DRG® Gliadin IgG ELISA (EIA-3371)

Revised 12 Apr. 2010 rm (Vers. 1.1) **IVD**

INTENDED USE

The DRG Gliadin IgG ELISA test system is intended for the qualitative and semi-quantitative detection of IgG-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 has 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 maiabsorption are not apparent (4).

PRINCIPLE OF THE ELISA ASSAY

The DRG Gliadin IgG ELISA test system is designed to detect IgG 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 IgG 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 IgG 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 un-reacted 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.

MATERIALS PROVIDED

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on packaging label.

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1. **Plate.** 96 wells configured in twelve 1x8-well strips coated with Gliadin antigen. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
2. **Conjugate.** Conjugated (horseradish peroxidase) goat anti-human IgG (y chain specific). Ready to use. One, 15 mL vial with a white cap. Preservative added.
3. **Positive Control (Human Serum).** One, 0.35 mL vial with a red cap. Preservative added.
4. **Calibrator (Human Serum).** One, 0.5 mL vial with a blue cap. Preservative added.
5. **Negative Control (Human Serum).** One, 0.35 mL vial with a green cap. Preservative added.
6. **Sample diluent.** One 30 mL bottle (green cap) containing Tween-20, bovine serum albumin and phosphate-buffered-saline, (pH 7.2 ± 0.2). Green solution, ready to use. Note: Shake Well Before Use. Preservative added.
7. **TMB:** One 15 mL amber bottle (amber cap) containing 3,3',5,5'-tetramethylbenadine (TMB). Ready to use. Contains DMSO ≤ 15% (w).
8. **Stop solution:** One 15 mL bottle (red cap) containing 1M H_2SO_4, 0.7M HCI. Ready to use.
9. **Wash buffer concentrate (10X):** dilute 1 part concentrate + 9 parts deionized or distilled water. One 100 mL bottle (clear cap) containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). Contains preservative NOTE: 1X solution will have a pH of 7.2 ± 0.2.

PRECAUTIONS

1. For *In Vitro* Diagnostic Use.
2. Normal precautions exercised in handling laboratory reagents should be followed. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective gloves, clothing and eye/face protection. Do not breathe in vapor. Dispose of waste observing all local, state and federal laws.
3. 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.
4. The human serum controls are POTENTIALLY BIOHAZARDOUS MATERIALS. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products 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 Microbiological and Biomedical Laboratories": current edition; and OSHA's Standard for Bloodborne Pathogens.
5. Adherence to specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20-25°C) before starting the assay.** Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize amount of any residual wash solution; (e.g. by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
7. The human serum controls, Sample Diluent, Conjugate, and Wash Buffer concentrate contain a preservative (thimerosal, 0.04% (w/v)) which may be toxic if ingested.
8. The Stop Solution is TOXIC. Causes burns. Toxic by inhalation, in contact with skin and if swallowed. In case

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of accident or if you feel unwell, seek medical advice immediately.
9. The TMB Solution is HARMFUL. Irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. Irritating ot eyes, respiratory system and skin.
11. Wipe bottom of plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Reagents from other sources or manufacturers should not be used.
14. 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.I. (Substrate Incubation) to determine amount of substrate solution to be used.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and /or samples could cause erroneous results.
18. Reusable glassware must be washed out and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will
22. protect the wells from condensation.
23. Wash Solution should be collected in a disposal basin. Treat the waste solution with 10% household bleach (0.5% sodium hypochlorite). Avoid exposure of reagents to bleach fumes.
24. Caution: Liquid waste at acid pH should be neutralized before adding to sodium hypochlorite (bleach).
25. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
26. 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.
27. 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 of450nm.
2. Microliter pipettes capable of accurately delivering 10 and 200 uL.
3. Adjustable multichannel pipette (50-200uL) 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.

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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.
12. Disposal basin and disinfectant, (Example: 0.5% sodium hypochlorite, 10% household bleach).

SPECIMEN COLLECTION

It is recommended that specimen collection be carried out in accordance with NCCLS document M29: Protection of Laboratory Workers from Infectious Disease.

No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.

Only freshly drawn and properly stored blood sera obtained by approved aseptic venipuncture procedures should be used in this assay (6,7). 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. Conjugated: Store at 2-8°C. DO NOT FREEZE.
4. Positive Control, Negative Control and Calibrator: 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 IX, 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.

TEST PROCEDURE

1. Remove the individual kit components and allow them to warm to room temperature (20-25°C).
2. Determine the number of microwells needed. Allow six Control/Calibrator determinations (one Blank, one Negative Control, three Calibrators and one Positive Control) per run. A Reagent Blank should be run on each

assay. Check software and reader requirements for the correct Controls/Calibrator configurations. 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.

EXAMPLE PLATE SET-UP

	1	2
A	Blank	Patient 3
B	Neg. Control	Patient 4
C	Calibrator	Etc.
D	Calibrator	
E	Calibrator	
F	Pos. Control	
G	Patient 1	
H	Patient 2	

3. Prepare a 1:21 dilution (e.g.: 10µl of serum + *200µL* of Sample Diluent. NOTE: Shake Well Before Use of the Negative Control, Calibrator, Positive Control, and each patient serum.
4. To individual wells, add 100µL of each diluted control, calibrator and sample. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100µL of Sample Diluent to well A1 as a reagent blank. Check software and reader requirements for the correct reagent blank well configuration.
6. Incubate the plate at room temperature (20-25°C) for 25 ± 5 minutes.
7. Wash the microwell strips 5X.

A. *Manual Wash Procedure:*

a. Vigorously shake out the liquid from the wells.
b. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
c. Repeat steps a. and b. for a total of 5 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 (bleach) at the end of the days run.

B. Automated Wash Procedure:

If using an automated microwell wash system, set the dispensing volume to 300-350µL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.

8. Add 100µL of the Conjugate to each well, including reagent blank well. at the same rate and in the same order as the specimens were added.
9. Incubate the plate at room temperature (20-25°C) for 25 ± 5 minutes
10. Wash the microwells by following the procedure as described in step 7.

11. Add 100uL of TMB to each well, including reagent blank well. at the same rate and in the same order as the specimens were added.
12. Incubate the plate at room temperature (20-25°C) for 10 to 15 minutes.
13. Stop the reaction by adding *50µL* of Stop Solution to each well, including reagent blank well, at the same rate and in the same order as the TMB was added. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.
14. Set the microwell 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 wells. If any of the three 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 Calibrator and the OD values for the Positive and Negative Controls should fall within the following ranges:

i.	OD RANGE Negative Control	≤ 0.250
ii.	Positive Calibrator	≥ 0.300
iii.	Positive Control	≥ 0.500

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 Positive Control and Negative Control are 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.
   Refer to NCCLS document C24: Statistical Quality Control for Quantitative Measurements for guidance on appropriate QC practices.

INTERPRETATION OF RESULTS

A. *Calculations*

1. Correction Factor

A cutoff 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 cutoff 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 is printed on the Component list located in the kit box.

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2. Cutoff OD Value

To obtain the cutoff OD value, multiply the CF by the mean OD of the Calibrator determined above. (CF x mean OD of Calibrator = cutoff 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 cutoff OD from step 2. Example:

Mean OD of Calibrator	=0.793
Correction Factor (CF)	=0.25
Cutoff OD	=0.793 x 0.25 = 0.198
Unknown Specimen OD	=0.432
Specimen Index Value or OD Ratio	0.432/0.198 = 2.18

B. Interpretations:

Index Values or OD Ratios are interpreted as follows:

	Index Value or OD Ratio
Negative Specimens	≤0.90
Equivocal Specimens	0.91 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, 159/255 (62.3%) was positive, 94/255 (36.9%) was negative, and 2/255 (0.8%) was equivocal.

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

PERFORMANCE CHARACTERISTICS

I. Comparative Study:
An in-house comparative study was performed to demonstrate the equivalence of the DRG Intl.,Inc. Scientific, Inc. Gliadin IgG ELISA test system to **another** commercially available Gliadin IgG ELISA test system. Performance was evaluated using 305 specimens and the results are summarized in Table 1 below:

Table 1

		DRG International, Inc. Gliadin IgG ELISA			
		-	±**	+	Totals
Commercial ELISA Test System	-	130	1	6	137
	-+**	8	1	13	22
	+	5	1	140	146
	Totals	143	3	159	305

Relative Sensitivity = 140/145 = 96.6% 95% Confidence Interval = 94% to 100%

Relative Specificity = 130/136 = 95.6% 95% Confidence Interval = 92% to 99%

Relative Agreement = 270/281 = 96.1% 95% Confidence Interval = 94% to 98%

** Data Excluded From Calculation

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

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)	
	Dav 1		Dav 2		Dav 3			
Sample Number	Mean AAU/mL	CV	Mean AAU/mL	CV	Mean AAU/mL	CV	Mean AAU/mL	CV
Sample #1	119	5.9%	117	5.7%	93	7.3%	110	12.5%
Sample #2	53	13.1%	44	6.5%	37	9.3%	44	18.2%
Sample #3	686	8.9%	652	5.2%	697	4.0%	678	6.8%
Sample #4	192	9.9%	165	5.2%	171	4.7%	176	9.8%
Sample #5	726	9.3%	677	5.5%	787	8.1%	730	9.8%
Sample #6	526	11.5%	481	4.0%	475	6.8%	494	9.2%
NC	13	20.8%	12	3.4%	6	24.4%	10	35.0%
HPC	1007	5.6%	981	4.1%	1131	7.3%	1040	8.6%

Kit 2

	Intra-Assay (n=8)						Inter-Assay (n=24)	
	Dav 1		Dav 2		Dav 3			
Sample Number	Mean AAU/mL	CV	Mean AAU/mL	CV	Mean AAU/mL	CV	Mean AAU/mL	CV
Sample#l	120	5.3%	113	7.1%	99	6.7%	110	10.1%
Sample #2	51	13.2%	41	6.2%	36	12.5%	42	18.4%
Sample #3	698	8.5%	681	3.0%	648	13.4%	676	9.3%
Sample #4	194	10.4%	170	8.7%	164	6.0%	176	11.4%
Sample #5	744	8.8%	720	6.0%	758	3.0%	741	6.5%
Sample #6	540	11.2%	485	11.2%	484	4.9%	503	10.7%
- NC	11	21.0%	8	11.2%	8	17.4%	9	24.9%
HPC	1033	5.6%	1091	5.1%	1029	5.5%	1051	5.8%

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 IgG test system. Twenty-four of twenty-six (24/26) were negative for gliadin IgG activity, while two of twenty-six (2/26) were positive. The results of this study indicate that the potential for interference due to cross reactivity with such auto antibodies is unlikely.

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