

For *In Vitro* Diagnostic Use

Product Code: 704145, 704150, 704155, 704255
504145, 504145.10, 504150, 504150.25,
504155.25, 504255.25

CLIA Complexity: High

Intended Use

Monkey oesophagus sections are intended for use as a substrate for the screening of antibodies to intra-epidermal antigens or basement membrane zones in human serum, as an aid in the diagnosis of pemphigus and bullous pemphigoid respectively. The sections can also be used for detection of endomysial antibodies as an aid in the diagnosis of coeliac disease.

Summary and Explanation of the test

Intra-epidermal IgG antibodies which characterise various clinical forms of pemphigus react with antigens present on the cell surface of epidermal keratinocytes. A positive result gives a characteristic “chicken wire” pattern. Tissue deposition of immunoglobulin occurs in almost all cases. It is recommended that both patient serum and tissue are always investigated with bullous disease. Basement membrane zone antibodies are classically found in bullous pemphigoid. In this disease direct biopsy reveals linear deposition of IgG and C3 along the dermo-epidermal junction. These autoantibodies are regularly, but not always, present in the serum of affected patients. However, antibody titre does not correlate with disease state and in clinical remission antibody may still be present in both tissues and serum. An anti-human IgG (H&L) FITC conjugate is provided in this kit to demonstrate both these autoantibodies. Endomysial antibodies are directed against “reticulin-like” fibres in connective tissue around smooth muscle fibers in the intestinal tracts of primates. An almost 100% sensitivity and specificity has been reported for IgA class endomysial antibodies active in coeliac disease^{1,2}. These antibodies are detected by the anti-human IgA FITC conjugate provided.

Principles of the Procedure

The kit uses an indirect immunofluorescence technique³ where patient samples and appropriate controls are incubated with the sections. The unreacted antibodies are washed off and then appropriate fluorescein labeled conjugates are applied. Unbound conjugate is washed off as before. Slides are viewed with a fluorescence microscope and positive samples produce apple-green fluorescence which corresponds to areas of the section where autoantibody has bound.

Reagents

1. Monkey oesophagus sections on 5- or 10-well slides with desiccant

Kits 704145 and 704150 only:

2. Two positive control sera (derived from human serum), one giving “chicken wire” pemphigus pattern and the other “reticulin like” endomysial pattern on monkey oesophagus sections, containing 0.09% sodium azide. **Prediluted, ready for use.**
3. Negative control serum, universally negative for all autoantibodies, containing 0.09% sodium azide. **Prediluted, ready for use.**
4. Phosphate buffered saline (PBS), provided as a 40-fold concentrate in liquid form.
5. Affinity purified sheep anti-human IgG (H&L), (monkey adsorbed) fluorescein conjugate and anti-human IgA fluorescein conjugate, containing 0.09% sodium azide. **Prediluted, ready for use.**
6. 1% Evans Blue, as an optional counterstain
7. Blotters
8. Mounting medium, containing an anti-fading agent
9. Coverslips

Kit 704155 only:

2. Positive control sera (derived from human serum), giving “reticulin like” endomysial pattern on monkey oesophagus sections, containing 0.09% sodium azide. **Prediluted, ready for use.**
3. Negative control serum, universally negative for all autoantibodies, containing 0.09% sodium azide. **Prediluted, ready for use.**
4. Phosphate buffered saline (PBS), provided as a 40-fold concentrate in liquid form.
5. Anti-human IgA fluorescein conjugate, containing 0.09% sodium azide. **Prediluted, ready for use.**
6. 1% Evans Blue, as an optional counterstain
7. Blotters
8. Mounting medium, containing an anti-fading agent
9. Coverslips

Kit 704255 only

“Please note these slides are of a different format to those described in the insert, being multichannel pipette compatible. This only affects the position of the wells. All other technical and performance characteristics of the slides remain the same.”

1. Monkey oesophagus sections on Multichannel 10-well slides with desiccant
2. Positive control sera (derived from human serum), giving “reticulin like” endomysial pattern on monkey oesophagus sections, containing 0.09% sodium azide. **Prediluted, ready for use.**
3. Negative control serum, universally negative for all autoantibodies, containing 0.09% sodium azide. **Prediluted, ready for use.**
4. Phosphate buffered saline (PBS), provided as a 40-fold concentrate in liquid form.
5. Anti-human IgA fluorescein conjugate, containing 0.09% sodium azide. **Prediluted, ready for use.**
6. 1% Evans Blue, as an optional counterstain
7. Mounting medium, containing an anti-fading agent
8. Coverslips

Warnings/Precautions

All donors of human serum supplied (kits only) have been serum tested and found to be negative for Hepatitis B surface antigen and antibodies to Hepatitis C virus and Human Immunodeficiency Virus (HIV 1 & 2). However, these tests cannot guarantee the absence of infective agents. Proper handling and disposal methods should be established as for all potentially infective material and only personnel adequately trained in such methods should be permitted to perform the procedures. The Evans Blue and the kit controls contain 0.09% sodium azide as a preservative and must be handled with caution – do not ingest or allow contact with the skin or mucous membranes. If contact does occur wash with a large volume of water and seek medical advice. Explosive metal azides may be formed with the lead and copper plumbing; on disposal of reagent, flush with a large volume of water to prevent azide build up. This product should only be used by suitably trained persons for the purposes stated. Adherence to the given procedure is recommended.

Storage Conditions

Unopened kits should be stored at 2-8°C and can be used until the given expiry date. **DO NOT FREEZE.** Once slides are removed from a foil bag, they should be used immediately. Diluted PBS buffer can be stored for up to one month at 2-8°C. All other reagents should be stored at 2-8°C.

Specimen Collection

Blood samples should be collected by venepuncture, allowed to clot naturally and the serum separated as soon as possible to prevent haemolysis. The serum may be stored at 2-8°C for up to 7 days prior to assay, or for prolonged storage, aliquoted and stored at -20°C or below⁸. **DO NOT freeze and thaw sera more than once.** Avoid using lipaemic, haemolysed or microbially contaminated sera as decreased titres or unclear staining patterns may occur.

Procedure

Materials provided (kits)

704145

1. 10 x Monkey Oesophagus – 5-well slide
2. 1 x 1mL Pemphigus Positive Control (prediluted)
3. 1 x 1mL Endomysial (Celiac) Positive Control (prediluted)
4. 1 x 1mL IFA System Negative Control (prediluted)
5. 1 x 7mL FITC IgG (H&L), monkey adsorbed Conjugate
6. 1 x 7mL Anti-Human IgA (α) AFF FITC
7. 1 x 3mL 1% Evans Blue Counterstain
8. 2 x 25mL PBS concentrate (x40)
9. 1 x 3mL Mounting Medium
10. 20 x Blotters
11. 10 x Coverslips
12. 1 x instruction leaflet

704150

1. 25 x Monkey Oesophagus – 10-well slide
2. 1 x 1mL Pemphigus Positive Control (prediluted)
3. 1 x 1mL Endomysial (Celiac) Positive Control (prediluted)
4. 1 x 1mL IFA System Negative Control (prediluted)
5. 1 x 15mL FITC IgG (H&L), monkey adsorbed Conjugate
6. 1 x 15mL Anti-Human IgA (α) AFF FITC
7. 1 x 3mL 1% Evans Blue Counterstain
8. 2 x 25mL PBS concentrate (x40)
9. 1 x 10mL Mounting Medium
10. 50 x Blotters
11. 25 x Coverslips
12. 1 x instruction leaflet

704155

1. 25 x Monkey Oesophagus – 10-well slide
2. 1 x 1mL Endomysial (Celiac) Positive Control (prediluted)
3. 1 x 1mL IFA System Negative Control (prediluted)
4. 2 x 15mL Anti-Human IgA (α) AFF FITC
5. 1 x 3mL 1% Evans Blue Counterstain
6. 2 x 25mL PBS concentrate (x40)
7. 1 x 10mL Mounting Medium
8. 50 x Blotters
9. 25 x Coverslips
10. 1 x instruction leaflet

704255

1. 25 x Monkey Oesophagus Multichannel Slides - 10 well
2. 1 x 1mL Endomysial (Celiac) Positive Control
3. 1 x 1mL IFA System Negative Control (prediluted)
4. 2 x 15mL Anti-Human IgA (α) AFF FITC
5. 1 x 3mL 1% Evans Blue Counterstain
6. 2 x 25mL PBS concentrate (x40)
7. 1 x 10mL Mounting Medium
8. 25 x Coverslips
10. 1 x instruction leaflet

Materials provided (slides)

504145	1 x Monkey Oesophagus Slide (5 well)
504145.10	10 x Monkey Oesophagus Slide (5 well)
504150	1 x Monkey Oesophagus Slide (10 well)
504150.25	25 x Monkey Oesophagus Slide (10 well)
504155.25	25 x Monkey Oesophagus Slide (10 well)
504255.25	25 x Monkey Oesophagus Multichannel Slides (10 well)

Additional Materials Required But Not Provided

Distilled water to dilute PBS concentrate

Container for PBS buffer

Micropipettes and disposable tips to apply patient samples

Humid chamber for incubation steps

Fluorescence microscope with 495nm exciter filter and 515nm barrier filter

Plastic squeeze bottle for initial wash in PBS

Additional components may be obtained from INOVA Diagnostics: PBS (508002), IFA System Negative Control (508186), Pemphigus Positive Control (504051), Pemphigoid Positive Control (504055), Endomysial Positive Control (504050), FITC (H+L), monkey adsorbed Conjugate (504011, 504071), Anti-Human IgA (α chain) AFF FITC (504023, 504045), Anti-Human IgA (α chain) monkey adsorbed AFF FITC (504015), 1% Evan's Blue (504049) and Mounting Medium (508001, 508005, 508006).

Test Procedure

Quality control

Positive and negative controls should be used every time samples are tested.

1. **Dilute PBS concentrate.** Dilute PBS concentrate with distilled water (1 part PBS concentrate + 39 parts distilled water) and mix. NB: only make up the entire amount of kit PBS if the entire kit is to be used within one month. The PBS is used for diluting patient samples and as a wash buffer.
2. **Dilute patient samples.** Dilute patient samples 1/10 by adding 20 μ L of serum to 180 μ L of PBS buffer.
3. **Substrate slides.** Allow substrate slide(s) to reach room temperature (18-28°C) prior to removal from pouch(es). Label slides appropriately, place in the humid chamber and add one drop of positive and negative controls to the appropriate wells. Add 50 μ L of diluted patient sample to the remaining wells.
4. **Slide incubation.** Incubate slides for 30 minutes in a humid chamber at room temperature (18-28°C). (Moistened paper towels at the bottom of the chamber will maintain humidity.)
5. **PBS wash.** Remove slides from humid chamber and rinse briefly with PBS squeeze bottle. Do not squirt directly on to the wells. Place slides in a rack and immerse in PBS and agitate or stir for 5-10 minutes.
6. **Addition of fluorescein conjugate.** Shake off excess PBS and blot around wells using blotters provided. Return slides to humid chamber and immediately cover each well with a drop of the appropriate fluorescein conjugate. The anti-human IgG (H&L) AFF FITC is for use in detecting skin autoantibodies and the anti-human IgA (α chain) AFF FITC for use in detecting endomysial autoantibodies. **DO NOT LEAVE WELLS UNCOVERED FOR LONGER THAN 15 SECONDS.**
7. **Slide incubation.** Incubate slides for 30 minutes in a humid chamber at room temperature (18-28°C) in the dark.
8. **PBS wash.** Wash again as described in step 5.
9. **Optional counterstain.** Add 2-3 drops of 1% Evans Blue per 100mL of PBS prior to slide immersion.

10. **Mounting with coverslip.** Remove one slide at a time from PBS wash. Quickly dry around the wells and add a drop of mounting medium to each well. Carefully lower the slide onto the coverslip, avoiding air bubbles, but if present do not attempt to remove. Wipe excess medium from around edge of coverslip.
11. **View slides under fluorescence microscope.** Slides may be stored for up to 3 days at 2-8°C, in the dark, without significant loss of fluorescence.

Results

Quality Control

The positive controls should give a bright apple-green staining pattern in the intercellular cement of the epithelium (“chicken-wire pattern”) for pemphigus and “reticulin-like” fibres in connective tissue around smooth muscle for the endomysial control. The negative control should show dull green staining in all the tissue, with no discernible fluorescence. If the controls do not appear as described, the test is invalid and should be repeated.

Interpretation of Results

See reference 5 for colour photographic examples of these patterns. Results are reported as positive or negative.

Positive pemphigus antibody

Staining of the intercellular cement of the stratified epithelium.

Positive pemphigoid antibody

Staining of the basement membrane zone along the dermo-epidermal junction.

Positive endomysial antibody⁴

Staining of the “reticulin-like” fibers in connective tissue around smooth muscle fibers.

NB: Each laboratory should establish at which point a positive result is considered clinically significant.

Limitations of the Procedure

1. The light source, filters and optics of different makes of fluorescence microscopes will influence the sensitivity of the assay. The performance of the microscope is significantly influenced by correct maintenance especially centering of the mercury vapour lamp and changing of the lamp after the recommended period of time.
2. Monkey oesophagus tissue is inherently prone to auto-fluorescence, independent of the manufacturing process used to produce the tissue slides. This tends to be most pronounced in the lamina propria and submucosal regions. Care should therefore be taken when interpreting samples giving weak positive staining patterns in the muscularis mucosa, i.e. endomysial and smooth muscle staining.
3. Antinuclear (ANA), anti-mitochondrial (AMA), anti-smooth muscle (ASMA) and skeletal muscle antibodies may react with the oesophagus substrate. The presence of these autoantibodies should be confirmed on the appropriate substrate.
4. Cross reactivity due to anti-A and anti-B blood group antibodies may occur, as oesophagus mucosa can contain certain blood group substances⁶. In some patients the staining pattern can mimic pemphigus antibodies, although blood group antibodies will also stain capillaries in the oesophageal musculature.
5. Due to the short distance between wells on the 10-well slides it is possible for cross contamination of samples and controls to occur. Care must be taken, especially at the washing stages, to ensure that this does not happen.
6. This test alone should not be considered diagnostic. All other factors including the clinical history of the patients and other serological or biopsy results must also be taken into account.
7. Suitability for use with other manufacturers' IFA reagents has not been assessed but use with such reagents should not necessarily be excluded.

“Please note the multi-channel slides are of a different format to those described in this insert, being multichannel pipette compatible. This only affects the position of the wells. All other technical and performance characteristics of the slides remain the same.”

Slides sold separately are classified as “Analyte Specific Reagents”. Except as a component of the kit, analytical and performance characteristics are not established.

Expected Values

Monkey oesophagus slides were used to test pemphigus and pemphigoid skin disease patients as well as 20 random blood donors. The results were:

Patient group	Number	Number positive	
		Intercellular	Basement membrane zone
Pemphigus	12	12	0
Pemphigoid	11	0	9
Normals	20	0	0

IgA class endomysial antibodies

Subjects/diagnosis	Positive/total	% Positive
Confirmed coeliacs		
On gluten	38/38	100
On gluten-free diet	17/37	46
Suspected coeliacs		
On gluten	27/30	90
On gluten-free diet	5/30	17
Dermatitis herpetiformis (total)	203/253	80
(sub-total) confirmed villous atrophy	42/42	100
Normal blood donors	0/87	0
Disease controls (gastro intestinal)		
Ulcerative colitis	0/59	0
Crohns disease	0/41	0
Liver diseases	0/20	0
Infectious diarrhoea	0/210	0
Toddler's diarrhoea	0/170	0
Recurrent diarrhoea	0/124	0
Milk protein sensitivity	0/60	0
Disease controls (skin)		
Linear IgA bullous dermatitis	0/41	0
Non dermatitis herpetiformis skin diseases	0/180	0

Data compiled from different studies, Chorzelski *et al* 1990⁷.

Specific Performance Characteristics

A comparison study was performed on 43 serum samples (35 clinical, 8 normal) using this kit and two commercially available reference methods, one for pemphigus and pemphigoid positive samples, and the other endomysial positive samples. Overall, there was good agreement between all of the kits. For pemphigus, 10 out of 13 samples tested positive on both kits. Two of the discrepant samples were borderline positive on the kit but negative on the reference kit. For pemphigoid, all four positive samples tested agreed, although the staining was slightly weaker on the competitor kit. For the endomysial positive samples tested, all eighteen were positive by both methods. The eight normal samples tested were negative on all three kits.

Summary of the Procedure

1. Dilute PBS with distilled water.
2. Dilute patient sera 1/10 with PBS.
3. Equilibrate substrate slides to room temperature (18-28°C).
4. Apply 50µL positive and negative controls and diluted patient sera to appropriate wells.
5. Incubate in a humid chamber for 30 minutes.
6. Wash for 5-10 minutes in PBS.
7. Blot around each well and immediately cover each well with a drop of conjugate.
8. Incubate as in Step 5.
9. Wash as Step 6.
10. Mount.
11. View slide under fluorescence microscope.

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References

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April 2011
Revision 1

