher titer than EMA. The most common cause of the interference phenomenon in EMA tests is the coexistence of smooth muscle antibodies. It is recommended that patients sera which also contain ASMA be tested further at higher dilutions. IgA class ASMA are not a common occurrence. IgG class ASMA do not block IgA-EMA as the former react with the sarcoplasm of smooth muscle bundles and the latter react with the endomysium of the sarcolemma around the smooth muscle bundles. Anti-reticulin antibodies do not interfere with the reaction of EMA because they do not react with primate smooth muscle tissue. The coexistence of IgG class EMA may interfere with the detection of IgA class EMA. However, this rarely occurs as:

1. IgG class - EMA are present in only 25% of celiac disease patients,
2. IgG class - EMA titers are usually much lower than IgA-EMA titers and
3. IgA antibodies are usually of higher avidity than IgG antibodies.

In some patients with celiac disease and IgA deficiency, the IgA-class endomysial antibodies are absent. However, such patients are usually positive for IgG class EMA.

Patients with celiac disease on a gluten free diet for >9 months invariably are negative for EMA.

When making a diagnosis, results of all laboratory testing must always be evaluated along with the total clinical history of the patient.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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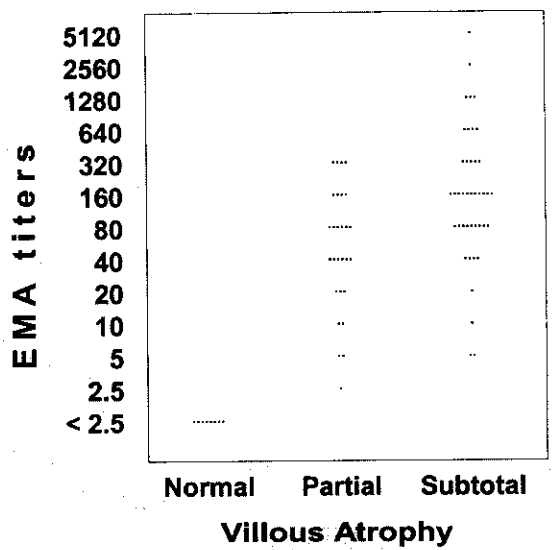
EXPECTED VALUES

As seen in Table 1, EMA, as detected on primate smooth muscle are highly specific markers for celiac disease and dermatitis herpetiformis. The presence of EMA seems to be related to the intestinal pathology both in celiac disease and dermatitis herpetiformis rather than to the skin lesions in the latter, as depicted in Figure 2:

Figure 2: Correlation of IgA-EMA titers in Villous Atropy



From Chorzelski TP et al[1] and Kumar V et al[8]

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Table 1. Incidence of IgA Class EMA

Clinical Condition	No. Tested	% Positive
Confirmed Celiacs		
On gluten	185	99
On gluten free diet	190	9
Suspected Celiacs		
On gluten	82	83
On gluten free diet	30	16
Dermatitis Herpetiformis (DH)	253	80
DH with Subtotal Villous Atrophy	42	100
DH on gluten free diet	36	3
Disease Controls (GI)		
Infectious Diarrhea	210	0
Recurrent Diarrhea	124	0
Toddlers Diarrhea	170	0
Milk Protein Intol.	69	0
Ulcerative Colitis	69	0
Crohn's Disease	65	0
Liver Diseases	21	0
Disease Controls (Skin)		
Linear IgA Bullous Dermatosis	4	0
Other Skin Diseases	180	0

Compiled from the literature as per Chorzelski TP et al[1].

PERFORMANCE CHARACTERISTICS

The ImmuGlo™ Anti-Endomysial Antibody (EMA) Test kit, using primate smooth muscle substrate and a polyvalent conjugate, was compared with another commercially available kit also using a polyvalent conjugate and monkey esophagus as a substrate. The comparison included a total of 68 sera: 20 from patients with clinically suspected celiac disease and 48 from normal controls. Sera were tested according to the procedure recommended by the manufacturer. A screening dilution of 2.5 was used and all sera positive for EMA were titrated to endpoint. The results were as follows:

		ImmuGlo™ EMA		
		Positive	Negative	Total
Other	Positive	18	0	18
	Negative	2	48	50
	Total	20	48	68

Agreement: 97%
Sensitivity: 100%
Specificity: 96%

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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REFERENCES

1. Chorzelski TP, Beutner EH, Kumar V and Zalewski TK (Eds). Serologic Diagnosis of Celiac Disease. CRC Press Boca Raton; 1990.
2. Levenson SD, Austin RK, Dietler MD, Kasarda DD and Kagnoff MF. Specificity of antigliadin antibody in celiac disease. Gastroenterology; 1985, 89:1-5.
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4. Hallström O. Comparison of IgA-class reticulin and endomysium antibodies in coeliac disease and dermatitis herpetiformis. Gut; 1989, 30:1225-1232.
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9. Calabuig M, Torregosa R, Polo P, Tuset L et al. Serological markers and celiac disease: a new diagnostic approach. J Paediatric Gastroenterol Nutr; 1990, 10:435-442.
10. Karpati S, Bürgin-Wolff A, Krieg T, Maurer M et al. Binding to human jejunum of serum IgA antibody from children with coeliac disease. Lancet; 1990, 336: 1335-1338.
11. Kumar V, Hemedinger E, Chorzelski TP et al. Reticulin and endomysial antibodies in bullous disease - comparison of specificity and sensitivity. Arch Dermatol; 1987, 123: 1179-1182.
12. Peters MS and McEvoy MT. IgA antiendomysial antibodies in dermatitis herpetiformis. J Am Acad Dermatol; 1989, 21: 1225-1231.
13. Walker-Smith JA, Quandalini S, Schmitz J et al. Revised criteria for diagnosis of celiac disease. Report of working group of European Society of Paedriatric Gastroenterology and Nutrition. Arch Dis Child; 1990, 65:909-911.
14. Beutner EH, Kumar V, Krasny SA and Chorzelski TP. Defined immunofluorescence in immunodermatology. In "Immunopathology of the Skin", Beutner EH, Chorzelski Tp and Kumar V, Eds, John Wiley and Sons New York; 1987, 3rd Ed, 3-40.
15. Biosafety in Microbiological and Biomedical Laboratories. Centers for Disease Control, National Institutes of Health, 1993 (HHS Pub. No. {CDC} 93-8395).

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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For technical assistance please contact:

IMMCO Diagnostics, Inc.
60 Pineview Drive
Buffalo, NY 14228-2120
Telephone: (716) 691-0091
Fax: (716) 691-0466
Toll Free USA/Canada: 1-800-537-TEST
E-Mail: info@immcodiagnostics.com

or your local product distributor

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

(b)(4)

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APPENDIX F

Interference Studies

(b)(4)

[redacted]

Cross Reactivity Studies

(b)(4)

[redacted]

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration
Memorandum

From: Reviewer(s) - Name(s) Rufina Carlos

Subject: 510(k) Number K032571 / S1

To: The Record - It is my recommendation that the subject 510(k) Notification:

- ☐ Refused to accept.
- ☐ Requires additional information (other than refuse to accept).
- ☒ Is substantially equivalent to marketed devices. 12/18/03
- ☐ NOT substantially equivalent to marketed devices.
- ☐ Other (e.g., exempt by regulation, not a device, duplicate, etc.)

Is this device subject to Section 522 Postmarket Surveillance?	☐ YES	☒ NO
Is this device subject to the Tracking Regulation?	☐ YES	☒ NO
Was clinical data necessary to support the review of this 510(k)?	☐ YES	☒ NO
Is this a prescription device?	☒ YES	☐ NO
Was this 510(k) reviewed by a Third Party?	☐ YES	☒ NO
Special 510(k)?	☐ YES	☒ NO
Abbreviated 510(k)? Please fill out form on H Drive 510k/boilers	☐ YES	☒ NO

Truthful and Accurate Statement ☐ Requested ☒ Enclosed

☐ A 510(k) summary OR ☒ A 510(k) statement

☐ The required certification and summary for class III devices N/A

☒ The indication for use form

Combination Product Category (Please see algorithm on H drive 510k/Boilers) N

Animal Tissue Source ☐ YES ☒ NO Material of Biological Origin ☒ YES ☐ NO

The submitter requests under 21 CFR 807.95 (doesn't apply for SEs):

☐ No Confidentiality ☐ Confidentiality for 90 days ☐ Continued Confidentiality exceeding 90 d

Predicate Product Code with class: 866.5660 Multiple Autoantibodies Immunological Test

Additional Product Code(s) with panel (optional):

MVM Class II

Review: Maria Chan (Branch Chief) IMDB (Branch Code) 12/22/03 (Date)

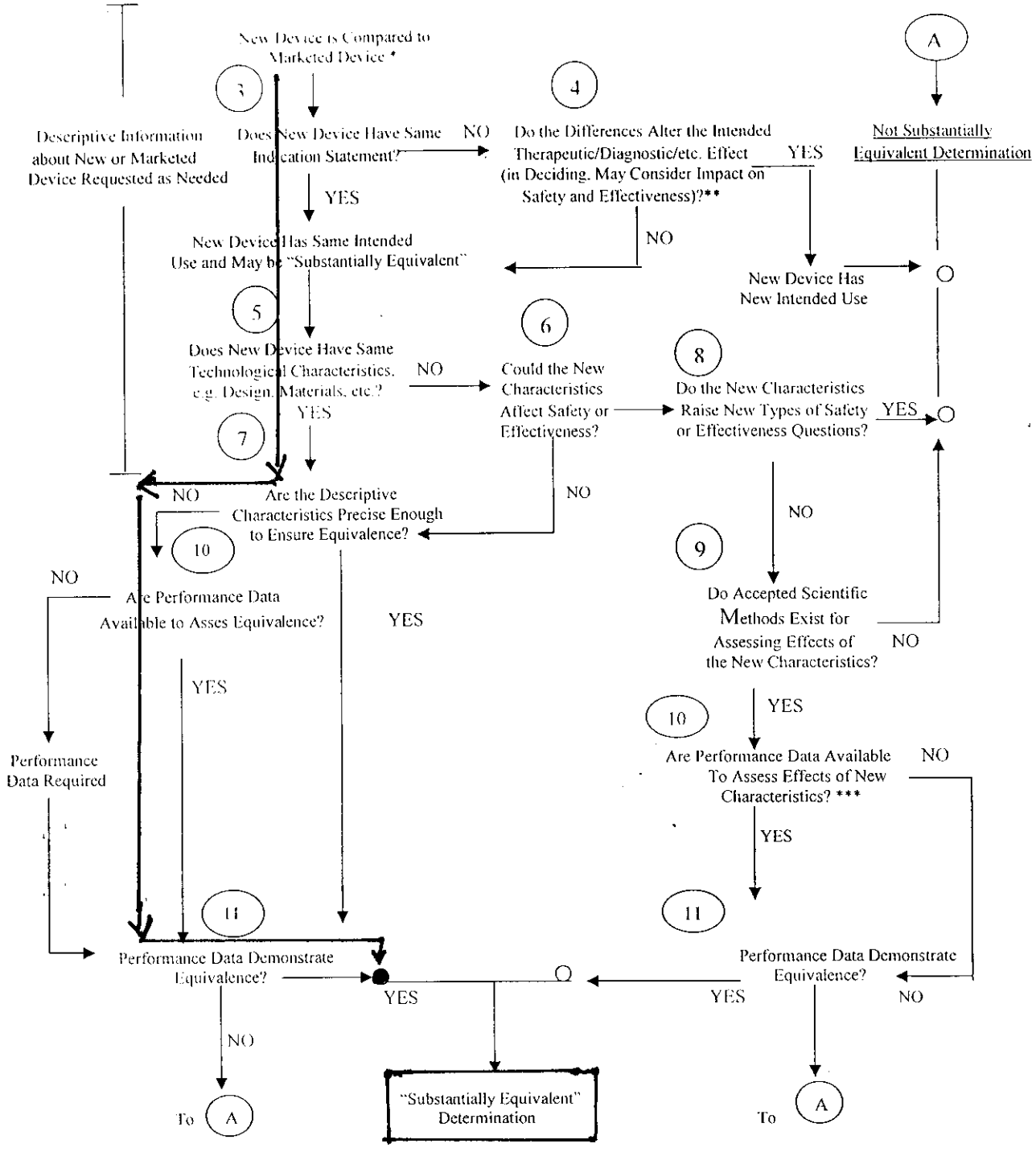
Final Review: S Butler (Division Director) 12/24/03 (Date)

Revised:4/2/03

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request 2014-6998; Released 10/30/14

510(k) "SUBSTANTIAL EQUIVALENCE" DECISION-MAKING PROCESS



* 510(k) Submissions compare new devices to marketed devices. FDA requests additional information if the relationship between marketed and "predicate" (pre-Amendments or reclassified post-Amendments) devices is unclear.

** This decision is normally based on descriptive information alone, but limited testing information is sometimes required.

*** Data maybe in the 510(k), other 510(k)s, the Center's classification files, or the literature.

5

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request 2014-6998; Released 10/30/14

"SUBSTANTIAL EQUIVALENCE" (SE) DECISION MAKING DOCUMENTATION

K032571

Reviewer: **Rufina Carlos**

Division/Branch: **OIVD/DIHD/IMDB**

Device Name: **ImmuLisa Anti-Human Tissue Transglutaminase (hu-tTG) Antibody IgA ELISA**

.

Product To Which Compared (510(K) Number If Known) **IMMCO ImmuLisa Anti-tTG Antibody ELISA and INOVA Anti-tTG Antibody Kit K99278 and K01156**

	YES	NO	
1. Is Product A Device	X		If **NO** = Stop
2. Is Device Subject To 510(k)?	X		If **NO** = Stop
3. Same Indication Statement?	X		If **YES** = Go To 5
4. Do Differences Alter The Effect Or Raise New Issues of Safety Or Effectiveness?			If **YES** = Stop **NE**
5. Same Technological Characteristics?	X		If **YES** = Go To 7
6. Could The New Characteristics Affect Safety Or Effectiveness?			If **YES** = Go To 8
7. Descriptive Characteristics Precise Enough?		X	If **NO** = Go To 10 If **YES** = Stop **SE**
8. New Types Of Safety Or Effectiveness Questions?			If **YES** = Stop **NE**
9. Accepted Scientific Methods Exist?			If **NO** = Stop **NE**
10. Performance Data Available?	X		If **NO** = Request Data
11. Data Demonstrate Equivalence?	X		Final Decision: **SE**

Note: In addition to completing the form on the LAN, "yes" responses to questions 4, 6, 8, and 11, and every "no" response requires an explanation.

6

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request 2014-6998; Released 10/30/14

1. Intended Use:
   An enzyme linked immunosorbent assay (ELISA) for the detection and semi-quantitation of anti-human Tissue Transglutaminase IgA antibodies in human serum to aid in the diagnosis of patients with celiac disease and dermatitis herpetiformis.
2. Device Description:
   The anti-hu tTG antibody test is performed as solid phase immunoassay (ELISA). Microwells are coated with recombinant hu tTG antigen followed by blocking the unreacted sites to reduce nonspecific binding. Controls, calibrators and patient serum samples are incubated in the antigen coated wells which allows anti-tTG antibodies present in the serum to bind. Unbound antibody and other serum proteins are removed by washing the microwells. Antibodies bound to the microwells are detected by adding enzyme labeled anti-human IgA conjugates to the wells. These enzyme conjugated antibodies bind specifically to the human immunoglobulin of the appropriate class. Unbound enzyme conjugate is removed by washing. Specific enzyme substrate (pNPP) is then added to the wells and the presence of antibodies to hu tTG is detected by a color change produced by the conversion of pNPP substrate. The reaction is stopped and the intensity of the color change, which is proportional to the concentration of the antibody, is read by a spectrophotometer at 405 nm. Results are expressed in ELISA units per milliliter (EU/mL).

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request 2014-6998; Released 10/30/14

EXPLANATIONS TO "YES" AND "NO" ANSWERS TO QUESTIONS ON PAGE 1 AS NEEDED

1. Explain why not a device:

2. Explain why not subject to 510(k):

3. How does the new indication differ from the predicate device's indication:

4. Explain why there is or is not a new effect or safety or effectiveness issue:

5. Describe the new technological characteristics:

6. Explain how new characteristics could or could not affect safety or effectiveness:

7. Explain how descriptive characteristics are not precise enough: Several issues regarding population breakdown in method comparison, linearity and detection limit, several typo errors were raised. Responses were adequate to support claims of substantial equivalence. These issues were noted in the reviewer's memorandum.

8. Explain new types of safety or effectiveness questions raised or why the questions are not new:

9. Explain why existing scientific methods can not be used:

10. Explain what performance data is needed:

11. Explain how the performance data demonstrates that the device is or is not substantially equivalent: The sponsor has submitted sufficient performance data to demonstrate substantial equivalence.(See reviewer's memo)

ATTACH ADDITIONAL SUPPORTING INFORMATION

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request 2014-6998; Released 10/30/14

DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service

Food and Drug Administration
Center for Devices and
Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
9200 Corporate Blvd.
Rockville, Maryland 20850

December 10, 2003

IMMCO DIAGNOSTICS, INC.
60 PINEVIEW DR.
BUFFALO, NY 14228
ATTN: KEVIN LAWSON

510(k) Number: K032571
Product: IMMULISA ANTI-HUMAN TISSUE TRANSGLUTAMINASE

The additional information you have submitted has been received.

We will notify you when the processing of this submission has been completed or if any additional information is required. Please remember that all correspondence concerning your submission MUST be sent to the Document Mail Center (HFZ-401) at the above letterhead address. Correspondence sent to any address other than the one above will not be considered as part of your official premarket notification submission. Also, please note the new Blue Book Memorandum regarding Fax and E-mail Policy entitled, "Fax and E-Mail Communication with Industry about Premarket Files Under Review. Please refer to this guidance for information on current fax and e-mail practices at www.fda.gov/cdrh/ode/a02-01.html.

The Safe Medical Devices Act of 1990, signed on November 28, states that you may not place this device into commercial distribution until you receive a letter from FDA allowing you to do so. As in the past, we intend to complete our review as quickly as possible. Generally we do so 90 days. However, the complexity of a submission or a requirement for additional information may occasionally cause the review to extend beyond 90 days. Thus, if you have not received a written decision or been contacted within 90 days of our receipt date you may want to check with FDA to determine the status of your submission.

If you have procedural or policy questions, please contact the Division of Small Manufacturers International and Consumer Assistance (DSMICA) at (301) 443-6597 or at their toll-free number (800) 638-2041, or contact me at (301) 594-1190.

Sincerely yours,

Marjorie Shulman
Supervisory Consumer Safety Officer
Premarket Notification Section
Office of Device Evaluation
Center for Devices and
Radiological Health

9

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE

A. 510(k) Number:
k032571

B. Analyte:
Anti-tissue transglutaminase (tTG)

Type of Test:
Semi-quantitative- ELISA

Applicant:
IMMCO Diagnostics, Inc.

Proprietary and Established Names:
ImmuLisa Anti-Human Tissue Transglutaminase (hu-tTG) Antibody IgA ELISA

C. Regulatory Information:

1. Regulation section:
   866.5660 Multiple Autoantibodies Immunological Test
2. Classification:
   Class II
3. Product Code:
   MVM
4. Panel:
   Immunology (82)

D. Intended Use:

An enzyme linked immunosorbent assay (ELISA) for the detection and semi-quantitation of anti-human Tissue Transglutaminase IgA antibodies in human serum to aid in the diagnosis of patients with celiac disease and dermatitis herpetiformis

1. Indication(s) for use:
   An enzyme linked immunosorbent assay (ELISA) for the detection and semi-quantitation of IgA antibodies to human tissue transglutaminase, as an aid in diagnosing patients with Gluten Sensitive Enteropathy (celiac disease and dermatitis herpetiformis).
2. Special condition for use statement(s):
   Not applicable
3. Special instrument Requirements:
   - Microplate reader capable of reading absorbance values at 405 nm. If dual wavelength microplate is available, the reference filter should be set at 600-650 nm.
   - Automatic microplate washer capable of dispensing 200 uL

E. Device Description:

The IMMCO kit has a set of four calibrators, a positive and a negative control, microplate with individual breakaway microwells coated with hu TG antigen, anti-human Alkaline Phosphatase conjugate, serum diluent, enzyme substrate, stop solution and wash buffer. Except for the wash buffer, the reagents are ready to use

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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and has its own identifying color. A kit contains sufficient reagents to perform 96 determinations.
This device is a modification of a previously cleared device (K992878). The modification is a change of target antigen from a guinea pig to a human recombinant antigen. The sponsor states that guinea pig tTG antibody ELISA method has limitations with regards to sensitivity. More false negatives are observed on the guinea pig tTG antibody method. These claims of improved performance are not in the labeling or the Indications for Use. The name of the device reflects the human antigen.

F. Substantial Equivalence Information:

1. Predicate device name(s):
   1. IMMCO ImmuLisa Anti-tTG Antibody IgA ELISA
   2. INOVA QUANTA-Lite h-tTG (human tissue transglutaminase) IgA ELISA
2. Predicate K number(s):
   1. K992878
   2. K011566
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
	ImmuLisa hu-tTG Antibody IgA ELISA	1. ImmuLisa Anti-tTG Antibody IgA ELISA 2. INOVA QUANTA-Lite h-tTG IgA ELISA
Methodology	ELISA	Same
Detection of antibodies	tTG	Same
Quantitation	Semi-quantitative	Same
Conjugate specificity	IgA	Same
Sample	Serum	Same
Differences		
Item	**Device**	**Predicate**
Conjugate	Alkaline Phosphatase	1. Alkaline Phosphatase 2. Horseradish Peroxidase
Substrate	P-NPP	1. p-NPP 2. TMB
Absorbance	405 nm	1. 405 nm 2. 450 nm
Source of tTG antigen	human	1. guinea pig 2. human

G. Standard/Guidance Document Referenced (if applicable):
Not applicable

H. Test Principle:

11

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The anti-hu tTG antibody test is performed as solid phase immunoassay (ELISA). Microwells are coated with recombinant hu tTG antigen followed by blocking the unreacted sites to reduce nonspecific binding. Controls, calibrators and patient serum samples are incubated in the antigen coated wells which allows anti-tTG antibodies present in the serum to bind. Unbound antibody and other serum proteins are removed by washing the microwells. Antibodies bound to the microwells are detected by adding enzyme labeled anti-human IgA conjugates to the wells. These enzyme conjugated antibodies bind specifically to the human immunoglobulin of the appropriate class. Unbound enzyme conjugate is removed by washing. Specific enzyme substrate (pNPP) is then added to the wells and the presence of antibodies to hu tTG is detected by a color change produced by the conversion of pNPP substrate. The reaction is stopped and the intensity of the color change, which is proportional to the concentration of the antibody, is read by a spectrophotometer at 405 nm. Results are expressed in ELISA units per milliliter (EU/mL).

I. Performance Characteristics (if/when applicable):

1. Analytical performance:

(b)(4)

[redacted]

12

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<20 EU/mL	Negative
20-25 EU/mL	Indeterminate (borderline)
>25 EU/mL	Positive

2. Comparison studies:

(b)(4)

[redacted]

13

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b. *Matrix comparison:*
Not applicable.

3. Clinical studies:
   a. *Clinical sensitivity:*
   Not applicable
   b. *Clinical specificity:*
   Not applicable
   c. *Other clinical supportive data (when a and b are not applicable):*
   Not applicable
4. Clinical cut-off:
   Not applicable
5. Expected values/Reference range:

(b)(4)

[redacted]

J. Conclusion:

Based on the review of the information provided in this 510(k), the ImmuLisa Anti-Human Tissue Transglutaminase (hu-tTG) Antibody IgA ELISA appears to be **Substantially Equivalent** to devices regulated under 21CFR 866.5660, Multiple Autoantibodies Immunological Test System, Product code MVM, Class II.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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K. Other Supportive Information:

(b)(4)

[redacted]

L. Administrative Information:

1. Applicant contact information:

   a. *Name of applicant*:
   IMMCO Diagnostics
   b. *Mailing address*:
   60 Pineview Drive
   Buffalo, NY 14228

15

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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c. *Phone #*:
(716) 691-0091 ext 110
d. *Fax #*:
(716) 691-0466
e. *E-mail address (optional)*:
kevinlawson@immcodiagnostics.com
f. *Contact*:
Kevin J. Lawson

2. Review documentation:

9/08/03	assigned to /received by reviewer
9/24/03	deficiency issues emailed to sponsor
10/15/03	telephone hold
12/11/03	response received from sponsor through DMC
12/15/03	phone call to sponsor re: additional questions
12/17/03	response received via email
12/18/03	SE decision

M. Reviewer Name and Signature:

Rufina Carlos

Rufina Carlos
CDRH/OIVD/DIHD/IMDB

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request 2014-6998; Released 10/30/14

INDICATIONS FOR USE STATEMENT

510(k) Number (if known): K 032571

Device Name: ImmuLisa Anti-human tissue Transglutaminase (human tTG) IgA ELISA

Indications For Use: An enzyme linked immunoassay (ELISA) for the detection and semi-quantitation of IgA antibodies to human tissue transglutaminase, as an aid in diagnosing patients with Gluten Sensitive Enteropathy (celiac Disease and Dematitis herpetiformis)

(PLEASE DO NOT WRITE BELOW THIS LINE - CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)

Prescription Use ✓ OR Over-The-Counter Use _____
(Per 21 CFR 801.109) (Optional Format 1-2-96)

Mark M Chan
Division Sign-Off

Office of In Vitro Diagnostic Device Evaluation and Safety

510(k) 032571

17

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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PREMARKET NOTIFICATION
TRUTHFUL AND ACCURATE STATEMENT
[As required by 21 CFR 807.87(j)]

I certify that, in my capacity as Director of Regulatory Affairs of IMMCO Diagnostics, I believe to the best of my knowledge, that all data and information submitted in the premarket notification are truthful and accurate and that no material fact has been omitted.

Kevin Lawson
(Signature of Certifier)

Kevin Lawson
(Typed Name)

8/14/03
(Date)

K032571
(Premarket Notification [510(k)] Number)

18

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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510(k) STATEMENT
(As Required By 21 CFR 807.93)

I certify that, in my capacity as Director of Regulatory Affairs of IMMCO Diagnostics, Inc., I will make available all information included in this premarket notification on safety and effectiveness within 30 days of request by any person if the device described in the premarket notification submission is determined to be substantially equivalent. The information I agree to make available will be a duplicate of the premarket notification submission, including any adverse safety and effectiveness information, but excluding all patient identifiers, and trade secret and confidential commercial information, as defined in 21 CFR 20.61.

Kevin Lawson
(Signature of Certifier)

Kevin Lawson
(Typed Name)

8/14/03
(Date)

K032571
*(Premarket Notification [510(k)] Number)

*For a new submission, leave the 510(k) number blank.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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MEMORANDUM- electronic (email correspondence)

Date: September 24, 2003

From: Rufina Carlos, Scientific Reviewer, DIHB,OIVD
Tel No. (301) 594-1293 ext. 167
E-mail: rxc@cdrh.fda.gov
Fax: (301) 594-5941

Subject: K032571 ImmuLisa Anti-Human Tissue Transglutaminase (hu-tTG) Antibody IgA ELISA
Review Issues

To: IMMCO Diagnostics, Inc.
c/o Kevin Lawson
e-mail: kevinlawson@immcodiagnostics.com
Tel (716) 691-0091 ext 110
Fax (716) 691-0466

- Please include a brief description of *dermatitis herpetiformis* in the Summary and Explanation section of the PI.
- Please provide the following:
  1. a certificate of analysis and Quality Control document for the alkaline phosphatase labeled conjugate
  2. quality control documents for the positive and negative controls
  3. summary data of linearity/assay reportable range, and stability studies. What is the detection limit of the assay?
  4. concentration of samples found on Table E: Normal Sera Studies (O.D.s were provided) and breakdown of population included in the study.
- Please modify the title name of the PI to include IgA i.e., "Anti-human Tissue Transglutaminase (hu-tTG) Antibody IgA ELISA".
- Make corrections of a few typo errors: Table of Normal Data is Appendix E (p.12), Interference Studies is Appendix F (p.16)

This submission will be held at the reviewer's desk for two weeks pending receipt of the requested information. If not received by that time, it will be placed on hold with the DMC.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

(b)(4)

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Recombinant human tTG Stability Study

(b)(4)

[redacted]

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

(b)(4)

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Carlos, Rufina

From: Kevin Lawson [kevinlawson@immcodiagnostics.com]
Sent: Wednesday, December 17, 2003 5:30 PM
To: Carlos, Rufina
Cc: Vijay Kumar
Subject: Re: K032571 hu-tTG

Dear Ms. Carlos,

In response to your phone call, I have provided the following additional information:

Comparative Sensitivity and Specificity Population breakdown:

(b)(4)

[redacted]

Linearity Study Narrative:

(b)(4)

[redacted]

Please let me know if there are any questions.

Sincerely,

Kevin J. Lawson
Director of Regulatory Affairs
IMMCO Diagnostics
phone 800.537.8378
fax 716.691.0466

106

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request 2014-6998; Released 10/30/14

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration
Memorandum

From: Reviewer(s) - Name(s) Rufina Carlos

Subject: 510(k) Number K032571

To: The Record - It is my recommendation that the subject 510(k) Notification:

- ☐ Refused to accept.
- ☒ Requires additional information (other than refuse to accept). 10/14/03 RXC
- ☐ Is substantially equivalent to marketed devices.
- ☐ NOT substantially equivalent to marketed devices.
- ☐ Other (e.g., exempt by regulation, not a device, duplicate, etc.)

Is this device subject to Section 522 Postmarket Surveillance?	☐ YES	☐ NO
Is this device subject to the Tracking Regulation?	☐ YES	☐ NO
Was clinical data necessary to support the review of this 510(k)?	☐ YES	☐ NO
Is this a prescription device?	☐ YES	☐ NO
Was this 510(k) reviewed by a Third Party?	☐ YES	☐ NO
Special 510(k)?	☐ YES	☐ NO
Abbreviated 510(k)? Please fill out form on H Drive 510k/boilers	☐ YES	☐ NO

Truthful and Accurate Statement ☐ Requested ☐ Enclosed

☐ A 510(k) summary OR ☐ A 510(k) statement

☐ The required certification and summary for class III devices

☐ The indication for use form

Combination Product Category (Please see algorithm on H drive 510k/Boilers) _____

Animal Tissue Source ☐ YES ☐ NO Material of Biological Origin ☐ YES ☐ NO

The submitter requests under 21 CFR 807.95 (doesn't apply for SEs):

☐ No Confidentiality ☐ Confidentiality for 90 days ☐ Continued Confidentiality exceeding 90 days

Predicate Product Code with class: _____

Additional Product Code(s) with panel (optional): _____

Review: _____
(Branch Chief) (Branch Code) (Date)

Final Review: _____
(Division Director) (Date)

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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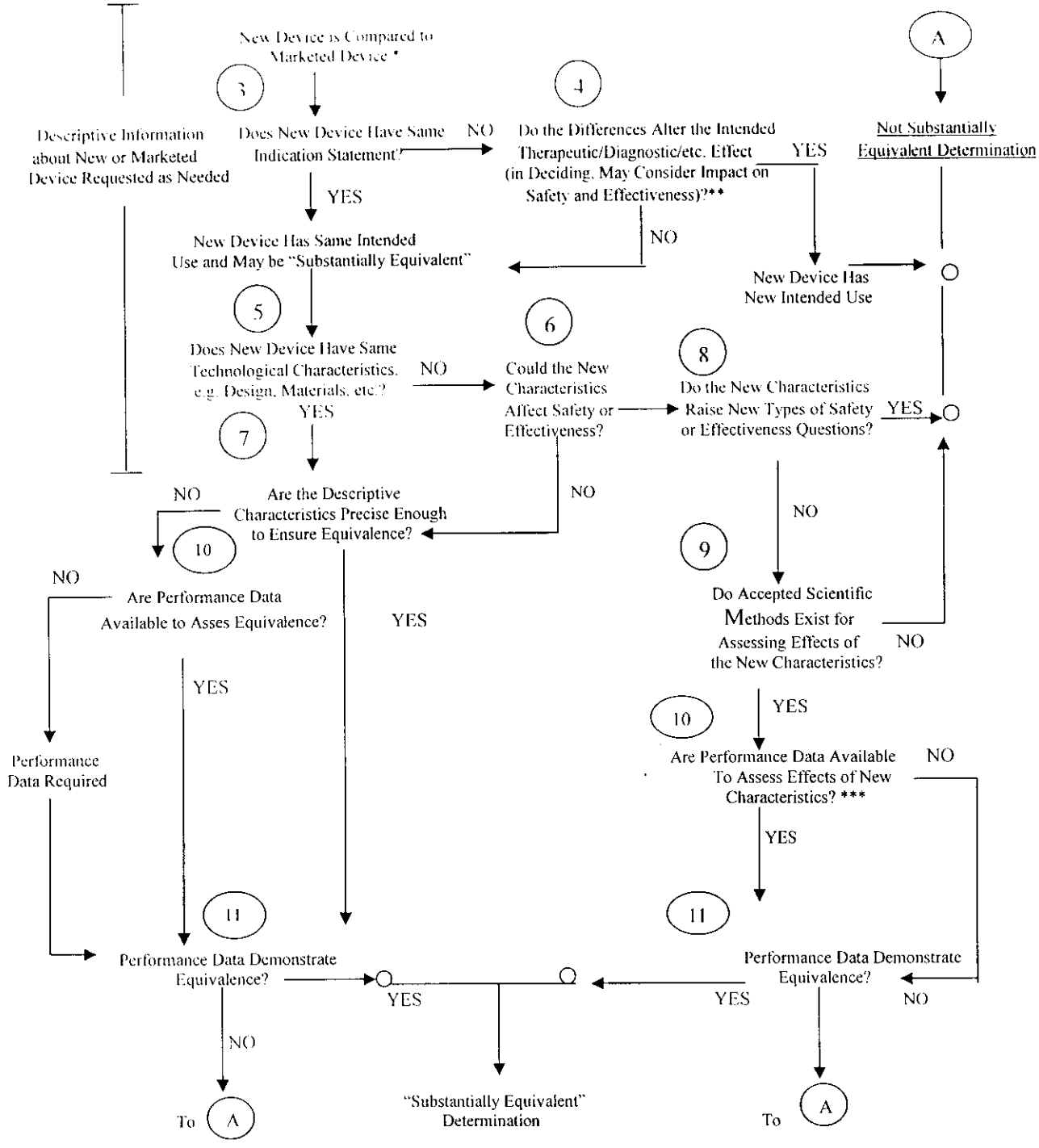
510(k) "SUBSTANTIAL EQUIVALENCE" DECISION-MAKING PROCESS



* 510(k) Submissions compare new devices to marketed devices. FDA requests additional information if the relationship between marketed and "predicate" (pre-Amendments or reclassified post-Amendments) devices is unclear.

** This decision is normally based on descriptive information alone, but limited testing information is sometimes required.

*** Data maybe in the 510(k), other 510(k)s, the Center's classification files, or the literature.

110

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Date: _____

The deficiencies identified above represent the issues that we believe need to be resolved before our review of your 510(k) submission can be successfully completed. In developing the deficiencies, we carefully considered the statutory criteria as defined in Section 513(i) of the Federal Food, Drug, and Cosmetic Act for determining substantial equivalence of your device. We also considered the burden that may be incurred in your attempt to respond to the deficiencies. We believe that we have considered the least burdensome approach to resolving these issues. If, however, you believe that information is being requested that is not relevant to the regulatory decision or that there is a less burdensome way to resolve the issues, you should follow the procedures outlined in the "A Suggested Approach to Resolving Least Burdensome Issues" document. It is available on our Center web page at: http://www.fda.gov/cdrh/modact/leastburdensome.html

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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REVISED:3/14/95

THE 510(K) DOCUMENTATION FORMS ARE AVAILABLE ON THE LAN UNDER 510(K) BOILERPLATES TITLED "DOCUMENTATION" AND MUST BE FILLED OUT WITH EVERY FINAL DECISION (SE, NSE, NOT A DEVICE, ETC.).

"SUBSTANTIAL EQUIVALENCE" (SE) DECISION MAKING DOCUMENTATION

K_____

Reviewer:_____

Division/Branch:_____

Device Name:_____

Product To Which Compared (510(K) Number If Known):_____

	YES	NO	
1. Is Product A Device			If NO = Stop
2. Is Device Subject To 510(k)?			If NO = Stop
3. Same Indication Statement?			If YES = Go To 5
4. Do Differences Alter The Effect Or Raise New Issues of Safety Or Effectiveness?			If YES = Stop NE
5. Same Technological Characteristics?			If YES = Go To 7
6. Could The New Characteristics Affect Safety Or Effectiveness?			If YES = Go To 8
7. Descriptive Characteristics Precise Enough?			If NO = Go To 10 If YES = Stop SE
8. New Types Of Safety Or Effectiveness Questions?			If YES = Stop NE
9. Accepted Scientific Methods Exist?			If NO = Stop NE
10. Performance Data Available?			If NO = Request Data
11. Data Demonstrate Equivalence?			Final Decision:

Note: In addition to completing the form on the LAN, "yes" responses to questions 4, 6, 8, and 11, and every "no" response requires an explanation.

112

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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1. Intended Use:

2. Device Description: Provide a statement of how the device is either similar to and/or different from other marketed devices, plus data (if necessary) to support the statement. Is the device life-supporting or life sustaining? Is the device implanted (short-term or long-term)? Does the device design use software? Is the device sterile? Is the device for single use? Is the device over-the-counter or prescription use? Does the device contain drug or biological product as a component? Is this device a kit? Provide a summary about the devices design, materials, physical properties and toxicology profile if important.

EXPLANATIONS TO "YES" AND "NO" ANSWERS TO QUESTIONS ON PAGE 1 AS NEEDED

1. Explain why not a device:

2. Explain why not subject to 510(k):

3. How does the new indication differ from the predicate device's indication:

4. Explain why there is or is not a new effect or safety or effectiveness issue:

5. Describe the new technological characteristics:

6. Explain how new characteristics could or could not affect safety or effectiveness:

7. Explain how descriptive characteristics are not precise enough:

8. Explain new types of safety or effectiveness questions raised or why the questions are not new:

9. Explain why existing scientific methods can not be used:

10. Explain what performance data is needed:

11. Explain how the performance data demonstrates that the device is or is not substantially equivalent:

ATTACH ADDITIONAL SUPPORTING INFORMATION

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Internal Administrative Form

	YES	NO
1. Did the firm request expedited review?		✓
2. Did we grant expedited review?		✓
3. Have you verified that the Document is labeled Class III for GMP purposes?		
4. If, not, has POS been notified?		
5. Is the product a device?	✓	
6. Is the device exempt from 510(k) by regulation or policy?		✓
7. Is the device subject to review by CDRH?	✓	
8. Are you aware that this device has been the subject of a previous NSE decision?		✓
9. If yes, does this new 510(k) address the NSE issue(s), (e.g., performance data)?		
10. Are you aware of the submitter being the subject of an integrity investigation?		✓
11. If, yes, consult the ODE Integrity Officer.		
12. Has the ODE Integrity Officer given permission to proceed with the review? (Blue Book Memo #I91-2 and Federal Register 90N0332, September 10, 1991.		

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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K032571/S1

FDA/CDRH/OCE/PMO
2003 DEC 10 A 11:19

November 21, 2003

Food and Drug Adminstration
Office of Device Evaluation
Center for Devices and Radiological Health
Document Mail Center, HFZ-401
Attn: Rufina Carlos
9200 Corporate Blvd.
Rockville, MD 20850

Subject: K032571 hu-tTG response

Dear Ms. Carlos:

I have provided response to the issues you raised below.

(b)(4)

[redacted]

60 Pineview Drive · Buffalo, NY · 14228 · USA
Toll Free 800.537.8378 · International Phone 716.691.0091 · Facsimile 716.691.0466
www.immcodiagnostics.com

SK21
20

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(b)(4)

[redacted]

Please contact us if you have any further comments.

Sincerely,

Kevin Lawson

Kevin J. Lawson
Director of Regulatory Affairs

60 Pineview Drive · Buffalo, NY · 14228 · USA
Toll Free 800.537.8378 · International Phone 716.691.0091 · Facsimile 716.691.0466
www.immcodiagnostics.com

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Attachment 1

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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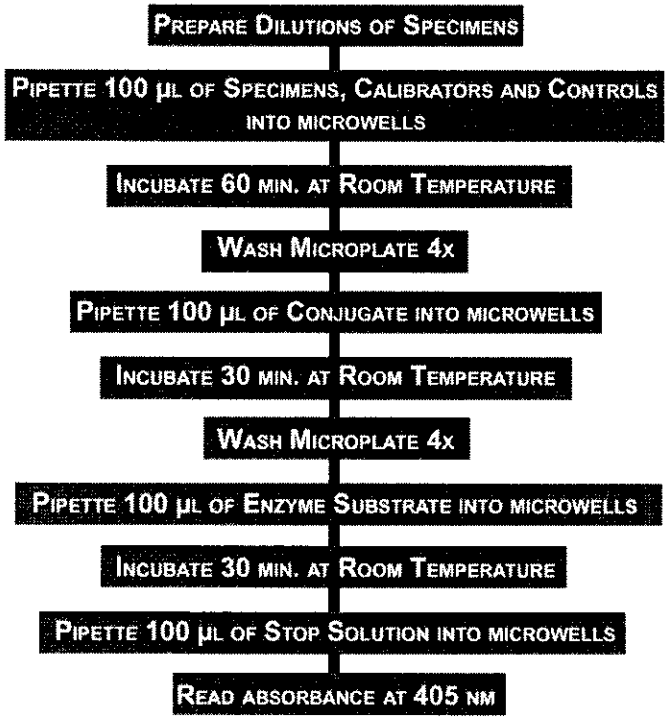
ImmuLisa™ Procedure at a Glance



For technical assistance please contact:

IMMCO Diagnostics, Inc.
60 Pineview Drive
Buffalo, NY 14228-2120
Telephone: (716) 691-0091
Fax: (716) 691-0466
Toll Free USA/Canada: 1-800-537-TEST
E-Mail: info@immcodiagnostics.com

or your local product distributor

OCT2003

Document No. PI4144

IMMCO DIAGNOSTICS

ImmuLisa™ Anti-human Tissue Transglutaminase (hu tTG) Antibody IgA ELISA

For *In vitro* diagnostic use
CLIA Complexity: High
CDC Analyte Identification Code: 0546
CDC Test System Identification Code: 28569

Code 1144 **PRODUCT INSERT** 96 Determinations

INTENDED USE

An enzyme linked immunosorbent assay (ELISA) for the detection and semi-quantitation of anti-human Tissue Transglutaminase IgA antibodies in human serum to aid in the diagnosis of patients with celiac disease and dermatitis herpetiformis.

SUMMARY AND EXPLANATION

Celiac Disease (CD) is an autoimmune gastrointestinal disorder that may occur in genetically susceptible individuals triggered by the ingestion of gluten-containing grains such as wheat, barley and rye. Of the many autoimmune disorders, CD represents one of the few where the etiological agent is known and the disease subsides and goes in remission once the etiological agent is withdrawn from the diet. *Dermatitis Herpetiformis* (DH) is an autoimmune bullous disorder related to CD that is also triggered by ingestion of gluten and responds similarly to a gluten-free diet.

CD is characterized by malabsorbtion resulting from inflammatory injury to the small intestinal mucosa and, when prolonged, can cause malnutrition. The classical symptoms of CD include diarrhea, weight loss and malnutrition.

Only a small percentage of patients with CD presents with classical symptoms. Consequently, the clinical spectrum of CD has grown much broader than in the past to include patients that do not present with classical symptoms. It is not uncommon for the initial symptoms to be non-gastrointestinal or for gastrointestinal symptoms, if present, to be mild or intermittent. Some common non-gastrointestinal manifestations include short stature, iron and folate deficiency, anemia, bone loss, aphthous stomatitis, arthralgia, dental enamel defects, etc. The need to examine a wider range of clinical presentation has led to greater numbers of individuals diagnosed with CD later in life than ever before. Adults may present with iron deficiency, macrocytic anemia and hypocalcaemia.

Studies have found the prevalence of CD to be highly variable from population to population.[1] The true prevalence has been difficult to ascertain. Disparate criteria used in diagnosis of CD are often the cause. If clinical criteria alone are used in determining prevalence, the incidence of CD is much lower as compared with incidence established by serological methods.[1,2] Using serological methods, the incidence of CD in the general population is approximately one in 200.

Diagnosis of CD based on clinical criteria thus can be misleading and may lead to serious delays in proper diagnosis. Frequently, delays in diagnosis extend 10-13 years from the first clinical presentation of symptoms.

Failure to diagnose CD early on may predispose an individual to long-term complications such as splenic atrophy and intestinal lymphoma. The incidence of lymphoma arising in the context of CD is difficult to ascertain. One study has shown incidence of lymphoma involving the gastrointestinal (GI) tract in patients with CD to range from 3.6 percent to 40 percent.[3] In another recent study,[4] CD is associated [illegible] significantly

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

elevated risk for intestinal lymphoma, especially for non-Hodgkin's. A gluten-free diet (GFD) normalizes the mucosa and helps reduce the malignant potential.

Histological examination of the small intestinal biopsy remains the gold standard for diagnosing CD, but it has its own limitations. These include some patients with latent or even active CD that may have normal histopathology.[5]

The revised European Society of Paediatric Gastroenterology and Nutrition (ESPGHAN) criteria include only a single biopsy with clear-cut remission of clinical symptoms on GFD[6]. Positive serology at the time of diagnosis with disappearance on GFD contributes to the diagnosis. The various serological tests employed in the work-up of patients suspected to have CD include anti-gliadin antibody (AGA), anti-endomysial antibody (EMA), anti-reticulin antibody (ARA) and anti-tissue transglutaminase (tTG) antibody tests. Antibodies to gliadin and tTG are detected by ELISA, whereas endomysium and reticulin antibodies are detected by indirect immunofluorescence. EMA are very specific indicators of CD. However, the EMA test is an immuno-histochemical method that requires experience in reading immunofluorescence reactions. Rosario et al[7] concludes that "EmA-IgA is 100 percent sensitive and specific in active, untreated IgA-sufficient CD patients when performed by an established laboratory." EMA and tTG antibodies are also present in 60% - 70% of patients with DH.

Since identification of tTG as the endomysial antigen, ELISA methods have been described for detecting antibodies in the sera of patients with CD. The advantage of the anti-tTG antibody assay is that it is automatable and less subjective than EMA. For this reason, many laboratories have opted to use the tTG antibody method as the screening method. In these laboratories, it may be the only assay used for detection of CD cases. In various studies on the efficacy of the tTG antibody method for screening for CD, the specificity and sensitivity of this method has been found to range from 90 percent to 95 percent.[8] Human tTG has been described to improve the sensitivity of the tTG antibody assay for CD.

PRINCIPLES OF PROCEDURES

The anti-hu tTG antibody test is performed as a solid phase immunoassay (ELISA). Microwells are coated with recombinant hu tTG antigen followed by blocking the unreacted sites to reduce nonspecific binding. Controls, calibrators and patient serum samples are incubated in the antigen coated wells which allows anti-tTG antibodies present in the serum to bind. Unbound antibody and other serum proteins are removed by washing the microwells. Antibodies bound to the microwells are detected by adding enzyme labeled anti-human IgA conjugates to the wells. These enzyme conjugated antibodies bind specifically to the human immunoglobulin of the appropriate class. Unbound enzyme conjugate is removed by washing. Specific enzyme substrate (pNPP) is then added to the wells and the presence of antibodies to hu tTG is detected by a color change produced by the conversion of the pNPP substrate. The reaction is stopped and the intensity of color change, which is proportional to the concentration of antibody, is read by a spectrophotometer at 405 nm. Results are expressed in ELISA units per milliliter (EU/ml).

REAGENTS

Storage and Preparation

Store all reagents at 2-8°C. **Do not freeze.** Do not use if reagent is not clear or if a precipitate is present. All reagents must be brought to room temperature (20-25°C) prior to use. When stored at 2-8°C, the reconstituted wash buffer is stable until the kit expiration date. Reconstitute the wash buffer to 1 liter with distilled or deionized water. Coated microwell strips are for one time use only.

REFERENCES

1. Guandalini S, Gupta P. Celiac disease- A diagnostic challenge. *Clin Appl Immun Rev* 2002;2:293-305.
2. Fasano A, et al. Prevalence of celiac disease in at risk and not-at-risk groups in the United States 2003; 163:286-292.
3. Wolber R, et al. Lymphocytic gastritis in patients with celiac sprue or spruelike intestinal disease. *Gastroenterology* 1990;98:310-315.
4. Catassi, et al. Risk of non-Hodgkin lymphoma in celiac disease. *JAMA* 2002;287:1413-1419.
5. Arranz E, Ferguson A. Intestinal antibody pattern of celiac disease: Occurrence in patients with normal jejunal biopsy histology. *Gastroenterology* 1993;104:1263-72.
6. Hill ID, et al. Celiac disease: Working group report of the first world congress of pediatric gastroenterology, hepatology, and nutrition. *J. Pediatr Gastroenterol Nutr* 2002; 35:S78-S88
7. Rosario D, et al. Further studies of anti-endomysium and anti-gliadin antibodies in patients with suspected celiac disease. *J Pediatr Gastroenterol Nutr* 1998;27:191-195.
8. Vitoria JC, et al. Antibodies to gliadin, endomysium, and tissue transglutaminase for the diagnosis of celiac disease. *J Pediatr Gastroenterol Nutr* 1999;29:571-4.
9. Biosafety in Microbiological and Biomedical Laboratories. Centers for Disease Control National Institutes of Health; 1993 (HHS Pub No [CDC] 93-8395).
10. Sulkanen S, Haltunen T, Laurila K, et al. Tissue Transglutaminase autoantibody enzyme-linked immunosorbent assay in detecting celiac disease. Gastroenterology; 1998, 115:1322-1328.
11. Dietrich W, Laag E, Schopper H, et al. Autoantibodies to tissue transglutaminase as predictors of celiac disease; Gastroenterology 1998, 115:1317-1321.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Precision:

Based on 10 replicates, the intra-assay and inter-assay Coefficient of Variation (CV) of the IgA anti-hu tTG ELISA test were calculated.

	Inter-assay CV	Intra-assay CV
High (199 EU/ml)	6.2%	2.9%
Medium (85 EU/ml)	8.4%	6.5%
Low (41 EU/ml)	15.6%	9.6%

Recovery:

Samples with known anti-hu tTG concentrations were mixed with appropriate dilutions of another positive sample with known amounts of anti-tTG. Anti-hu tTG antibody levels of the mixed samples were determined and from the values obtained the percent recovery calculated. The results are as follows:

	anti-hu tTG Ab. conc. added (EU/ml)	anti-hu tTG Ab. conc. obtained (EU/ml)	% Recovery
Sample 1	107.5	112	104.2
Sample 2	86	94.6	110
Sample 3	64	70.9	109.9

Precautions

For *in vitro* diagnostic use. All human derived components used have been tested for HBsAg, HCV, HIV-1 and 2 and HTLV-I and found negative by FDA licensed tests. However, human blood derivatives and patient specimens should always be considered potentially infectious. Follow good laboratory practices in storing, dispensing and disposing of these materials[9].

WARNING - Sodium azide (NaN_3) may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal of liquids, flush with large volumes of water to prevent azide buildup. Sodium azide may be toxic if ingested. If ingested, report incident immediately to laboratory director or poison control center.

Instructions should be followed exactly as they appear in this kit insert to ensure valid results. Do not interchange kit components with those from other sources other than the same lot number from IMMCO Diagnostics. Follow good laboratory practices to minimize microbial and cross contamination of reagents when handling. Do not use beyond expiration date on the label.

Materials Provided

Kit contains sufficient reagents to perform 96 determinations each.

12 x 8	Ready to use **Microplate** with individual breakaway microwells coated with hu tTG antigen.
1 x 1.5 ml	Ready to use **Positive Control*** *(red cap)*. Contains human serum positive for anti-tTG antibodies. The expected concentration range in EU/ml is printed on the label.
1 x 1.5 ml	Ready to use **Negative Control*** *(white cap)*. Contains human serum.
4 x 1.5 ml	Ready to use **set of 4 Calibrators***; Calibrator A *(green cap)*, Calibrator B *(violet cap)*, Calibrator C *(blue cap)* and Calibrator D *(yellow cap)*. Human serum containing antibodies to hu tTG. Concentrations in EU/ml are printed on the labels.
1 x 12 ml	Ready to use **anti-human Alk. Phos. Conjugate***. Color coded pink.
1 x 60 ml	Ready to use **Serum Diluent***. Color coded blue.
1 x 12 ml	Ready to use **Enzyme Substrate***. Contains pNPP. **Protect from light.**
1 x 12 ml	Ready to use **Stop Solution**.
2 vials	Powder **Wash Buffer**. Reconstitute to one liter each.
2 x	Protocol Sheets

*Contains <0.1% NaN_3

Materials Required But Not Provided

- Deionized or distilled water
- Squeeze bottle to hold diluted wash buffer
- Pipettors capable of delivering 5 µl to 1000 µl
- Disposable pipette tips
- Clean test tubes 12 x 75 mm and test tube rack
- Timer
- Absorbent paper
- Microplate reader capable of reading absorbance values at 405 nm. If dual wavelength microplate reader is available, the reference filter should be set at 600-650 nm.
- Automatic microplate washer capable of dispensing 200 µl

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Other available serological tests to aid in the Diagnosis of CD

ImmuGlo™ anti-endomysial antibody (EMA) IFA	*Code 1114*
ImmuGlo™ anti-reticulin antibody (ARA) IFA	*Code 1115*
ImmuLisa™ anti-gliadin antibody (AGA) IgA ELISA	*Code 1117A*
ImmuLisa™ anti-gliadin antibody (AGA) IgG ELISA	*Code 1117G*
ImmuLisa™ anti-gliadin antibody (AGA) screen ELISA	*Code 1117S*
ImmuLisa™ anti-tTG antibody IgA ELISA	*Code 1157*
ImmuLisa™ anti-tTG antibody IgG ELISA	*Code 1157G*

SPECIMEN COLLECTION AND HANDLING

Only serum specimens should be used in this procedure. Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of the test and should not be used. Store specimens at 2-8°C for no longer than one week. For longer storage, serum specimens should be frozen. Avoid repeated freeze and thaw of samples.

PROCEDURE

Procedural Notes

- Before starting with the assay read carefully the product insert.
- Let serum specimens and test reagents equilibrate at room temperature for at least 30 minutes before starting with the test procedure. Return all unused specimens and reagents to refrigerator immediately after use.
- All dilutions of the patient samples should be prepared prior to starting with the assay.
- Good washing technique is critical. If washing is performed manually, adequate washing is accomplished by directing a forceful stream of wash buffer with a wide tip wash bottle across the entire microplate. **An automated microplate washer is recommended.**
- Use a multichannel pipette capable of delivering 8 wells simultaneously. This speeds the process and provides for a more uniform incubation time.
- For all steps, careful control of timing is important. The start of all incubation periods begins with the completion of reagent addition.
- Addition of all samples and reagents should be performed at the same rate and in the same sequence.
- Remove required microwell strips from the pouch and carefully reseal the pouch to prevent condensation in the unused wells. Return pouch immediately to refrigerator.

Test Method

Step 1 Let all reagents and specimens equilibrate at room temperature.

Step 2 Label protocol sheet to indicate sample placement in the wells. It is good laboratory practice to run samples in duplicate.

Step 3 For a **qualitative determination** use only the Ready to Use Low Calibrator D (*vial with yellow cap*).

or For a **semi-quantitative determination** use the Ready to Use Calibrators A through D as depicted in the sample layout below.

PERFORMANCE CHARACTERISTICS

The utility of the ImmuLisa™ anti-hu tTG IgA Antibody ELISA was determined by comparing the results with:

a) another commercially available anti-hu tTG IgA ELISA method and

b) ImmuGlo™ anti-endomysial antibody immunofluorescence method.

Normal Range: The normal range was established by testing 64 serum samples from apparently healthy donors obtained from the Red Cross. The mean plus three standard deviations of the mean of this normal population was used to determine the cut-off between normal and borderline positive individuals.

Comparative Specificity and Sensitivity

A. Endomysial Antibodies vs. ImmuLisa™ hu tTG IgA Antibody ELISA: A total of 274 samples were tested for antibodies to endomysium using ImmuGlo™ anti-endomysial antibody kit and the results of anti-endomysial antibodies were compared with ImmuLisa anti-hu tTG ELISA method. The results are summarized below:

B. ImmuLisa™ anti-hu tTG IgA ELISA vs. Another Commercial anti-hu tTG IgA ELISA Method: A total of 74 samples were tested on the ImmuLisa™ anti-hu tTG IgA antibody kit and another commercially available anti-hu tTG IgA kits. The results of these studies follow:

ImmuLisa™ anti-hu tTG IgA

ImmuGlo™ EMA	Positive	Negative	Total
Positive	109	3	112
Negative	5	157	162
Total	114	160	274

Relative Agreement: 97%
Relative Sensitivity: 98%
Relative Specificity: 94%

ImmuLisa™ anti-hu tTG IgA

Other ELISA	Positive	Negative	Total
Positive	57	1	58
Negative	1	15	16
Total	58	16	74

Relative Agreement: 97%
Relative Sensitivity: 96%
Relative Specificity: 98%

C. Cross Reactivity: A total of 65 disease controls from autoimmune vesiculobullous cases such as pemphigus were tested. None were found positive.

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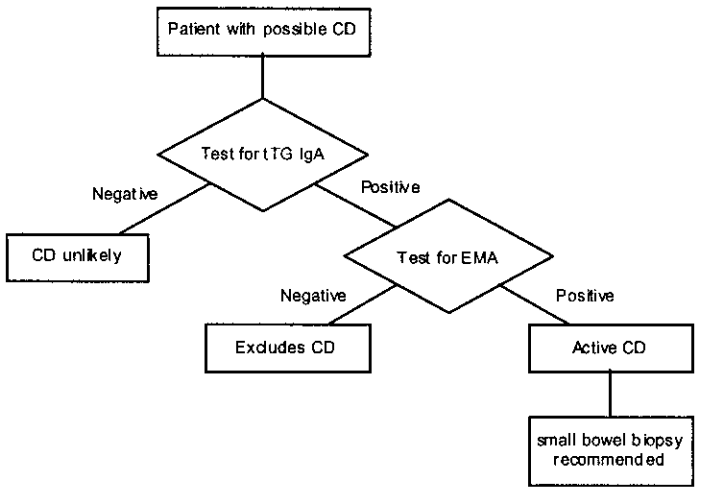
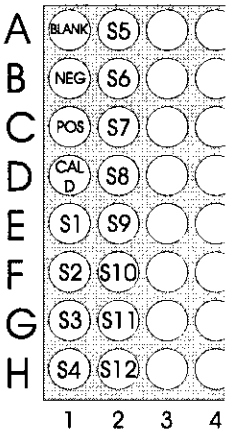
As there are several antibody markers associated with CD, the following algorithm may serve as a guide in the interpretation of various serological markers seen in CD:

EMA	ARA	AGA IgG	AGA IgA	anti-tTG	Interpretation
+	+	+	+	+	CD - certain
+	+	-	+	+	CD - certain
+	+	-	-	+	CD - certain
+	-	+	+	+	CD - certain
+	-	-	-	+	CD - highly probable
-	+	+	+	+	CD - highly probable
-	-	+	+	+	CD- possible
-	-	+	-	-	CD - unlikely but may not be precluded
-	-	-	+	-	
-	-	-	-	-	CD - excluded

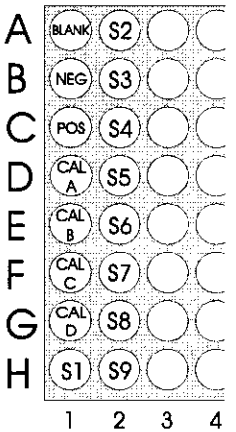
Some investigators have taken the following approach to establish the diagnosis of CD using serological methods:



QUALITATIVE DETERMINATION



SEMI-QUANTITATIVE DETERMINATION



Specimen Layout

Step 4 Prepare a **1:51** dilution of the patient samples by mixing **10 µl** of the patient sera with **0.5 ml** of Serum Diluent.

Step 5 Pipette **100 µl** of Ready to Use Calibrators, Positive and Negative controls and diluted patient samples to the appropriate microwells as per protocol sheet.

Note: Include one well which contains **100 µl** of the Serum Diluent as a reagent blank. Zero the ELISA reader against the reagent blank. The absorbance of the reagent blank should not be more than 0.3 when read against air.

Step 6 Incubate **60 minutes** (± 5 min) at room temperature.

Step 7 Wash **4x** with wash buffer. For manual washing, fill each microwell with reconstituted wash buffer. Discard the fluid by inverting and tapping out the contents of each well or by aspirating the liquid from each well. To blot at the end of the last wash, invert strips and tap the wells vigorously on absorbent paper towels. For automatic washers, program the washer as per manufacturer's instructions.

Step 8 Pipette **100 µl** of Conjugate into microwells.

Step 9 Incubate **30 minutes** (± 5 min) at room temperature.

Step 10 Wash all microwells as in Step 7.

Step 11 Pipette **100 µl** of Enzyme Substrate into each microwell in the same order and timing as for the Conjugate.

Step 12 Incubate **microwells 30 minutes** (± 5 min) at room temperature.

Step 13 Pipette **100 µl** of Stop Solution into each microwell using the same order and timing as for the addition of the Enzyme Substrate. Read absorbance within 1 hour of adding Stop Solution.

Step 14 Read absorbance of each microwell at **405 nm** using a single or 405/630nm dual wavelength microplate reader against the reagent blank set at zero absorbance.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Quality Control

Calibrators, Positive and Negative Controls and a reagent blank must be run with each assay to verify the integrity and accuracy of the test. The absorbance reading of the reagent blank should be <0.3. Calibrator A should have an absorbance reading >1.0, otherwise the test must be repeated. The negative control must be <20 EU/ml. If the test is run in duplicate, take the mean of the two readings to determine the concentration of anti-hu tTG. While performing qualitative determinations, the optical density of Calibrator D must be greater than that of the negative control and less than the positive control. For semi-quantitative determinations, the positive control must give values in the range stated on the vial. We recommend borderline samples be tested with a fresh sample taken at a later date to ensure accuracy.

RESULTS

Calculations

Concentrations of the patient samples can be determined by either of two methods:

1. QUALITATIVE DETERMINATION

Results obtained by this method should be reported as positive or negative.

$$\frac{\textbf{Abs. of Test Sample}}{\textbf{Abs. of Calibrator D}} \times \textbf{EU/ml of Calibrator D} = \textbf{EU/ml Test Sample}$$

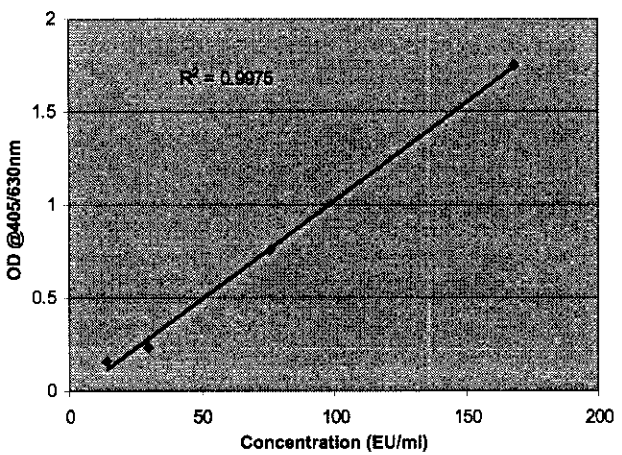
2. SEMI-QUANTITATIVE DETERMINATION

Plot absorbance of Calibrator A through D against their respective concentration on linear-linear graph paper. Plot the concentration in EU/ml on the X-axis against the absorbance on the Y-axis and draw the best fit curve. Determine the concentrations of patient samples on the curve against the corresponding absorbance value.

Calibrator

The ready to use Calibrators are included to provide semi-quantitation and must be used with each run. Patient samples containing higher antibody levels may give absorbance values greater than that of the Calibrator A. For determining accurate semi-quantitative values such serum samples should be further diluted so they fall within the range of the calibrator curve when retested. For determining EU/ml, multiply the units obtained by the dilution factor.

Anti-hu tTG ImmuLisa™ Standard Curve



Interpretation

The following serves only as a guide in the interpretation of the laboratory results. These values were determined by testing 113 adult normal blood donors. The values depicted below are the mean of the normal subjects plus 3SD. Each laboratory must determine its own normal values.

anti-hu tTG value	Interpretation
<20 EU/ml	Negative
20-25 EU/ml	Indeterminate (Borderline)
>25 EU/ml	Positive

LIMITATIONS OF THE PROCEDURE

The ImmuLisa™ anti-hu tTG assay should not be performed on grossly hemolyzed, microbially contaminated or lipemic samples. The method should be used for testing human serum samples only. Results obtained serve only as an aid in the diagnosis and should not be interpreted as diagnostic in themselves. In IgA deficient CD patients the anti-hu tTG IgA ELISA may be negative.

EXPECTED VALUES

The expected values in a normal population are negative (<20 EU/ml for adults and children). However, it has been determined that some apparently healthy, asymptomatic individuals may test positive for IgA anti-tTG antibodies.

The incidence of anti-tTG antibodies has been studied by various investigators and the findings are summarized below:

Incidence of EMA and tTG Antibodies

Study Group	No.	EMA	tTG
Dermatitis herpetiformis	44		
on gluten diet	33	33 (100%)	33 (100%)
on gluten-restricted diet	11	0 (0%)	3 (27%)
Celiac Disease	58		
on gluten diet	34	34 (100%)	31 (91%)
on gluten-restricted diet	24	0 (0%)	1 (4%)
Controls	189	0 (0%)	0 (0%)
Pemphigus	80	0 (0%)	1 (1%)
Linear IgA dermatosis	3	0 (0%)	0 (0%)
Normal	106	0 (0%)	1 (1%)

Kumar V et al; Clin Immunol 2001; 98: 378-382

The incidence and the levels of anti-tTG antibodies is dependent upon the diet status. The levels of these antibodies decrease and eventually will become negative in patients with CD who are on a gluten-free diet. Similarly, the levels of these antibodies will increase and may become positive when patients with CD who were on a gluten-free diet ingest a gluten-containing diet[10,11].

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Attachment 2

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

(b)(4)

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Attachment 3

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

(b)(4)

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Attachment 4

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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TITLE: QC Test for ELISA Positive Controls, Negative Controls and Calibrators

RETESTING

If any of the acceptance criteria does not conform, the Director of Manufacturing or immediate Supervisor should be informed and necessary action taken. If the non-confirmation compromises the efficacy of the kit performance, then the lot should be rejected with proper documentation.

Product is:

✓ released for use in packaging
____ Rejected
____ Hold – reason _____

Performed by: (b)(4) [redacted] Date 7/21/03

Reviewed by: (b)(4) [redacted] Date 7/22/03

QC Approval: (b)(4) [redacted] (b)(4) [redacted] Date 8/19/03

64

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Attachment 5

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

(b)(4)

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Recombinant human tTG Stability Study

Accelerated:

Materials stored at 37°C. Recommended storage temperature is 2-8°C. In accelerated conditions one day at 37°C is determined equivalent to one month at 2-8°C. Anticipated shelf life: 18 months.

(b)(4)

[redacted]

Real Time:

Real Time stability studies are ongoing. Performance within quality specifications through (b)(4) [redacted].

(b)(4)

[redacted]

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

(b)(4)

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Attachment 6

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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August 14, 2003
Food and Drug Adminstration
Office of Device Evaluation
Center for Devices and Radiological Health
Document Mail Center (HFZ-440)
Attn: Rufina Carlos
9200 Corporate Blvd.
Rockville, MD 20850

Re: ImmuLisa Anti-Human Tissue Transglutaminase (hu-tTG) Antibody IgA ELISA 510(k) submission

Dear Ms. Carlos:

Enclosed you will find two copies of the 510(k) submission for our device listed below:

Submitter:	IMMCO Diagnostics, Inc. 60 Pineview Drive Buffalo, NY 14228
Contact:	Kevin J. Lawson Address same as above (716) 691-0091 ext. 110 kevinlawson@immcodiagnostics.com
Proprietary Name:	ImmuLisa Anti-Human Tissue Transglutaminase (hu- tTG) Antibody IgA ELISA
Classification Name:	SYSTEM, TEST, ANTIBODIES, ANTI-TISSUE TRANSGLUTAMINASE IGA
FDA Registration No.:	1315336
Manufactured by IMMCO:	same registration number
Device Class:	2
Intended Use:	An enzyme linked immunoassay (ELISA) for the detection and semi-quantitation of IgA antibodies to Human Tissue Transglutaminase (hu-tTG), as an aid towards the diagnosis of Celiac Disease

A 510(k) statement is contained within.

Please review this device at your earliest opportunity to enable us to market the device as soon as possible. Thank you for your attention to this matter.

Sincerely,

Kevin Lawson
Director of Regulatory Affairs

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request 2014-6998; Released 10/30/14

TABLE OF CONTENTS

Truthful and Accurate Statement	3
Compliance with Special Controls or Performance Standards	4
Proposed Labels, Labeling and Advertisement	5
Information and Data Supportive of Substantial Equivalency Claim	6
General Description	6
Principles of Procedures	7
Similarities with Other Commercially Available Devices	8
Differences from Other Commercially Available ELISA Devices	9
Comparison Summary Table	9
Performance Comparison	10
Manufacturing Information	14
Quality Information	16
510(k) Statement	19
Indications for Use Statement	20
Appendix A	21
Appendix B	22
Appendix C	23
Appendix D	24
Appendix E	25
Appendix F	26

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PREMARKET NOTIFICATION
TRUTHFUL AND ACCURATE STATEMENT
[As required by 21 CFR 807.87(j)]

I certify that, in my capacity as Director of Regulatory Affairs of IMMCO Diagnostics, I believe to the best of my knowledge, that all data and information submitted in the premarket notification are truthful and accurate and that no material fact has been omitted.

_____
(Signature of Certifier)

Kevin Lawson
(Typed Name)

_____
(Date)

_____
(Premarket Notification [510(k)] Number)

78

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COMPLIANCE WITH SPECIAL CONTROLS
OR PERFORMANCE STANDARDS

To date, no special controls or performance standards which affect this system have been finalized under Section 514 of the Act.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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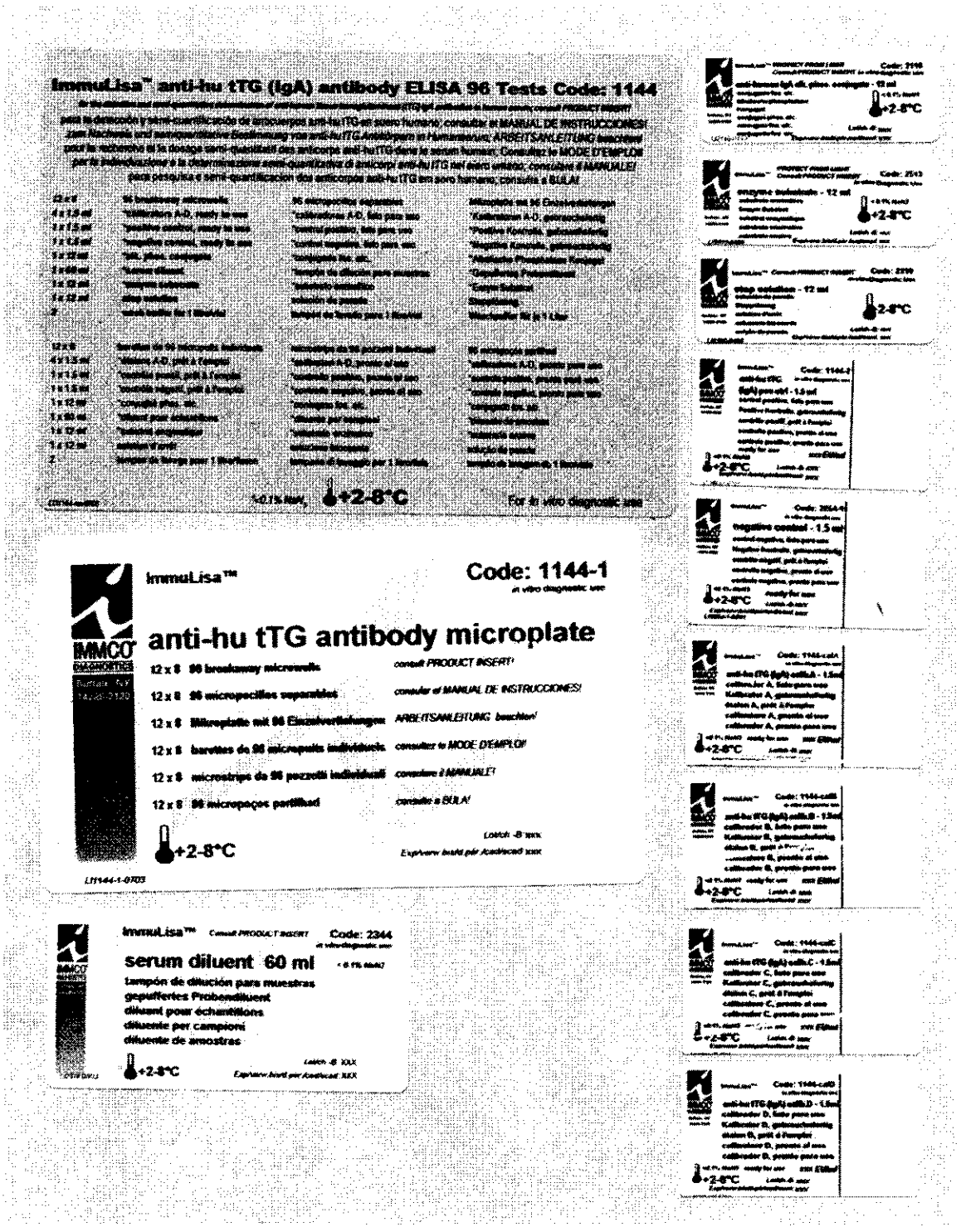
PROPOSED LABELS, LABELING AND ADVERTISEMENT



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Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request 2014-6998; Released 10/30/14

INFORMATION AND DATA SUPPORTIVE
OF SUBSTANTIAL EQUIVALENCY CLAIM

GENERAL DESCRIPTION

Celiac Disease (CD) is an autoimmune gastrointestinal disorder that may occur in genetically susceptible individuals triggered by the ingestion of gluten-containing grains such as wheat, barley and rye. Of the many autoimmune disorders, CD represents one of the few disorders where the etiological agent is known and the disease subsides and goes in remission once the etiological agent is withdrawn from the diet.

CD is characterized by malabsorbtion resulting from inflammatory injury to the small intestinal mucosa and, when prolonged, can cause malnutrition. The classical symptoms of CD include diarrhea, weight loss and malnutrition.

Only a small percentage of patients with CD, however, presents with classical symptoms. Consequently, the clinical spectrum of CD has grown much broader than in the past to include patients that do not present with classical symptoms. It is not uncommon for the initial symptoms to be non-gastrointestinal or for gastrointestinal symptoms, if present, to be mild or intermittent.

Some of the common non-gastrointestinal manifestations include short stature, iron and folate deficiency, anemia, bone loss, aphthous stomatitis, arthralgia, dental enamel defects, etc. The need to examine a wider range of clinical presentation has led to greater numbers of individuals diagnosed with CD later in life than ever before. Adults may present with iron deficiency, macrocytic anemia and hypocalcaemia.

Studies have found the prevalence of CD to be highly variable from population to population (Guandalini S, Gupta P. Celiac disease- A diagnostic challenge. *Clin Appl Immun Rev* 2002;2:293-305). The true prevalence has been difficult to ascertain. The disparate criteria used in the diagnosis of CD are often the cause. If only the clinical criteria are used in determining prevalence, the incidence of CD is much lower as compared with incidence established by serological methods (Guandalini S, Gupta P. Celiac disease- A diagnostic challenge. *Clin Appl Immun Rev* 2002;2:293-305; Fasano A, et al. Prevalence of celiac disease in at risk and not-at-risk groups in the United States 2223; 163:286-292). Using serological methods the incidence of CD in the general population is approximately one in 200.

Diagnosis of CD based on clinical criteria thus can be misleading and may lead to serious delays in proper diagnosis. Frequently, delays in diagnosis extend 10-13 years from the first clinical presentation of symptoms.

Failure to diagnose CD early on may predispose an individual to long-term complications such as splenic atrophy and intestinal lymphoma. The incidence of lymphoma arising in the context of CD is difficult to ascertain. One study has shown incidence of lymphoma involving the gastrointestinal (GI) tract in patients with CD to range from 3.6 percent to 40 percent (. Wolber R, et al. Lymphocytic gastritis in patients with celiac sprue or spruelike intestinal disease. *Gastroenterology* 1990;98:310-315). In another recent study, CD is associated with significantly elevated risk for intestinal lymphoma, especially for non-Hodgkin's (Catassi, et al. Risk of non-Hodgkin lymphoma in celiac disease. *JAMA* 2002;287:1413-1419). A gluten-free diet (GFD) normalizes the mucosa and helps reduce the malignant potential.

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Histological examination of the small intestinal biopsy remains the gold standard for diagnosing CD, but it has its own limitations. Of these include some patients with latent or even active CD that may have normal histopathology (Arranz E, Ferguson A. Intestinal antibody pattern of celiac disease: Occurrence in patients with normal jejunal biopsy histology. *Gastroenterology* 1993;104:1263-72).

The revised European Society of Paediatric Gastroenterology and Nutrition (ESPGAN) criteria include only a single biopsy with clear-cut remission of clinical symptoms on GFD (Hill ID, et al. Celiac disease: Working group report of the first world congress of pediatric gastroenterology, hepatology, and nutrition. *J. Pediatr Gastroenterol Nutr* 2002; 35:S78-S88). Positive serology at the time of diagnosis with disappearance on GFD contributes to the diagnosis. The various serological tests employed in the work-up of patients suspected to have CD include anti-gliadin antibody (AGA), anti-endomysial antibody (EMA), anti-reticulin antibody (ARA) and anti-tissue transglutaminase (tTG) antibody tests. Antibodies to gliadin and tTG are detected by ELISA, whereas endomysium and reticulin antibodies are detected by indirect immunofluorescence. EMA are very specific indicators of CD. However the EMA test is an immuno-histochemical method and requires experience in reading immunofluorescence reactions. Rosario et al (*J Pediatr Gastroenterol Nutr* 1998;27:191-195) concludes that "EmA-IgA is 100 percent sensitive and specific in active, untreated IgA-sufficient CD patients when performed by an established laboratory."

Since identification of tTG as the endomysial antigen, ELISA methods have been described for detecting antibodies in the sera of patients with CD. The advantage of the anti-tTG antibody assay is that it is automatable and less subjective than EMA. For this reason, many laboratories have opted to use the tTG antibody method as the screening method. In these laboratories, it may be the only assay used for detection of CD cases. In various studies on the efficacy of the tTG antibody method for screening for CD, the specificity and sensitivity of this method has been found to range from 90 percent to 95 percent (Vitoria JC, et al. Antibodies to gliadin, endomysium, and tissue transglutaminase for the diagnosis of celiac disease. *J Pediatr Gastroenterol Nutr* 1999;29:571-4). Human tTG has been described to improve the sensitivity of the tTG antibody assay for CD.

PRINCIPLES OF PROCEDURES

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SIMILARITIES WITH OTHER COMMERCIALLY AVAILABLE DEVICES

The ImmuLisa anti-human tTG antibody IgA ELISA test kit is substantially equivalent in principle and methodology and performance to the currently marketed IMMCO ImmuLisa anti-tTG antibody IgA ELISA antibody test kit (K992878) and INOVA anti-tTG antibody kit (K011566) for the detection of IgA antibodies to tissue Transglutaminase. Copies of the package inserts for each assay have been included in the appendix of this submission. In addition, the ImmuLisa hu tTG antibody kit is also equivalent to the Immuflour EMA test kit (K912551)

Similarities among the IMMCO ImmuLisa anti hu tTG antibody IgA ELISA, the ImmuLisa anti-tTG antibody ELISA and the INOVA anti-tTG antibody IgA ELISA kits are listed below:

- All three kits are enzyme linked immunosorbant assays (ELISA) based assays.
- All three kits detect IgA autoantibodies to tTG in human sera
- All three kits allow semiquantitation of antibodies to tTG.
- All three kits follow the same basic procedure for testing antibodies in patient sera.
- All three kits provide pre-diluted controls.
- All three kits use enzyme substrate that changes color when hydrolyzed by the enzyme to a color product. The color change is directly proportional to the concentration of the antibody in patient's serum and hence is the basis for quantitation.
- All three kits use stop solution to stop the enzymatic reaction and further color change.
- All kits are recommended to be stored at 2-8°C.
- All three kits have very similar labeling content.

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DIFFERENCES FROM OTHER COMMERCIALLY AVAILABLE ELISA DEVICES

- The IMMCO kit has a set of four calibrators and a positive and negative control, while the INOVA kit has high positive, low positive and negative controls, but no calibrators.
- The enzyme conjugate in the IMMCO kit is alkaline phosphatase labeled, while the INOVA kit conjugate is horseradish peroxidase labeled.
- The wash buffer in the IMMCO kits is powder to be reconstituted to one liter in water, while INOVA provides a 40x liquid concentrate to be diluted before use.

COMPARISON SUMMARY TABLE

	IMMCO anti-tTG	IMMCO anti-human tTG	INOVA anti-human tTG
Methodology	ELISA	ELISA	ELISA
Detection of antibodies	tTG	tTG	tTG
Screening dilution	1:51	1:51	1:101
Quantitation	Semi	Semi	Semi
Conjugate	alkaline phosphatase	alkaline phosphatase	horseradish peroxidase
Conjugate specificity	IgA	IgA	IgA
Substrate	p-NPP	p-NPP	TMB
Positive control	yes	yes	yes
Negative control	yes	yes	yes
Calibrators	yes	yes	no
Serum diluent	yes	yes	yes
Wash buffer	yes	yes	yes
Stop solution	yes	yes	yes
Absorbance	405nm	405nm	450nm
Storage	2-8°C	2-8°C	2-8°C

84

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PERFORMANCE COMPARISON

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85

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(b)(4)

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Based upon the studies above, we find the ImmuLisa human tTG antibody test kit to be equivalent to the INOVA anti-human tTG antibody kit, the IMMCO anti-guinea pig tTG antibody kit and the immunofluorescence EMA test kit.

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Precision Study:

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(b)(4)

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88

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Records processed under FOIA Request 2014-6998; Released 10/30/14

MANUFACTURING INFORMATION

Information provided following FDA guidelines for calibration and quality control labeling for in vitro diagnostic use devices.

The IMMCO anti-human tissue Transglutaminase antibody assay is manufactured according to FDA QSR guidelines and in accordance with its registered ISO9000 quality system.

As new lots of calibrators are developed, studies are performed to calibrate values against original calibrators. Each lot of calibrator is also tested using normal human sera, clinical samples and internal standards as in the initial Cutoff Study described in **Performance Comparison: Controls and Calibrators**, above.

QUALITY INFORMATION

Information provided following FDA guidelines for calibration and quality control labeling for in vitro diagnostic use devices.

Quality control studies are run on new microplate lots. Inter- and intra-plate CV is determined selecting plates from the beginning, middle and end of production and accepted or rejected according to manufacturing acceptance criteria. New lots of calibrators and conjugate are subjected to additional quality control and tested in conjunction with each plate lot. Each calibrator vial is labeled with the EU value of its contents based on these studies.

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INTERPRETATION OF RESULTS

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90

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Records processed under FOIA Request 2014-6998; Released 10/30/14

QUALITY CONTROL IN PERFORMING TEST

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LIMITATION OF PROCEDURE

The ImmuLisa™ Anti-human tissue Transglutaminase test should not be performed on grossly hemolyzed, microbially contaminated or lipemic samples. Such samples may not provide accurate results. Data produced using compromised specimens has been included in the interference studies found in Appendix F.

This method should be used for testing human serum samples only. Furthermore, a diagnosis cannot be made on the basis of anti- human tissue transglutaminase antibody results alone. The results of other laboratory tests and clinical findings must be considered.

In IgA-deficient CD patients, the anti-human tTG IgA ELISA may be negative.

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EXPECTED VALUES

The expected values in a normal population are negative (<20 EU/ml). However, it has been determined that some apparently healthy, asymptomatic individuals may test positive for IgA anti-human tTG antibodies. It has been suggested that all anti-tTG antibody positive samples need to be confirmed by the immunofluorescence EMA test (Fasano A, et al. Prevalence of celiac disease in at risk and not-at-risk groups in the United States 2003; 163:286-292).

The incidence of anti-tTG antibodies has been studied by various investigators and the findings of the specificity and sensitivities of the various studies are summarized below:

Reported sensitivities and specificities of various IgA t-TG antibody assays

Method	Sensitivity (%)	Specificity (%)
Guinae pig tTG		
Dieterich et al. (1998)	98	95
Sulkanen et al (1998)	95	94
Lampasona et al (1998)	84	92
Bazzigaluppi et al. (1999)	85	91
Troncone et al (1999)	92	98
Biagi et al (1999)	95	90
Lock et al (1999)	85	97
Piaggio et al (1999)	100	100
Amin et al (1999)	87	97
Miller et al (1999)	100	97
Koop et al (2000)	90	76
Sblattero et al (2000)	84	98
Hansson et al (2000)	95	96
Human erythrocyte tTG		
Bazzigaluppi et al (1999)	97	96
Sardy et al (1999)	98	98
Sblattero et al (2000)	92	99
Reombinant tTG		
Lampassona et al (1998)	98	97
Seissler et al (1999)	96	100

Adapted from Leon F et al Scand J. Gastroenterol 2001;36:849-53.

92

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The incidence and the levels of anti-tTG antibodies are dependent upon the diet status. The levels of these antibodies decrease and eventually will become negative in patients with CD who are on a gluten-free diet. Similarly, the levels of these antibodies will increase and may become positive when patients with CD who were on a gluten-free diet ingest a gluten-containing diet (Sulkanen S, Haltunen T, Laurila K, et al. Tissue Transglutaminase autoantibody enzyme-linked immunosorbent assay in detecting celiac disease. Gastroenterology; 1998, 115: 1322-1328. Dietrich W, Laag E, Schopper H, et al. Autoantibodies to tissue transglutaminase as predictors of celiac disease; Gastroenterology 1998, 115: 1317-1321.).

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510(k) STATEMENT
(As Required By 21 CFR 807.93)

I certify that, in my capacity as Director of Regulatory Affairs of IMMCO Diagnostics, Inc., I will make available all information included in this premarket notification on safety and effectiveness within 30 days of request by any person if the device described in the premarket notification submission is determined to be substantially equivalent. The information I agree to make available will be a duplicate of the premarket notification submission, including any adverse safety and effectiveness information, but excluding all patient identifiers, and trade secret and confidential commercial information, as defined in 21 CFR 20.61.

_____
(Signature of Certifier)

Kevin Lawson_____
(Typed Name)

_____
(Date)

_____
*(Premarket Notification [510(k)] Number)

*For a new submission, leave the 510(k) number blank.

94

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INDICATIONS FOR USE STATEMENT

510(k) Number (if known): K_____

Device Name: ImmuLisa Anti-human tissue Transglutaminase (human tTG) IgA ELISA

Indications For Use: An enzyme linked immunoassay (ELISA) for the detection and semi-quantitation of IgA antibodies to human tissue transglutaminase, as an aid in diagnosing patients with Gluten Sensitive Enteropathy (celiac Disease and Dematitis herpetiformis)

(PLEASE DO NOT WRITE BELOW THIS LINE - CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)

Prescription Use _____ OR Over-The-Counter Use _____
(Per 21 CFR 801.109) (Optional Format 1-2-96)

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APPENDIX A

IMMCO Anti-hu tTG Antibody ELISA Product Insert

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APPENDIX B

IMMCO Anti-tTG Antibody ELISA Product Insert

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APPENDIX C

INOVA tTG Product Insert

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Appendix D

ImmuGlo EMA Product Insert

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APPENDIX F

Interference Studies

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Cross Reactivity Studies

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