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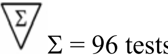
Gliadin IgA ELISA

For in vitro diagnostic use.
Catalog No. 1022-2

IVD	See external label	2°C-8°C	 Σ = 96 tests	REF #1022-2

INTENDED USE
The Diagnostic Automation, Inc. Gliadin IgA ELISA test system is intended for the qualitative and semi-quantitative detection of IgA-class antibodies to gliadin in human serum. The test system is intended to be used as an aid in the diagnosis of gastrointestinal disorders, mainly Coeliac Disease. This test is for *in vitro* diagnostic use.

SIGNIFICANCE AND BACKGROUND

Coeliac disease is an inflammatory disorder of the small intestine induced by the prolamines of certain cereals, mainly the gliadins of wheat. This permanent intolerance to gliadin results in intestinal villous flattening and crypt hyperplasia in susceptible individuals. Immune reactions to gliadin are likely to play a role in the pathogenesis of the disease since both humoral and cell-mediated responses have been demonstrated in the peripheral blood and in the gut of coeliac patients (1).

Classic signs of coeliac disease in adult include malabsorption characterized by weight loss, abdominal distension, diarrhea and steatorrhoea occurs because of the loss of absorptive area and the immaturity of surface epithelial cells. By the early 1980s, clinical features of coeliac disease have changed (2,3). There had been a shift towards milder symptoms such as indigestion in adults and recurrent abdominal pain in children. The classic symptoms and signs had become rare. And, despite manifest mucosal lesion, the disease can be even symptom-free, clinically silent. In children, it has become evident that the disease exists or appears late even though classical forms with malabsorption are not apparent (4).

PRINCIPLE OF THE ELISA ASSAY

The Diagnostic Automation, Inc. Gliadin IgA ELISA test system is designed to detect IgA class antibodies to gliadin in human sera. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in microwells coated with gliadin. Anti-gliadin specific IgA antibodies in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase conjugated goat anti-human IgA is added to the wells and the plate is incubated. The conjugate will react with antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted conjugate.

3. The microtiter wells containing immobilized peroxidase conjugate are incubated with peroxidase substrate

solution. Hydrolysis of the substrate by peroxidase produces a color change. After a period of time, the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the test sample.

KIT COMPONENTS

Reactive Reagents

1. Twelve, 1 x 8-well strips coated with Gliadin (antigen). The strips are packaged in a strip holder and sealed in an envelope with desiccant
2. Horseradish peroxidase conjugated goat anti-human IgA. Ready to use. One 15mL vial.
3. Human positive serum control. One 0.35mL vial, red cap.
4. Human positive calibrator. One 0.5mL vial, blue cap.
5. Human negative serum control. One 0.35mL vial, greem cap.
6. Sample diluent. One 30 mL(green Cap) bottle containing Tween-20, bovine serum albumin, and phosphate-buffered-saline, (pH 7.2 ± 0.2). Ready to use.

7.TMB. One 15 mL (amber cap) bottle containing 3,3',5,5'-tetramethylbenzidine (TMB). Ready to use.

1. Stop solution. One vial containing 15 mL bottle of 1.0M H_2SO_4, 0.7M HCl. Ready to use. (Clear solution with a red cap).
2. Wash Buffer concentrate. One 100mL clear bottle containing phosphate-buffered-saline and Tween-20. 10X concentrate. (Blue solution with a clear cap). **NOTE:** 1X solution will have a pH of 7.2 ± 0.2.

Non-reactive components

1. Sample dilution plate. One 96 well microtiter plate for preparing serum dilutions.
2. Two mylar plate sealers.

PRECAUTIONS

1. For *In Vitro* Diagnostic Use.
2. The microwell strips do not contain any viable, infectious agents. However, the strips should be considered potentially infectious and handled accordingly. Wash solutions should be collected in a disposable basin and treated with 0.5% sodium hypochlorite (10% household bleach) at the end of the days run.
3. Do not use the ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
4. Wipe bottom of plate free of residual liquid and/or fingerprints which can alter optical density (OD) readings.
5. Control sera, conjugate, and wash buffer contain preservative which may be toxic if ingested; Thimerosal at a concentration of 0.04% (w/v). The sample diluent contains sodium azide at a concentration of 0.1% (w/v).
6. Sodium azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosion on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing sodium azide.
7. Dilution or adulteration of these reagents may result in loss of sensitivity.
8. Do not substitute reagents from kits with different lot numbers or from other manufacturers.
9. Each donor unit used in the preparation of the controls was found to be negative when tested by an FDA approved method for the presence of HBsAg, and for antibodies to HIV-1, HIV-2, and HCV.

WARNING - POTENTIAL BIOHAZARDOUS MATERIAL

Because no test method can offer complete assurance that human immunodeficiency virus, hepatitis B virus, or other infectious agents are absent, these specimens/reagents, as well as patient samples, should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiology and Biomedical Laboratories", 1984, p.12-16, 3rd edition- 1993, and OSHA Standard for Bloodborne Pathogens (7).

9. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin or mucous membranes.
10. Avoid microbial contamination of reagents. Incorrect results may occur.
11. Cross contamination of reagents and/or samples could cause false results.

12. TMB solution should be colorless, very pale yellow, very pale green, or very pale blue. Contamination of the TMB substrate solution with conjugate or other oxidants will cause the solution to change color prematurely. Do not use substrate solution if it is noticeably blue in color. To help eliminate the possibility of contamination, refer to Test Procedure, Section D.1. (Substrate Incubation) to determine amount of substrate solution to be used.
13. Reusable glassware must be washed out and thoroughly rinsed free of all detergents.
14. Strict adherence to the specified time and temperature of incubations is essential for accurate results. All reagents must be brought to 20-25°C before starting the assay.
15. Improper washing will cause false positive or false negative results. Be sure to blot the plates free of any residual wash solution before adding conjugate or substrate solution. Do not allow the wells to dry out between incubations.
16. Do not allow the stop solution to contact skin or eyes. If contact occurs, immediately flush with water.
17. Caution: Liquid waste at acid pH should be neutralized before adding to sodium hypochlorite (bleach).
18. Avoid splashing or generation of aerosols.
19. Do not expose reagents to strong light during storage or incubation.
20. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
21. Do not allow the conjugate to come in contact with containers which may have previously contained a solution utilizing sodium azide as a preservative. Residual amounts of sodium azide may destroy the conjugate's enzymatic activity.
22. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this kit.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

1. Microtiter plate reader capable of reading at a wavelength of 450nm.
2. Microliter pipettes capable of accurately delivering 10 and 200 µL.
3. Adjustable multichannel pipette (50-200µL) for dispensing conjugate, substrate and stop solution.
4. Reagent reservoirs for multichannel pipettes.
5. Wash bottle or plate washing system.
6. Distilled or deionized water.
7. One liter graduated cylinder.
8. Serological pipette: 1, and 10 or 25 mL.
9. Disposable pipette tips.
10. Paper towels.
11. Timer with alarm capable of measuring to an accuracy of ± 1 second.
12. Disposal basin and disinfectant, (Example: 0.5% sodium hypochlorite, 10% household bleach).

SPECIMEN COLLECTION

Only freshly drawn and properly stored blood sera obtained by approved aseptic venipuncture procedures should be used in this assay (5, 6). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.

Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored at 2-10° C for no longer than 48 hours. If delay in testing is anticipated, store test sera at -20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results.

STORAGE CONDITIONS

1. Store the unopened kit at 2-8°C.
2. Gliadin coated microwell strips: Extra strips should be immediately resealed with desiccant and returned to

storage at 2-8°C. Strips are stable for 60 days after the envelope has been opened and properly resealed, and the indicator remains blue.
3. Peroxidase conjugated goat anti-human IgA: Store at 2-8°C. DO NOT FREEZE.
4. Human control and calibrator sera: Store at 2-8°C.
5. TMB substrate solution: Store at 2-8°C.
6. Wash buffer: Store at 2-25°C. Stable for 30 days at 2-8°C after diluting to 1X, or 7 days when stored at room temperature.
7. Sample diluent: Store at 2-8°C.
8. Stop solution: Store at 2-25°C.

NOTE: All kit components are stable until the expiration date printed on the label provided the recommended storage conditions are strictly followed. Do not use beyond the expiration date.

PROCEDURE

PREPARATION OF REAGENTS
1. Wash Buffer: Dilute the 100mL of 10X concentrate with 900mL of distilled or deionized water. Mix thoroughly to dissolve any crystals that may be present.
2. Sample diluent, stop solution, conjugate, and substrate solutions are ready to use.

TEST PROCEDURE

A. Set-Up of the Assay

Remove the individual kit components and allow them to warm to room temperature (20-25°C). Determine the total number of samples and controls to be tested. The positive calibrator should be run in triplicate. Also, a positive control, negative control, and a reagent blank must be included each time the assay is run. Controls and samples should be run in duplicate until the laboratory becomes proficient with the test procedure. Each sample, calibrator, control, and reagent blank requires one antigen coated microwell.

Determine the number of microwells needed. After the strips and holder have warmed to room temperature cut open the protective envelope and remove the plate containing the antigen coated microwell strips. Strips that are not needed for the assay should be placed into the re-sealable pouch, sealed and returned to storage at 2-8° C.

B. Serum Incubation

Prepare a 1:21 dilution of the positive and negative controls, the positive calibrator and each patient serum as follows:
1. Add 10µL of each sample to a separate well of the dilution plate provided. Add 200µL of sample diluent to each well containing a sample.
2. Using a multichannel pipette, transfer 100µL of each diluted sample and control from the dilution plate to the test plate. Withdraw and expel the samples several times before the final transfer to ensure that the samples are properly mixed. Use a different pipette tip for each sample. Add 100µL of sample diluent to a well as a reagent blank.
3. Cover the wells with a plate sealer and incubate the plate at room temperature (20-25°C) for 25 $_{+/-}$ 5 minutes.
4. Wash the microwell strips 5X.
    a. Vigorously shake out the liquid from the wells.
    b. Fill each well with wash buffer. Make sure no air bubbles are trapped in the wells.
    c. Repeat steps **a.** and **b.** for a total of five washes.
    d. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no

residual wash solution remains. Collect wash solution in a disposable basin and treat with 0.5% sodium hypochlorite (10% household bleach) at the end of the days run.

NOTE: Autowash - If using an automated wash system, set the dispensing volume to 300-350/µLwell. Set the wash cycle for 5 washes with no delay between washes. Remove microtiter plate from washer, invert plate over paper towel and tap firmly to remove any residual wash solution from the wells.

C. Conjugate Incubation

1. Add 100µL of the conjugate solution to each well at the same rate and in the same order as the specimens were added.
2. Cover the plate with the plate sealer provided and incubate at room temperature (20-25°C) for 25 +/- 5 minutes.
3. Wash the plate by following the procedure in Step **B.4., a.** through **d.**

D. Substrate Incubation

1. Add 100µL of the TMB substrate solution to each well at the same rate and in the same order as the conjugate was added. (One mL of TMB substrate is sufficient for 8 wells).
2. Incubate the plate at room temperature (20-25°C) for 10 to 15 minutes.
3. Add 50µL of stop solution to each well at the same rate and the same order as the TMB solution was added. Positive samples will turn from blue to yellow. After adding stop solution, **tap plate several times to ensure that the samples are thoroughly mixed.**
4. Set the microplate reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the reagent blank. The plate should be read within 30 minutes after the addition of the stop solution.

QUALITY CONTROL

1. Each time the assay is run, the positive calibrator should be run in triplicate. A positive and negative control, and reagent blank must also be included in each assay.
2. Calculate the mean of the three positive calibrator determinations. If any of the three positive calibrator values differ by more than 15% from the mean, discard that value and calculate the mean of the remaining two values.
3. The mean OD value for the positive calibrator and the OD values for the positive and negative controls should fall within the following ranges:

	OD RANGE
Negative Control	≤ 0.25
Positive Calibrator	≥ 0.30
Positive Control	≥ 0.50

   a. The OD of the negative control divided by the mean OD of the positive calibrator should be ≤ 0.9.
   b. The OD of the positive control divided by the mean OD of the positive calibrator should be ≥ 1.25.
   c. If the control values are not within the above ranges, the test should be considered invalid and the test should be repeated.

4. The HPC is intended to monitor for substantial reagent failure and will not ensure precision at the assay cut-off.
5. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

INTERPRETATION OF RESULTS

A. Calculations

1. Correction Factor

a cut-off OD value for positive samples has been determined by the manufacturer and correlated to the calibrator. The correction factor (CF) will allow you to determine the cut0off value for positive samples and to correct for slight day-to-day variations in test results. The correction factor is determined for each lot of kit components and it printed on the component list located in the kit box.

2. Cut-off OD value

To obtain the cut-off OD value, multiply the CF by the mean OD of the calibrator determined above.
(CF X mean of Claibrator= cut-off OD value)

3. Index Values or OD ratios

Calculate the index Value or OD ratio for each specimen by dividing its OD value by the cut-off OD from step 2.

Example:

Mean OD of calibrator = 0.793
Correction factor(CF) =0.25
Cut-off OD = .793 x 0.25 = 0.198
Unknown Specimen OD = 0.432
Specimen Index Value or OD Ratio = 0.432/0.1980= 2.18

B. Interpretation

Index Values or OD ratios are interpreted as follows:The manufacturer has established the following guidelines for interpretation of patient results:

Negative specimens ≤ 0.90
Equivocal Specimens .91 to 1.09
Positive specimens ≥ 1.10

LIMITATION OF THE ASSAY

1. A diagnosis should not be made on the basis of Gliadin ELISA results alone. The results for gliadin should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedure.
2. False positives can occur as other gastrointestinal disorders, such as Crohn’s disease and food protein intolerance, may induce circulating antibodies to gliadin.
3. The clinical significance of any test result depends upon its relationship to other medical patient data. Disease diagnosis and management should be based on an evaluation of all relevant patient information.
4. Values for the pediatric population have not been established with this assay.
5. Gliadin IgA negative result in untreated patient does not rule out gluten-sensitive enteropathy when associated with high levels of gliadin IgG antibodies. The finding can often be explained by selective IgA deficiency, a relatively frequent finding in celiac disease.

EXPECTED VALUES

To establish or estimate the expected reactivity rate, the 305 specimens, which were tested in-house, were analyzed. This represented two groups of specimens; 255 clinical specimens which were either sent to the lab for routine gliadin serological analysis or were part of an external gliadin study, and 50 random normal donor specimens.

With respect to the clinical population, 107/255 (42.0%) were positive, 140/255 (54.9%) were negative, and 8/255 (3.1%) were equivocal.

With respect to the normal population, 49/50 (98.0%) were negative, 1/50 (2.0%) was positive.

PERFORMANCE CHARACTERISTICS

I. Comparative Study:

An in-house comparative study was performed to demonstrate the equivalence of the Diagnostic Automation, Inc. Gliadin IgA ELISA test system to another commercially available Gliadin IgA ELISA test system. Performance was evaluated using 305 specimens and the results are summarized in Table 1 below:

Table 1

		Diagnostic AutomationGliadin IgA ELISA			
		-	±**	+	**Totals**
Commercial ELISA Test System	**-**	175	2	7	184
	±**	8	2	9	19
	+	6	4	92	102
	Totals	189	8	108	305

Relative Sensitivity = 92/98 = 93.9%
95% Confidence Interval = 89% to 99%
Relative Specificity = 175/182 = 96.2%
95% Confidence Interval = 93% to 99%

Relative Agreement = 267/280 = 95.4%
95% Confidence Interval = 93% to 98%
** Data Excluded From Calculation

II. Reproducibility:

Reproducibility studies were conducted in-house using the same specimens. The study was conducted as follows:

Briefly, six specimens were tested on two different kit lots; values ranging from strong positive to negative. In addition to these six panel members, the kit positive control and the kit negative control were included as two additional precision members. Each specimen was tested in eight replicates, once per day, on each of three days. The resulting data was used to calculate both intra and inter-assay precision.

Kit 1

	Intra-Assay (n=8)						Inter-Assay (n=24)	
	Day 1		Day 2		Day 3			
Sample Number	Mean AAU/mL	CV	Mean AAU/mL	CV	Mean AAU/mL	CV	Mean AAU/mL	CV
Sample #1	8	32.4%	11	17.8%	7	15.8%	9	29.1%
Sample #2	81	5.0%	99	2.0%	87	4.3%	89	9.3%
Sample #3	160	4.6%	178	4.2%	168	2.8%	169	5.8%
Sample #4	178	9.0%	203	3.4%	182	8.1%	187	9.0%
Sample #5	165	5.1%	171	3.6%	175	7.8%	170	6.1%
Sample #6	269	7.8%	333	3.2%	318	5.1%	307	10.5%
NC	11	18.7%	15	9.2%	11	11.2%	12	18.7%
HPC	973	6.8%	1139	2.9%	1050	6.1%	1054	8.3%

Kit 2

Sample Number	Intra-Assay (n=8) Day 1 Mean AAU/mL	Day 1 CV	Day 2 Mean AAU/mL	Day 2 CV	Day 3 Mean AAU/mL	Day 3 CV	Inter-Assay (n=24) Mean AAU/mL	Inter-Assay CV
Sample #1	5	30.2%	6	21.4%	7	20.6%	6	26.5%
Sample #2	81	6.1%	77	4.3%	83	5.4%	80	6.1%
Sample #3	153	3.1%	158	4.5%	159	7.0%	157	5.3%
Sample #4	179	6.3%	162	2.2%	177	3.9%	173	6.3%
Sample #5	174	10.0%	154	6.9%	171	6.4%	166	9.4%
Sample #6	321	4.4%	282	4.1%	304	3.3%	302	6.6%
NC	10	10.9%	11	28.8%	11	9.1%	11	17.8%
HPC	1175	8.6%	1185	8.4%	1089	5.4%	1150	8.3%

CROSS REACTIVITY:

To investigate the potential for positive reactions due to cross reactive antibodies, twenty-six specimens which were reactive for various auto antibodies (ANA, PR3, MPO, cardiolipin, dsDNA, ENA, Jo-1, RF, Scl-70, Sm, Sm/RNP, SSA and SSB) were tested on the Gliadin IgA test system. All twenty-six (26/26) were negative for gliadin IgA activity. The results of this study indicate that the potential for interference due to cross reactivity with such auto antibodies is unlikely.

REFERENCES

1. Trocone R, Ferguson A: Anti-gliadin Antibodies. J. of Ped. Gastro and Nut. 12:150-158, 1991.
2. Swinson CM, Levi AJ: Is coeliac disease underdiagnoses? BMJ 281:1258-1260, 1980.
3. Logan RFA, tucker G, Rifkind EA, Heading RC,Ferguson A: Changes in clinical features of coeliac disease in adults in Edinburgh and the Lothianss 1960-79. BMJ 286:95-97, 1983.
4. Maki M, Kallonen K, Lahdeaho ML, Visakorpi JK: Changing pattern of childhood coeliac disease in Finland. Acta Paediar Scand 77:408-412, 1988.
5. Procedures for the collection of diagnostic blood specimens by venipuncture. Second Edition: Approved Standard (1984). Published by National Committee for Clinical Laboratory Standards.
6. Procedures for the Handling and Processing of Blood Specimens. NCCLS Document H18-A, Vol. 10, No. 12, Approved Guideline, 1990.
7. U.S. Department of Labor, Occupational Safety and Health Administration: Occupational Exposure to Bloodborne Pathogens, Final Rule. Fed. Register 56:64175-64182, 1991.

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ISO 13485-2003

Issue Date: 10/2003
F/Insert/List B/Gliadin IgA_1022-2