



# ImmuGlo™ Anti-Endomysial Antibody (EMA) Test System

For *in vitro* diagnostic use

## PRODUCT INSERT

Product Code: 1114A-PDE  
Product Code: 1114G-PDE

48 Determinations  
48 Determinations

## INTENDED USE

An indirect immunofluorescence antibody test for the qualitative and semi-quantitative detection of endomysial antibodies (EMA of IgA or IgG) in human serum as an aid in the diagnosis of celiac disease and *dermatitis herpetiformis* in combination with other clinical and other laboratory findings.

## 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. The classic symptoms of CD include diarrhea, weight loss and malnutrition. However, only a small percentage of patients with CD present with the classic symptoms. Consequently, the clinical spectrum of CD has grown much broader to include patients without classic symptoms. The inclusion of a wider range of clinical presentation has led to greater numbers of individuals diagnosed with CD later in life than ever before.

The revised European Society of Paediatric Gastroenterology and Nutrition (ESPGAN) criteria for diagnosis of CD include only a single biopsy with clear-cut remission of clinical symptoms on GFD<sup>1</sup>. Positive serology at the time of diagnosis with a decline in antibody levels on GFD contributes to the diagnosis<sup>2-14</sup>. 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-reticulín antibody (ARA) and anti-tissue transglutaminase (tTG) antibody tests<sup>2-26</sup>. 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 may occur when IgA class EMA are in high titer or in individuals who are IgA deficient.<sup>23,24</sup>

One limitation of certain serological methods is that they detect only the IgA isotype of the antibodies. IgA deficient CD patients may therefore yield false negative serology<sup>22</sup>. This may compromise the utility of these methods in detecting all CD. IgA deficient CD patients produce IgG class of EMA and not IgA. To prevent false negative results in IgA deficient cases of CD, it is necessary to include serological methods that can detect antibodies of IgG isotype<sup>22-26</sup>. Detection of IgA and IgG EMA helps identify all cases of CD.

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The levels of EMA are also of interest, as antibody levels tend to correlate with the severity of the disease. When a patient is on a gluten free diet, the levels of these antibodies will decrease and eventually disappear, suggesting that the patient is in remission<sup>20-22</sup>. Thereafter, tests for antibody levels may be considered to ensure dietary compliance. Intake of gluten in patients who are in remission will result in the re-appearance or increase of these antibodies in the serum.

EMA are detected on primate smooth muscle, distal esophagus and human umbilical cord.<sup>18-21</sup> Of these primate smooth muscle and primate distal esophagus, to be the most reliable in detecting EMA.

## PRINCIPLES OF PROCEDURE

Using this 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 (catalog number 1114A-PDE) or IgG (catalog number 1114G-PDE) class are detected by incubation of the substrate with fluorescein-labeled, anti-human immunoglobulin conjugates IgA or IgG respectively. Reactions are observed under a fluorescence microscope equipped with appropriate filters. The presence of EMA is demonstrated by an apple green fluorescence of the endomysium of smooth muscle bundles especially of the inner layer. When positive, the titer (the reciprocal of the highest dilution giving a positive reaction) of the antibody is then determined by testing serial dilutions.

## REAGENTS

### Storage and Preparation

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

### Materials Provided

Anti-Endomysial IgA Antibody (EMA) Test System	<i>Code: 1114A-PDE</i>
Anti-Endomysial IgG Antibody (EMA) Test System	<i>Code: 1114G-PDE</i>

Kit contains sufficient reagents to perform 48 determinations

- 8 x** 6-well - Primate Distal Esophagus Substrate Slides
- 1 x 0.5 ml** EMA Positive Control\* (1114A-PDE contains EMA IgA Positive Control; 1114G-PDE contains EMA IgG Positive Control)
- 1 x 0.5 ml** Negative Control\*
- 1 x 5.0 ml** Goat anti-human FITC Conjugate with Evan's Blue Counterstain. **Protect from light\*** (1114A-PDE contains IgA Conjugate; 1114G-PDE contains IgG Conjugate)
- 1 x 60 ml** Buffered Diluent. Ready to use\*

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		Clinical Diagnosis		
		CD	non-CD	Total
<b>Primate Distal</b>	Positive	14	0	14
<b>Esophagus</b>	Negative	0	10	10
<b>IgG IFA</b>	Total	14	10	24
	Clinical Sensitivity:		100%	
	Clinical Specificity:		100%	

EMA IgG on Primate Distal Esophagus vs. tTG IgG Antibody ELISA: A total of 50 samples were tested on each system. Samples were obtained from patients with CD, including IgA deficient patients, from IgA deficient non-CD cases, and from normal human sera. The results of this comparative study are summarized below:

		tTG IgG ELISA		
		Positive	Negative	Total
<b>Primate Distal</b>	Positive	15	1	16
<b>Esophagus</b>	Negative	3	31	34
<b>IgG IFA</b>	Total	18	32	50
	Positive Agreement:		83.3%	
	Negative Agreement:		96.9%	
	Overall Agreement:		92%	

Conclusion: These studies show IgG antibodies to EMA of IgG isotype to be sensitive and specific markers of CD in patients with IgA deficiency.

## REFERENCES

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- 2 vials** Phosphate Buffered Saline. **Dissolve each vial to 1 liter.**
- 1 x 5.0 ml** Mounting Medium. **Do not freeze\***
- 1 x 12** Cover Slips

### Optional Components:

Kits are available with alternate reagent set in which Conjugate and Counterstain are provided as individual components.

- Anti-EMA IgA Antibody (EMA) System with individual conjugate and counterstain *Code: 1114A-PDEX*
- Anti-EMA IgG Antibody (EMA) System with individual conjugate and counterstain *Code: 1114G-PDEX*
- 1 x 5.0 ml Goat anti-human IgA FITC Conjugate **Protect from light\*** *Code: 2107X*
- 1 x 5.0 ml Goat anti-human IgG FITC Conjugate **Protect from light\*** *Code: 2100X*
- 1 x 1.0 ml Evan's Blue Counterstain *Code: 2510*

\*CAUTION - Contains <0.1% NaN<sub>3</sub>

### Materials Required But Not Provided

- Fluorescence microscope
- Micropipette or Pasteur pipette
- Serological pipettes
- Staining dish (e.g. Coplin jar)
- Small test tubes (e.g. 13x75mm) and test tube rack
- Distilled or deionized water
- 1 liter container
- Wash bottle
- Paper towels
- Incubation chamber

### Warnings and 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<sup>27</sup>.

**WARNING - Sodium azide (NaN<sub>3</sub>) 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.

### 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

##### 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 0.05ml) 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 0.05ml) 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.

### Performance Characteristics

#### IgA-EMA

IgA-EMA on distal esophagus vs. clinical diagnosis of CD: a total of 60 samples were tested including 30 CD patients and 30 normal human sera.

		Clinical Diagnosis		
		CD	non-CD	Total
<b>Primate Distal</b>	Positive	30	0	30
<b>Esophagus</b>	Negative	0	30	30
<b>IgA IFA</b>	Total	30	30	60
		Clinical Sensitivity:		100%
		Clinical Specificity:		100%

EMA IgA on Primate Distal Esophagus vs. Primate Smooth Muscle: A total of 215 samples were tested on both substrates. Samples were obtained from patients with CD and normal human sera. The results of this comparative study are summarized below:

		Primate Smooth Muscle		
		Positive	Negative	Total
<b>Primate Distal</b>	Positive	174	0	174
<b>Esophagus</b>	Negative	2	39	41
<b>IgA IFA</b>	Total	176	39	215
		Positive Agreement:		98.9%
		Negative Agreement:		100%
		Overall Agreement:		99.1%

Conclusion: The results of these studies show that the two substrates are equivalent in their ability to detect EMA antibodies and that EMA are specific for CD.

#### IgG-EMA

IgG-EMA on distal esophagus vs. clinical diagnosis of CD: A total of 14 CD patients with IgA deficiency and 10 patients with IgA deficiency but not CD but with clinical symptoms that prompted testing for CD were tested for EMA of IgG isotype and the results are as follows:

## EXPECTED VALUES

As seen in Table 1, IgA class EMA are specific markers for celiac disease and dermatitis herpetiformis. In cases of CD with IgA deficiency, studies show IgG class EMA to be valuable aids in diagnosis (Table 2).

**Table 1. Incidence of IgA Class EMA**<sup>13,22</sup>

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 Dermatitis	4	0
Other Skin Diseases	180	0

**Table 2. IgG Class EMA in Patients with IgA deficiency**<sup>23-25</sup>

Study Group	Subjects	Endomysial IgG
CD Patients		
Korponay-Szabo	78	77
Kumar et al	14	14
Cataldo et al	20	20
CD Patients on GFD		
Korponay-Szabo	35	26
Kumar et al	1	0
Cataldo et	34	0
Non-CD Patients		
Korponay-Szabo	78	0
Kumar et al	10	0
Cataldo et al	10	0

CD=celiac disease, GFD=gluten free diet

- Step 11** Remove a slide from incubation chamber. 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**. 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 on 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: End Point Determination (Titration)

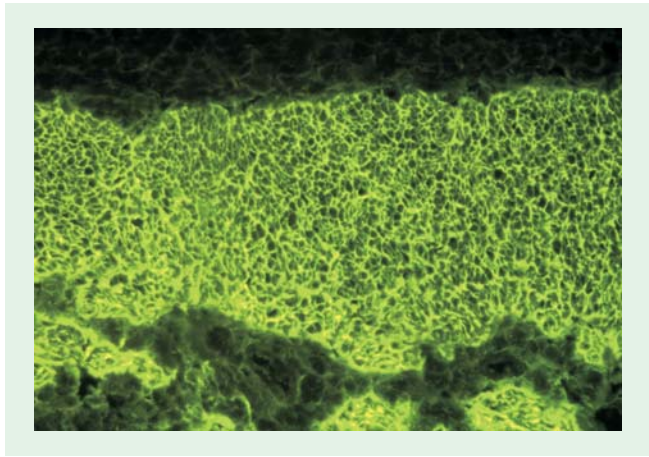
A serum positive in the screening test may be further tested following **Steps 5 through 13** to determine the titer. Each test run should include the Positive and Negative Controls. Make serial twofold dilutions starting at 1:5. The reciprocal of the highest dilution producing a positive reaction is the titer.

### Preparation of Serial Dilutions

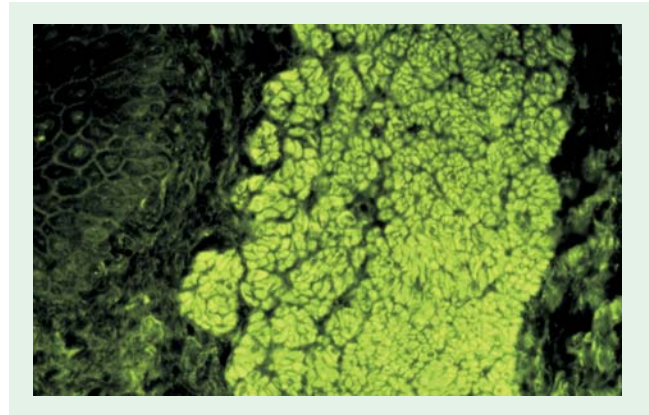
Number four tubes 1 through 4. Add 0.3 ml of Buffered Diluent to tube 1 and 0.2 ml to tubes 2 through 4. Pipette 0.2 ml of undiluted serum to tube 1 and mix thoroughly. Transfer 0.2 ml from tube 1 to tube 2 and mix thoroughly. Continue transferring 0.2 ml from one tube to the next after mixing to yield 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		0.2ml	0.2ml	0.2ml
Final dilution	1:5	1:10	1:20	1:40 etc.

**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.*



### Quality Control

Quality control studies are run on all new lots comparing retention of released component lots against the new lot and incorporating internal standards. Conjugate strength is formulated using checkerboard titrations. Positive controls are reacted and titer/equivalence with prior lots determined. Tissue substrates are examined for appropriate intensity and morphology of reaction as well as minimal background fluorescence.

### RESULTS

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

Read for specific staining of the endomysium lining of the smooth muscle bundles especially of the inner circular layer of the distal esophagus. **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 due to masking of EMA antibodies. If ASMA are detected, then the sample should be tested at higher dilutions. ANA reactions on smooth muscle tissue, when they occur, are usually weak and sparsely distributed and, therefore, unlikely to cause false negative results.

### LIMITATIONS OF THE PROCEDURE

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. As EMA of IgG isotype when present in a specimen are generally present in high titer, prozone phenomenon may occur more frequently with such sera.

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 (ASMA). It is recommended that patients sera which also contain ASMA be tested further at higher dilutions.

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 strict gluten free diet for an extended period may be negative for EMA. Studies have shown that EMA levels could be used to monitor patient response to gluten free diet.<sup>19,24</sup>

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