

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

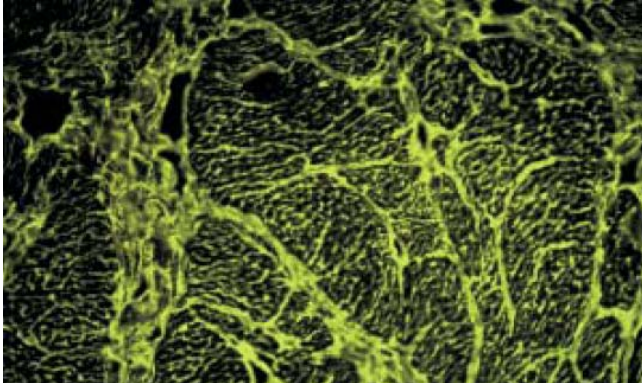
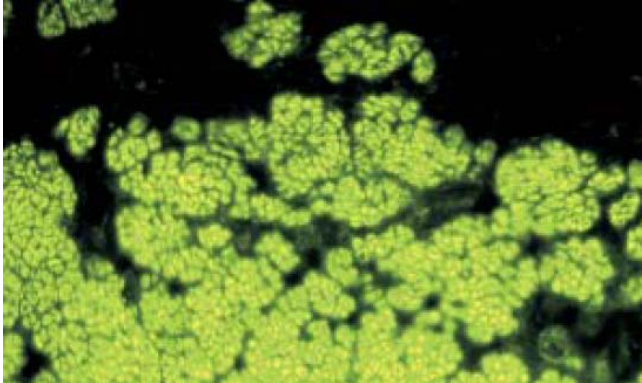


Photo 2. ASMA staining reaction on primate smooth muscle, 200X.  
Note staining of the smooth muscle sarcoplasm.



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**Cambridge Life Sciences Ltd.  
Instructions for Using Immco Slides:**

**ImmuGlo™ Anti-Endomysial  
Antibody (EMA) Test**

**Primate Oesophagus (distal) and Smooth  
Muscle Substrates**

**IVD** For *in vitro* diagnostic use

<b>REF</b>	Code: 2160	6 Well Substrate Slide	 6 Tests
<b>REF</b>	Code: 2155-1	6 Well Substrate Slide	 6 Tests
<b>REF</b>	Code: 2155-8	8 Well Substrate Slide	 8 Tests
<b>REF</b>	Code: 2155-1/10	10 Well Substrate Slide	 10 Tests
<b>REF</b>	Code: 2155-18	8 Well Substrate Slide	 8 Tests

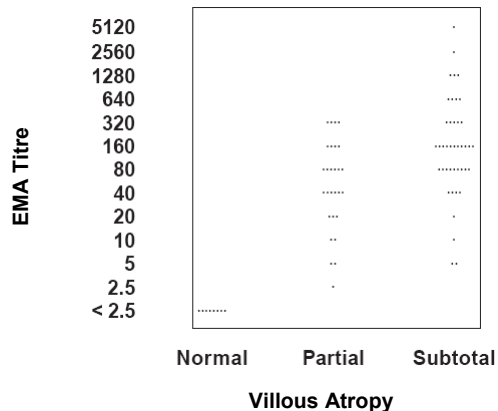


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**Figure 1. Correlation of IgA EMA titres in Villous Atrophy**



From Chorzelski TP et al<sup>1</sup> and Kumar V et al<sup>8</sup>.

### SPECIFIC 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 oesophagus 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:

Other EMA IFA	IMMCO EMA		
	Positive	Negative	Total
Positive	18	0	18
Negative	2	48	50
Total	20	48	68

Relative specificity: 97%  
Relative sensitivity: 100%  
Relative agreement: 96%

### 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 oesophagus 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<sup>1-12</sup>. 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<sup>13</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 also occur when IgA class EMA are in high titre 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 titres. Patients on a gluten free diet >9 months have reduced or negative EMA titres if they adhere to their diet restrictions<sup>1, 6-8, 10</sup>.

### PRINCIPLES OF PROCEDURE

In the indirect immunofluorescence method used, patient serum is incubated on tissue sections to allow binding of antibodies to the substrate. Any antibodies not bound are removed by washing. Bound antibodies of the IgA and IgG class are detected by incubation of the substrate with fluorescein-labelled, anti-human IgA or IgG 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 titre (the reciprocal of the highest dilution giving a positive reaction) is then determined by testing serial dilutions<sup>14</sup>.

### PRODUCT INFORMATION

#### Storage and preparation

Store all reagents at 2 - 8°C. Reagents are ready for use after equilibration to room temperature (18 - 25°C).

**Materials provided**

<b>SORB   SLD   6</b>	<b>Code: 2160</b>	6 well Primate Smooth Muscle Substrate Slide,
<b>SORB   SLD   6</b>	<b>Code: 2155-1</b>	6 well Primate Oesophagus (distal) Substrate Slide,
<b>SORB   SLD   8</b>	<b>Code: 2155-8</b>	8 well Primate Oesophagus (distal) Substrate Slide,
<b>SORB   SLD   10</b>	<b>Code: 2155-1/10</b>	10 well Primate Oesophagus (distal) Substrate Slide,
<b>SORB   SLD   8</b>	<b>Code: 2155-18</b>	8 well Primate Oesophagus (distal) Substrate Slide,

**Material required and available from CLS**

<b>CONTROL   +   EMA</b>	1 x 0.5mL EMA Positive Control. <b>Code 2250</b>
<b>CONTROL   +   EMA</b>	1 x 0.5mL EMA Low Titre Control. <b>Code 2250-1</b>
<b>CONTROL   -</b>	1 x 0.5mL Negative Control. <b>Code 2200</b>
<b>IgA-CONJ   FITC</b>	1 x 5mL Anti-human IgA FITC Conjugate. <b>Code 2107X</b> or
<b>IgA-CONJ   FITC   EB</b>	1 x 5mL Anti-human IgA FITC Conjugate containing Evan's Blue. <b>Code 2107</b>
<b>IgA/IgG-CONJ   FITC</b>	1 x 5mL Anti-human IgA/IgG FITC Conjugate. <b>Code 2113X</b> or
<b>IgA/IgG-CONJ   FITC   EB</b>	1 x 5mL Anti-human IgA/IgG FITC Conjugate containing Evan's Blue. <b>Code 2113</b>
<b>BUF</b>	1 x 60mL Buffered Diluent. <b>Code 2302</b>
<b>BUF   WASH</b>	1 vial Phosphate Buffered Saline (PBS). Dissolve each vial to 1 litre. <b>Code 2301</b>
<b>MOUNTING   MEDIUM</b>	1 x 5.0mL Mounting Medium. Do not freeze. <b>Code 2505</b>
<b>EVANS</b>	1 x 1.0mL Evan's Blue Counterstain. Not required if using 2100. <b>Code 2510</b>

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.

**EXPECTED VALUES**

As seen in Table 1, EMA, as detected on primate smooth muscle are highly specific markers for celiac disease and dermatitis herpetiformis.

**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 Diarrhoea	210	0
Recurrent Diarrhoea	124	0
Toddlers Diarrhoea	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 Dermatosi	4	0
Other Skin Diseases	180	0

Compiled from the literature as per Chorzeliski TP et al<sup>1</sup>.

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 1.

## RESULTS

The results of the tests for endomysial antibodies should be reported as negative (<2.5), positive greater or equal to 20, or preferably, positive with titre.

Read for specific staining of the endomysium lining of the smooth muscle bundles. **See Photo 1.** 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 Photo 2.**

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<sup>1</sup>. ANA reactions on smooth muscle tissue, when they occur, are usually weak and sparsely distributed and, therefore, unlikely to cause false negative results.

Consult Photo 1 and Photo 2 at the end of this document for example reactions.

## LIMITATION 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 titre determined.

The presence of two or more antibodies in a serum which are reactive with the same tissue may cause interference in their detection by immunofluorescence. This interference may cause either a failure to detect EMA or a suppression of its titre if the interfering antibody has a higher titre 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 titres are usually much lower than IgA-EMA titres and
3. IgA antibodies are usually of higher avidity than IgG antibodies.

## Material 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 75mm) and test tube rack  
Distilled or deionised water  
1 litre container  
Wash bottle  
Paper towels  
Incubation chamber  
Coverslip

## WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use only.

For Professional Use only.

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>14</sup>.

WARNING - Sodium azide (NaN<sub>3</sub>) is present in reagents at <0.1%. Sodium azide 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 build-up. Sodium azide may be toxic if ingested. If ingested, report incident immediately to laboratory director or poison control centre.

Instructions should be followed exactly as they appear in this insert to ensure valid results. Do not interchange components with those from other sources other than the same catalogue number from IMMCO/CLS. Do not use beyond expiration date given on the labels.

## SPECIMEN COLLECTION AND PREPARATION

Only serum specimens should be used for this procedure. Grossly haemolysed, lipaemic or microbial 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

1. Dilute each patient serum 1:2.5 with the Buffered Diluent (0.2mL serum + 0.3mL Diluent). **Do not dilute Positive or Negative Controls.** Save the undiluted sera to determine antibody titres if screening tests are found to be positive.
2. Allow pouches containing substrate slides to equilibrate to room temperature (18 – 25°C) for 10 - 15 minutes. Carefully remove the slides without touching the substrate.
3. Label the slides and place them in an incubation chamber lined with paper towels moistened with water to prevent drying.
4. Invert dropper vial and gently squeeze to apply 1-2 drops (approximately 50-100µL) of Negative Control to well #1. Similarly apply 1-2 drops of Positive Control to well #2. Avoid overfilling the wells.
5. Using a micropipette or Pasteur Pipette, apply 1-2 drops of patient's diluted serum (approximately 50-100µL) to the other wells. Avoid overfilling the wells.
6. Place the lid on the incubation chamber and incubate slides for 30 minutes at room temperature (18 – 25°C).
7. Remove slide from the incubation chamber. Hold slide at tab end and rinse gently with approximately 10mL PBS using a pipette, or rinse slide in beaker filled with PBS. Do not use wash bottle. Transfer slide immediately into Coplin jar and wash for 10 minutes. Repeat process with all remaining slides.
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 1-2 drops (approximately 50-100µL) to each well.
9. Replace the lid on the incubation chamber. Incubate for 30 minutes at room temperature (18 – 25°C).
10. 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.
11. 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 next step while slide is still wet.**
12. Mount the coverslip by applying 3 drops of Mounting Medium evenly on the coverslip and place coverslip over slide. Avoid applying undue pressure and prevent lateral movement of the coverslip.

13. Repeat steps 11 and 12 for each slide.
14. 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 for up to 48 hours. Slides should be stored in the dark at 2 - 8°C.

#### B. Endpoint Determination (titration)

A serum positive in the screening test may be further tested following steps 5 through 12 to determine the titre. Each test run should include the Positive and Negative Controls. Make serial two-fold dilutions starting at 1:2.5. The reciprocal of the highest dilution producing a positive reaction is the titre.

#### Preparation of Serial Dilutions

Number four tubes 1 through 4. Add 0.3mL of Buffered Diluent to tube 1 and 0.2mL to tubes 2 through 4. Pipette 0.2mL of undiluted serum to tube 1 and mix thoroughly. Transfer 0.2mL from tube 1 to tube 2 and mix thoroughly. Continue transferring 0.2mL from one tube to the next after mixing to yield the dilutions depicted in the following table:

Tubes	1	2	3	4 etc
Serum	0.2mL			
	+			
Buffered Diluent	0.3mL	0.2mL	0.2mL	0.2mL
		↗	↗	↗
Transfer		0.2mL	0.2mL	0.2mL
Final dilution	1:2.5	1:5	1:10	1:20 etc

Contact CLS for help with protocols for use with automated IFA instrumentation.

#### QUALITY CONTROL

Both a Positive and Negative Control should be included with each test run. The Negative Control should show no specific fluorescence of the endomyrial 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:

- Turbidity. Discard and use another control
- Problems with the optical system of the fluorescence microscope. These may include: improper alignment, bulb beyond useful life expectancy, etc.
- Allowing the slide to dry during the procedure.