Coeliac disease and autoimmune skin blistering diseases

IMMUNOFLUORESCENCE KITS FOR THE DIAGNOSIS OF COELIAC DISEASE AND AUTOIMMUNE DISEASES

Endomysium
Intercellular substance
Epidermal basement membrane

IIF kits are optimized and validated for detection
of IgA and IgG antibodies against endomysium on the monkey oesophagus

TestLine
Clinical
Diagnostics

Introduction

Coeliac disease is a common term for a worldwide occurring disease of children and adults. It is a hereditary autoimmune disease caused by gluten intolerance. The main symptoms include inflammatory changes in the small intestine mucosa with diarrhoea, anaemia, weight loss and general disorders of somatic and psychic development. If gluten is not completely and permanently removed from patient's food, their immune system gets exhausted, the disease affects other organs and further autoimmune diseases and complications may develop, most of them being life threatening.

The diagnosis of coeliac sprue is based on clinical manifestation, enterobiopsy and laboratory tests. Detection of highly specific IgA and IgG antibodies against deamidated gliadin, tissue transglutaminase and endomysium is very important not only for proper diagnosis of coeliac disease but also for monitoring of the gluten-free diet effects.

The detection of endomysial antibodies is done on the monkey oesophagus section in the part which contains smooth muscle (lamina muscularis mucosae). Endomysium is a thin layer of connective tissue which surrounds individual muscle fibres. In the case of positive reaction the antibodies react with endomysium, homogenous yellow-green fluorescence of connective tissue occurs and final image (fluorescence pattern) resembles an irregular network of thin lines. The cytoplasm of muscle cells, the "inside of the muscle fibre", does not show any fluorescence.

Positive fluorescence samples
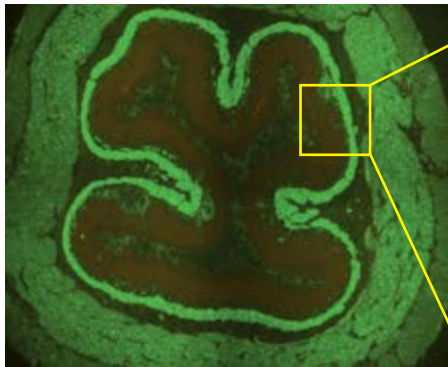
Monkey oesophagus section
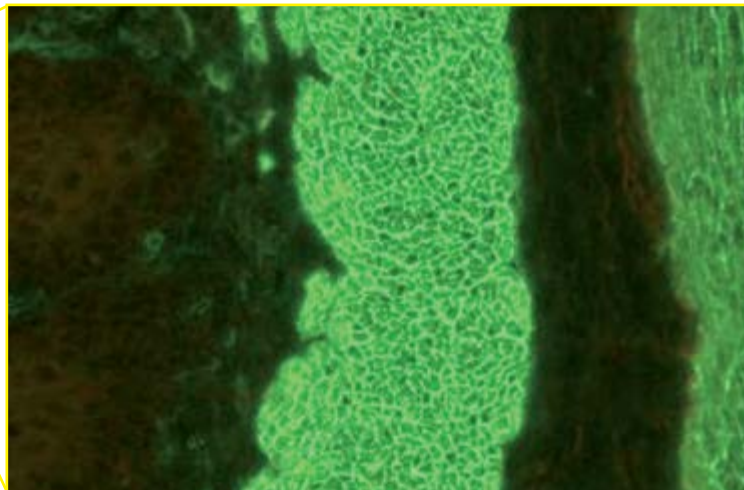
*Antibodies against endomysium*
"Irregular Network"
Antigen: Tissue transglutaminase

Autoimmune skin blistering diseases are rarely occurring diseases including pemphigus and pemphigoid, etc. During the illness autoantibodies react with adhesive molecules of outer (epithelium) and inner (connective tissue) layers of skin or mucous membrane, and acantholysis and blisters occur. The most frequent and serious illness is bullous pemphigus during which blisters burst and form large skin scabs and ulcers which can be easily infected with bacteria. Untreated pemphigus can result in sepsis and death.

The diagnosis of blistering skin diseases is based on evaluation of clinical manifestation, skin biopsy and laboratory tests. Evaluation of antibodies against intercellular substance (anti-ICS) and basement membrane zone (anti-BMZ) is, especially in the IgG class, a specific test which allows the diagnosis of these diseases.

The detection of anti-ICS and anti-BMZ autoantibodies is done on section of monkey oesophagus in the epithelial part. Anti-ICS antibodies react with adhesive molecules between individual epidermal cells and create the characteristic „chicken wire" fluorescence pattern. Anti--BMZ antibodies react with adhesive molecules between an epidermal cell and basement membrane, and a yellow-green fluorescent line is visible between surface epithelium and lamina propria on the section of monkey oesophagus.
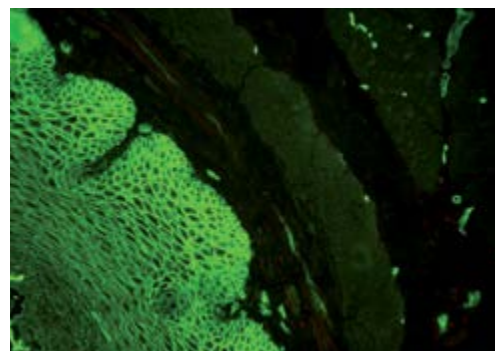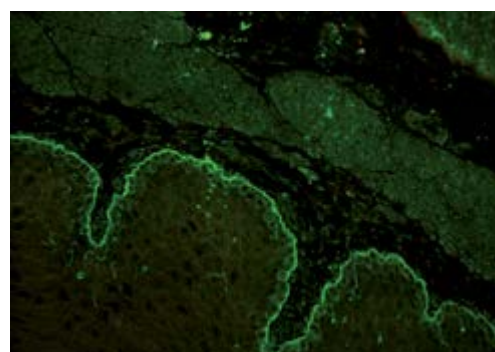

Antibodies against intercellular substance
"Chicken Wire" pattern
Antigen: Desmocolin 1, Desmoglein 1, Desmogleins 3

Antibodies against the epidermal basement membrane
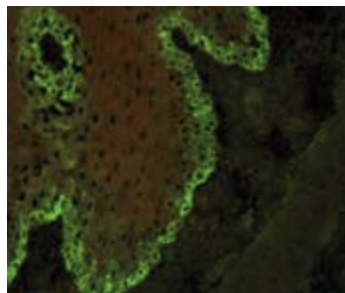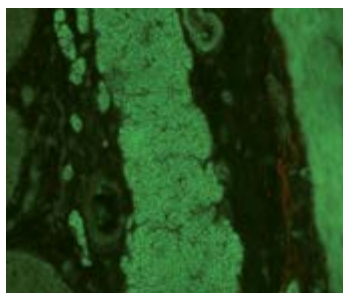*Antigen: BP180, BP230, Lamin 5, Collagen VII, Plectin*
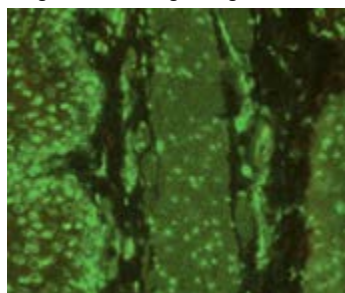
Negative fluorescence samples

Negative

Antibodies against basal cell layer
Antigen: Different target antigens

Antibodies against smooth muscle (SMA)
"Pomegranate Pulp"
Antigen: Actin, Myosin, Tropomyosin,
Troponin, Vimentin, Desmin, Cytokeratin,
Tubulin, Cytoskeletal antigens

Antinuclear antibodies (ANA)
Antigen: Nuclear antigens

Summary Protocol

| Step No.		Test steps
1		Dilute samples - screening serum/plasma 1:10 (10 µl + 90 µl)
2		Add Controls (1 drop) and diluted samples (35 µl)
3		Incubate at room temperature for 30 min
4		Rinse and wash the slide 2 x 5 min Shaker
5		Pipette Conjugate (1 drop)
6		Incubate at room temperature for 30 min
7		Rinse and wash of the slide 2 x 5 min Shaker
8		Dye of substrate in Evans blue 1 x 5 min Shaker
9		Add Mounting medium and cover with overslip
10		Evaluate using of fluorescence microscope

Test Characteristics

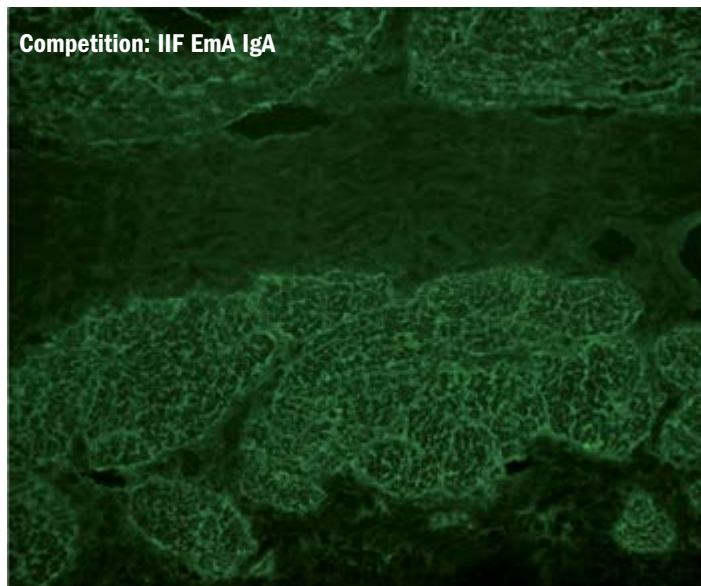|	Diagnostic Sensitivity	Diagnostic Specificity
IIF EmA IgA	96.55%	98.99%
IIF EmA IgG	95.24%	98.00%

Advantages

▶ Complete monkey oesophagus section

▶ Evans blue separately

▶ Components in sufficient volumes

▶ Specially modified IgA conjugate reducing nonspecific background (connective parts of the oesophagus)

TestLine: IIF EmA IgA
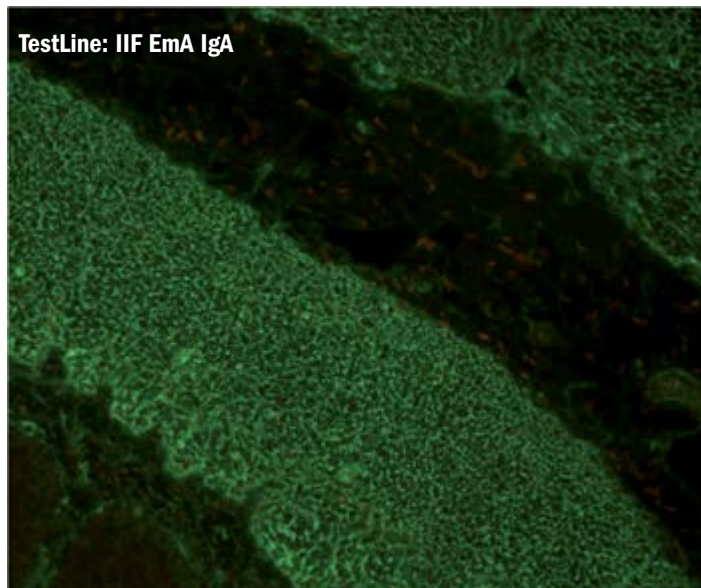
Competition: IIF EmA IgA

User Comfort

▶ Thicker teflon layer on the hydrophobic mask of the slide

▶ Reagents in dropper bottles

▶ Ideal shape of dropper bottles for comfort dispensing

▶ Blotting papers

Ordering Information

Cat. No.	Product	No. of Tests
EmAF100	IIF EmA IgA (Monkey Oesophagus)	100
EmGF100	IIF EmA IgG (Monkey Oesophagus)	100

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Company is certified to the quality management system standards ISO 9001 and ISO 13485 for in vitro diagnostics.

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