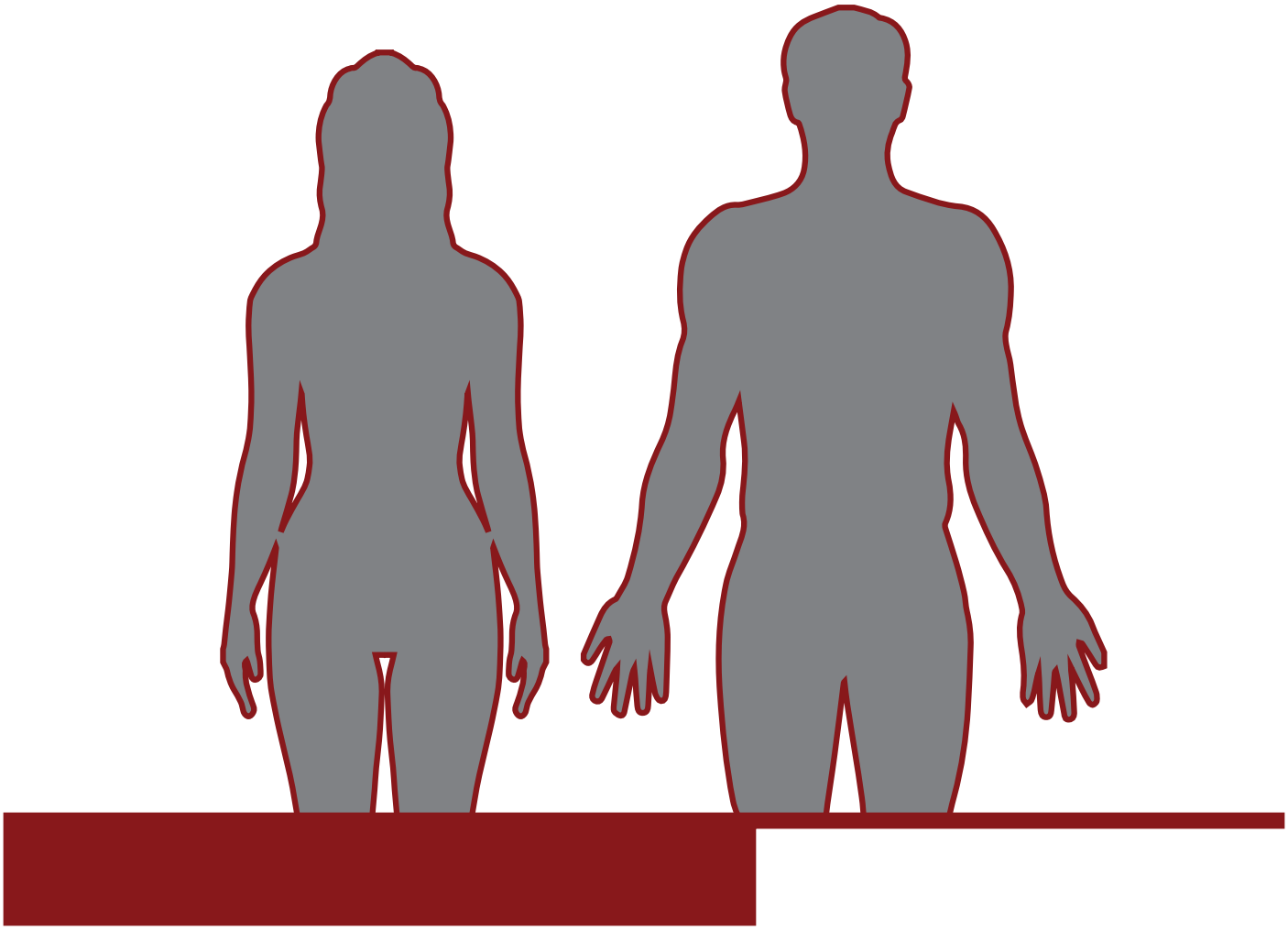
EUROIMMUN Medizinische Labordiagnostika AG

Autoimmune bullous dermatoses

Overview of serological diagnostics in autoimmune blister-forming diseases of the skin



- Pemphigoid diseases
- Pemphigus diseases
- Epidermolysis bullosa acquisita
- Dermatitis herpetiformis

The human skin

The skin shields the interior of a person from the external influences, protecting it from detrimental factors. It consists of three layers: epidermis, dermis and subcutis. The basal lamina links the deepest layer of the epidermis (basal layer, stratum basale) to the topmost connective tissue layer of the dermis (sublamina densa, stratum papillare). It consists of the lamina lucida and lamina densa.

The stability of the cell compound in the epidermis is essential for the protective function of the skin. Various cell contacts ensure a stable connection among the cells and with the basal lamina.

Desmosomes (Fig. 1) are responsible for the solid contact between the epidermal cells (keratinocytes) in the prickle-cell layer (statum spinosum). They tie the cytoskeletons of neighbouring cells to each other and are made of the transmembrane proteins desmoglein 1/3 and desmocollin, and intracellular plaque proteins (plakins).

So-called hemidesmosomes (Fig. 2) anchor the cells of the epidermal basal layer in the underlying basal lamina. They fix the cytoskeleton to the collagen fibrils of the basal lamina via the cytoplasmic proteins BP230 and plektin, and the transmembrane protein BP180 and integrin α6β4. Laminin 332 (laminin 5) acts as a link between BP180/integrin α6β4 and collagen type VII. By interaction between the collagens and anchoring fibrils of the sublamina densa the epidermis is anchored in the connective tissue layer.

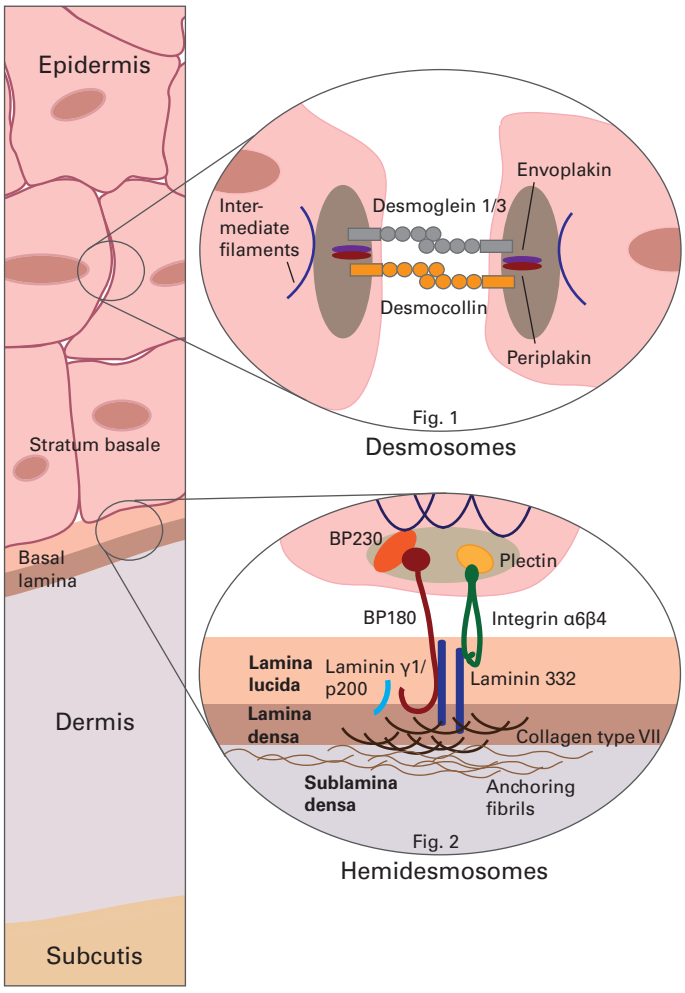


Fig. 1 Desmosomes

Fig. 2 Hemidesmosomes

Autoimmune bullous dermatoses

Bullous dermatoses are rare blistering diseases of the outer skin and neighbouring mucous membranes. These are auto-immune diseases in which the immune system produces anti-bodies against structural components of the desmosomes or hemidesmosomes. The immune response results in the loss of intercellular connections or in the peeling-away of the skin layers. Consequently, blisters form within the epidermis (Fig. 3) or between the epidermis and dermis (Fig. 4).

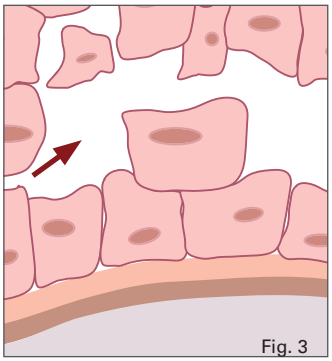


Fig. 3

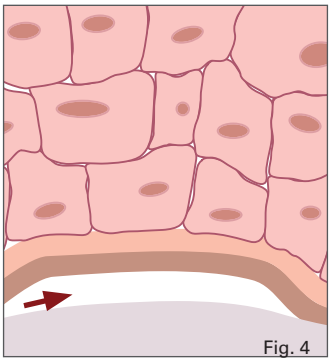


Fig. 4

Classification of autoimmune bullous dermatoses

Pemphigoid diseases

- Bullous pemphigoid
- Pemphigoid gestationis
- Mucosal pemphigoid
- Linear IgA dermatosis
- Anti-p200 pemphigoid

Pemphigus diseases

- Pemphigus vulgaris
- Pemphigus foliaceus
- Paraneoplastic pemphigus
- Further: IgA pemphigus P. vegetans, P. herpetiformis, P. erythematosus, drug-induced pemphigus

Epidermolysis bullosa acquisita

Dermatitis herpetiformis

Pemphigoid diseases

Pemphigoid diseases are a heterogeneous group of autoimmune diseases with subepidermal blister formation. Autoantibodies are directed against the components of hemidesmosomes and structural filaments. They cause the epidermis to peel away from the underlying dermis. The tissue-bound antibodies (immune complexes) can be detected along the basement membrane using direct immunofluorescence based on tissue samples of the skin. Indirect immunofluorescence for the specification of the autoantibody identity is often performed on oesophagus tissue sections and salt-split skin (Fig. 5). The target antigens BP180 and BP230, which are relevant in pemphigoid diseases, are located on the epidermal side of the salt-split skin. The antigens collagen type VII, laminin 332 and laminin γ1, however, remain on the dermal side after skin splitting.

Salt-split skin

Skin samples (primate) are incubated with 1 M NaCl. The salt dissolves the dermal/epidermal anchorage of the skin layers in the basal lamina. Indirect immunofluorescence on salt-split skin makes an important contribution to the specification of target antigens based on their localisation on the epidermal or dermal side of salt-split skin.

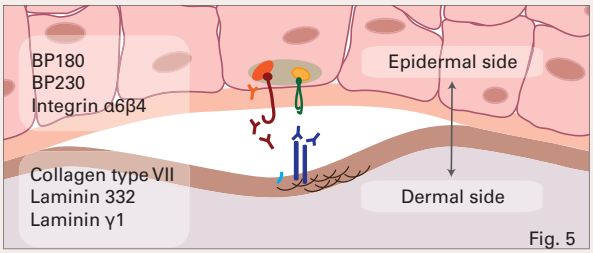


Fig. 5

Pemphigoid diseases	Characteristics	Target antigen (autoantibodies)
Bullous pemphigoid (BP)	Subepidermal blister formation in the outer skin, rarely in the mucous membranes; more frequently found in the elderly	BP180, BP230 (IgG, binds to the epidermal side of salt-split skin)
Pemphigoid gestationis (PG)	Is considered as manifestation of BP in pregnant women	BP180, BP230 (IgG, binds to the epidermal side of salt-split skin)
Mucous membrane pemphigoid (MMP)	Subepidermal blister formation, predominantly in the oral and ocular mucous membranes	BP180, rarely: integrin α6β4 (IgG, IgA, bind to the epidermal side of salt-split skin), laminin 332 (IgG, binds to the dermal side of salt-split skin)
Linear IgA dermatosis (LAD)	Formation of itching subepidermal blisters in the outer skin, most frequent form of autoimmune bullous dermatosis in children	Ectodomain of BP180 (LAD-1) (IgA, binds to the epidermal side of salt-split skin)
Anti-p200 pemphigoid	BP-similar subepidermal blister formation in the outer skin	p200 (laminin-γ1 chain) (IgG, binds to the dermal side of salt-split skin)

Pemphigus diseases

Pemhigus diseases are a group of autoimmune blistering diseases characterised by an intraepithelial disruption of the intercellular connections in the prickle-cell layer of the epidermis (acantholysis) of the outer skin and mucous membranes (Fig. 6). Acantholysis is caused by autoantibodies targeted against the desmosomes between keratinocytes, which they damage. Both in direct and indirect immunofluorescence the localisation of the immune complexes results in an intercellular, honeycomb-like fluorescence pattern on tissue samples of the skin and on oesophagus tissue sections. Target antigens in the desmosomes are especially desmoglein (Dsg) 1 and 3, as well as plakins and desmocollin (Dsc). Dsg1 is expressed particularly on the surface of the epidermis, whereas Dsg3 is mainly localised in the deep layers of the epidermis and in the mucous membranes. The localisation of Dsg1 and 3 explains the different manifestations of various forms of pemphigus.

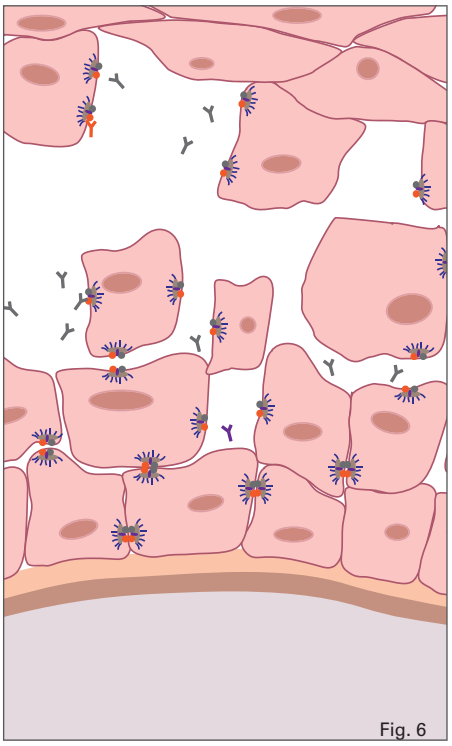


Fig. 6

Pemphigus disease	Characteristics	Target antigen
Pemphigus vulgaris (PV)	PV: Suprabasal blister formation in the outer skin and mucous membranes	Dsg1 and Dsg3
	Mucosal PV: Suprabasal blister formation, particularly in the mucous membranes	Dsg3
Pemphigus foliaceus (PF)	Blister formation in the upper epidermal layers of the outer skin; the mucous membranes are not involved	Dsg1
Paraneoplastic pemphigus (PNP)	Presence of a tumour (often haematological neoplasia) in addition to the skin disease; pronounced stomatitis	Plakins (envoplakin, periplakin, desmoplakins), Dsg3, Dsg1, plectin, BP230α-2-macroglobulin-like-1

Epidermolysis bullosa acquisita

Epidermolysis bullosa acquisita (EBA) is a severe autoimmune blistering dermatosis that affects the skin and the mucous membranes. The disease is divided into an inflammatory and a non-inflammatory form. The clinical manifestation of the inflammatory form is similar to that of BP, SHP and LAD. The target antigen of autoantibodies characteristic of EBA is collagen type VII (Fig. 7).

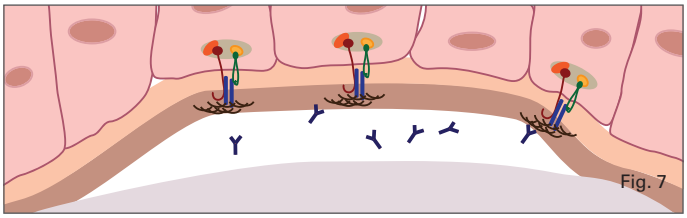


Fig. 7

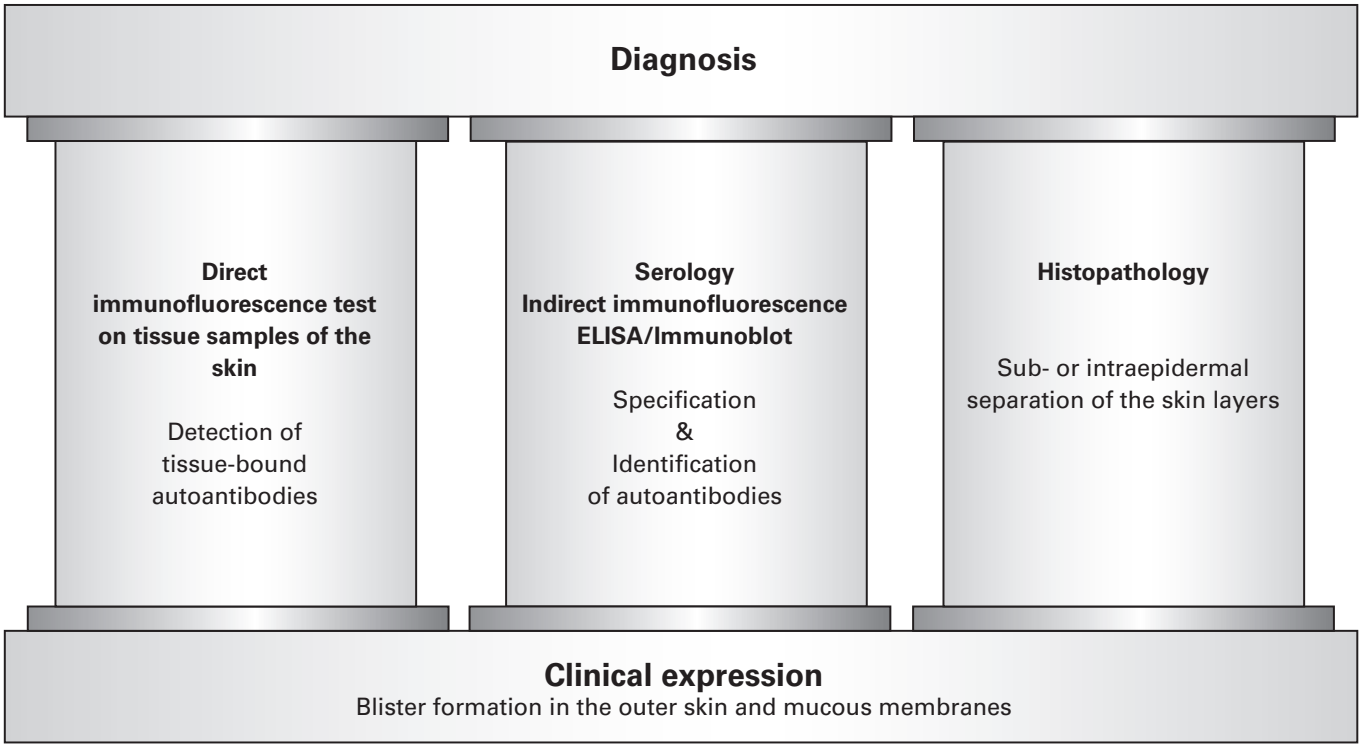
Disease	Characteristics	Target antigen
Epidermolysis bullosa acquisita (EBA)	Subepidermal blister formation in the outer skin and mucous membranes	Collagen type VII (IgG, binds to the dermal side of salt-split skin, Fig. 5)

Dermatitis herpetiformis

Dermatitis herpetiformis (DH) takes an exceptional position among autoimmune bullous dermatoses. Blisters are formed subepidermally as in pemphigoid diseases and EBA. The disease frequently affects the extensor sides of the extremities, but also the shoulders, the buttocks or the pelvic girdle. The mucous membranes generally do not show any blistering. DH is considered as the cutaneous manifestation of coeliac disease (gluten intolerance) and is also characterised by antibodies against endomysium (Ema, IgA), the body's own enzyme (tissue/epidermal) transglutaminase (anti-tTG/-eTG, IgA) and/or deamidated gliadin (IgA/IgG).

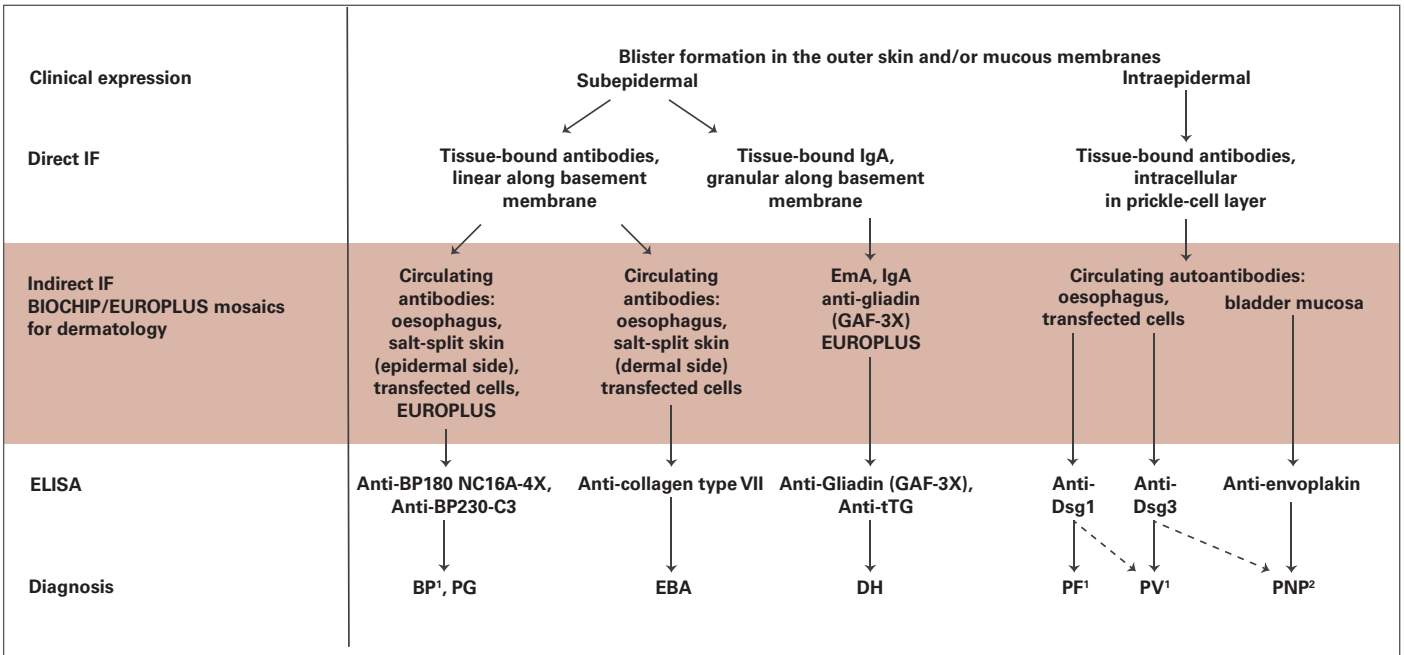
Disease	Characteristics	Target antigen
Dermatitis herpetiformis (DH)	Subepidermal blister formation; associated with gluten intolerance; improvement of symptoms with gluten-free diet	Deamidated gliadin peptides, (tissue/epidermal) transglutaminase, endomysium

The three pillars of autoimmune dermatoses diagnostics



Schmidt E et al., S2k-Leitlinie zur Diagnostik des Pemphigus vulgaris/foliaceus und des bullösen Pemphigoids [S2k guideline for the diagnosis of pemphigus vulgaris/foliaceus and bullous pemphigoid]. J Dtsch Dermatol Ges. 2015;13(7):713-27.

Diagnostics in autoimmune bullous dermatoses using EUROIMMUN dermatology test systems



[1] Criteria for securing diagnosis in Schmidt E et al., S2k-Leitlinien zur Diagnostik des Pemphigus vulgaris/foliaceus und des bullösen Pemphigoids [S2k guidelines for the diagnosis of pemphigus vulgaris/foliaceus and bullous pemphigoid]. J Dtsch Dermatol Ges. 2015; 13(7): 713-27.

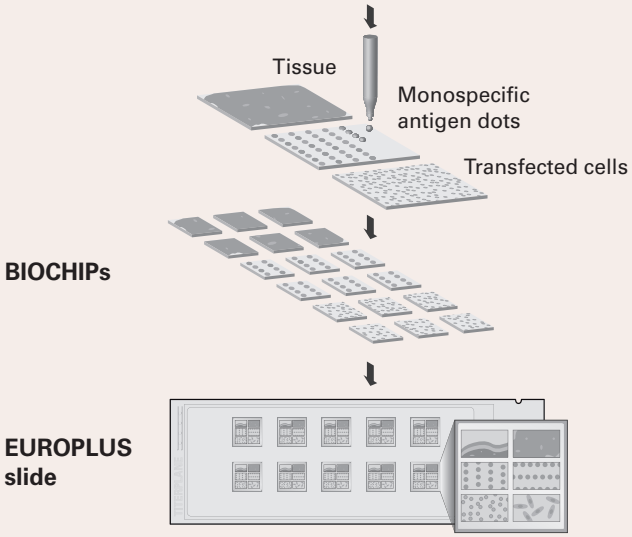
[2] Detection of neoplasia is obligatory for diagnosis.

Schematic illustration according to Otten et al. Current Molecular Medicine 2014, 14: 89-95.

Indirect immunofluroescence using EUROPLUS / BIOCHIP technology

In the EUROPLUS system the EUROIMMUN BIOCHIP mosaics (combination of tissue sections, cell culture substrates and transfected cells on a single reaction field) are supplemented by further BIOCHIPs coated with specific single antigens.

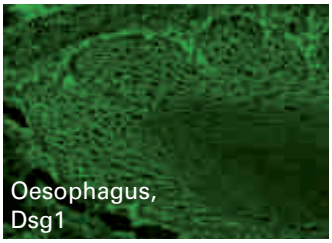
The native and recombinant antigens are applied to the cover glasses as droplets or diamonds. In a positive reaction the substrates show a clear fluorescence. As transfected cells, EUROPLUS substrates enable monospecific antibody detection in parallel to the screening on tissue/cell substrates in the same incubation.



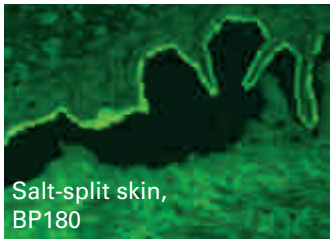
Indirect immunofluorescence tests (IIFT) for dermatology

EUROIMMUN offers a wide range of different IIFT substrates as BIOCHIP/EUROPLUS mosaics for the differentiation of the various autoimmune bullous dermatoses: tissues, transfected cells and EUROPLUS substrates.

A study from van Beek et al. (2012, Orphant J Rare Dis 7) on the Dermatology Mosaic 7 confirmed the high sensitivity and specificity of the substrates. 98.8% of 42 BP sera reacted with the basement membrane of the tissue substrates (oesophagus, salt-split skin), 100% with the EUROPLUS substrate BP180-NC16A-4X and 55% with BP230 (globular C-terminal domain, gC)-transfected cells. The Dsg1-transfected cells had a sensitivity of 90% for PF (n=50) , whereas 98.5% of PV sera (n = 65) reacted with Dsg3-transfected cells. The specificity of the substrates was between 98.2% and 100%.

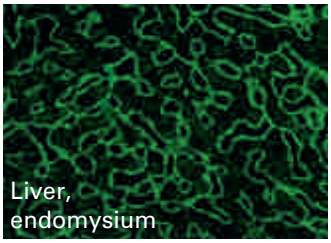


Oesophagus: detection of antibodies against **prickle-cell desmosomes** (pemphigus) and **basal lamina** (pemphigoid).

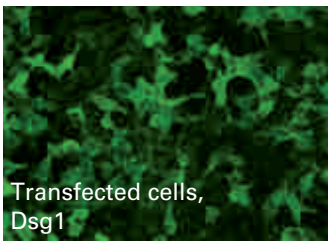


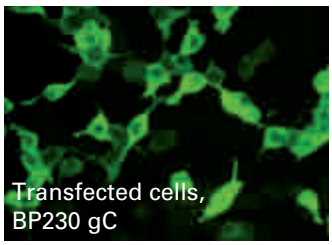
Salt-split skin: differentiation of autoantibodies against antigens of the epidermal (**BP180, BP230**) and dermal (**collagen type VII, laminin 332, p200**) sides of the skin.

Bladder mucosa: detection of autoantibodies against **plakins** (paraneoplastic pemphigus).

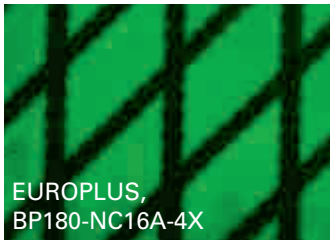


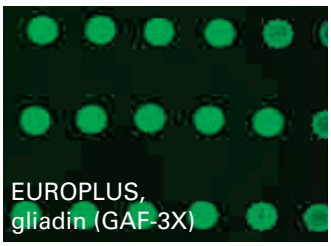
Endomysium: Detection of **EmA**, associated with coeliac disease and DH.





Transfected cells: Monospecific detection of antibodies against **Dsg1**, **Dsg3** (pemphigus), **BP230 gC** (pemphigoid), and **collagen type VII** (EBA).

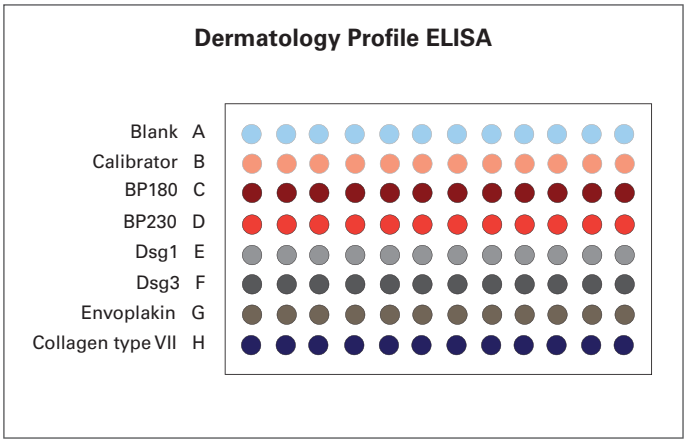




EUROPLUS substrates: Monospecific detection of antibodies against **BP180-NC16A-4X** (pemphigoid) and deamidated **gliadin (GAF-3X)** (coeliac disease, DH).

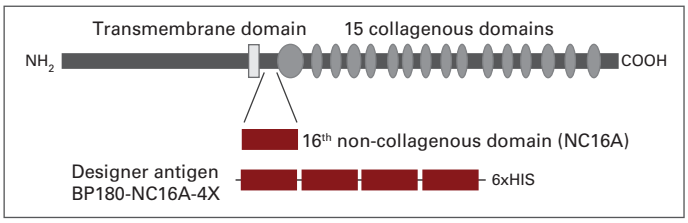
Enzyme-linked immunosorbent assays (ELISA) for dermatology

Since the corresponding autoantibodies are highly specific for the autoimmune diseases with which they are associated, monospecific antibody detection with the EUROIMMUN ELISAs helps to establish a reliable diagnosis. These tests also allow quantitative determination of antibody titers, which correlate with the disease activity in some diseases. Therefore, ELISAs are also often used for therapy monitoring. The new Dermatology Profile ELISA was developed as multiparameter test to quickly and reliably investigate patients with suspected autoimmune bullous dermatosis for various dermatological autoimmune diseases at the same time. The Profile ELISA comprises the antigens BP180, BP230, Dsg1, Dsg3, envoplakin and collagen type VII.

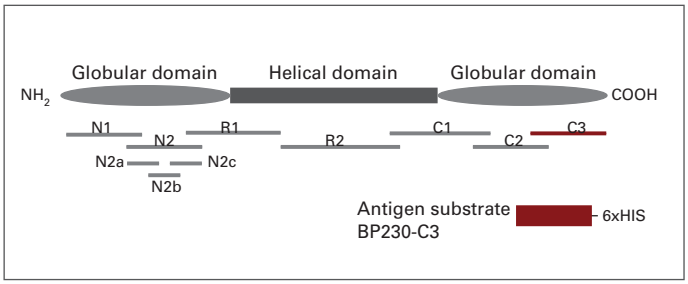


The Dermatology Profile ELISA – Combination of six important antigens

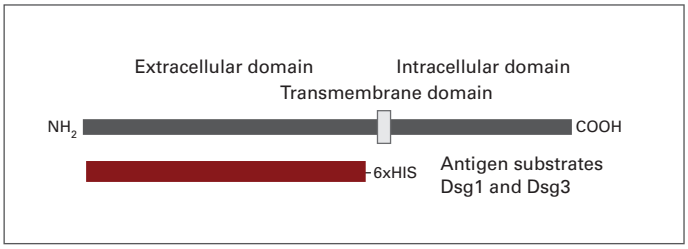
BP180-NC16A-4X: extracellular 16th non-collagenous domain (NC16A) as antigen substrate with the most important epitopes for autoantibodies in BP and PG in tetrameric form (4X) • Monospecific ELISA with **89.8% sensitivity** (118 BP sera, 20 PG sera) and **97.8% specificity** (229 control sera, 494 blood donors) • Antibody titer correlates with disease activity • Sitaru C et al. 2007, Exp Derma 16.



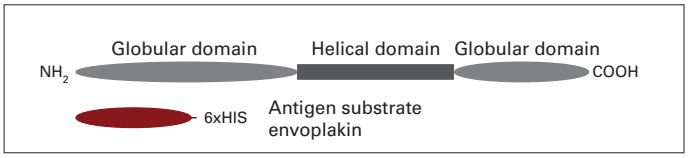
BP230-C3: BP230-C3 peptide (AS 2326-2649) as antigen substrate with highest reactivity in BP sera, selected from 10 tested overlapping BP230 fragments • Monospecific ELISA with **56.8% sensitivity** (118 BP sera) and **97.6% specificity** (276 control sera, 483 blood donors) • 4.2% additional diagnostic value for the identification of BP patients if anti-BP230 autoantibodies are tested in addition to anti-BP180 antibodies • Blöcker IM et al. 2012, Br J Dermatol 166.



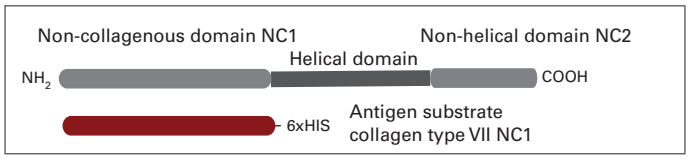
Dsg1 and Dsg3: ectodomains of Dsg1 and Dsg3 as antigen substrates for the diagnosis of PF and PV • Anti-Dsg1 ELISA: **96% sensitivity** for PF (50 PF sera) and **97.9%** (48 BP sera)/**99.3%** (401 blood donors) **specificity** • Anti-Dsg3 ELISA: **100% sensitivity** for PV (71 PV sera) and **97.9%** (48 BP sera)/**99.8%** (401 blood donors) **specificity** • Antibody titers correlate with the disease activity of pemphigus • Schmidt E et al. 2010, Exp Derma 19.



Envoplakin: N terminus (AS 1-481) as antigen substrate with highest reactivity in PNP sera • Monospecific ELISA with **80.6% sensitivity** (31 PNP sera) and **98.8% specificity** (30 PV sera, 50 BP sera) • Probst C et al. 2009, Clin Chim Acta 410.



Collagen type VII: N-terminal collagenous domain NC1 as most reactive antigen substrate in EBA sera • Monospecific ELISA with **94.5% sensitivity** (73 EBA sera) and **98.7% specificity** (395 control sera, 254 blood donors) • Komorowski L et al. 2012, J Am Acad Dermatol 68.



ORDERING

Indirect immunofluorescence tests (IIFT)			
Antibodies against	**Disease**	**Order no.**	**Substrates**
Epidermis (prickle-cell desmosomes, basal membrane)	Autoimmune bullous dermatoses	FA 1501	Oesophagus (primate)
Pemphigoid antigens	Pemphigoid diseases	FA 150b	Salt-split skin (primate)
BP180-NC16A-4X, BP230 gC	Bullous pemphigoid	FA 1502-1	BP180-NC16A-4X, transfected cells (BP230 gC)
Desmoglein 1 and 3	Pemphigus diseases	FA 1495-1	Transfected cells (desmoglein 1, desmoglein 3)
Transitional epithelium (plakins)	Paraneoplastic pemphigus	FA 1507	Bladder mucosa (rat)
Collagen type VII NC1	Epidermolysis bullosa acquisita	FA 1947-50	Transfected cells, control transfection

Dermatology mosaics	Disease	Order no.	Substrates
Dermatology Mosaic 7	Autoimmune bullous dermatoses (bullous pemphigoid, pemphigus diseases, paraneoplastic pemphigus, epidermolysis bullosa acquisita)	FA 1501-7	Oesophagus (primate), salt-split skin (primate), transfected cells (BP230 gC, desmoglein 1, desmoglein 3), EUROPLUS (BP180-NC16A-4X)
Dermatology Mosaic 11		FA 1501-11	Oesophagus (primate), liver (primate), bladder mucosa (rat), salt-split skin (primate), transfected cells (BP230 gC, desmoglein 1, desmoglein 3), control transfection, HEp-2 cells, EUROPLUS (BP180-NC16A-4X, gliadin (GAF-3X))
Dermatology Mosaic 20		FA 1501-20	Oesophagus (primate), salt-split skin (primate)

Additional reagents	Disease	Order no.	Application
AB adsorbent (Dermatology IIFT)	Autoimmune bullous dermatoses (bullous pemphigoid, pemphigus diseases, paraneoplastic pemphigus, epidermolysis bullosa acquisita)	ZF 1280-0105	For adsorption of antibodies against AB0 blood group antigens in IIFT on oesophagus tissue (primate)
FITC-labelled anti-human IgG (goat), primate-adsorbed		AF 302	Alternative IIFT conjugate for marking of specifically bound antibodies on e.g. oesophagus tissue (primate) and salt-split skin (primate)

ELISA			
Name	**Disease**	**Order no.**	**Recombinant antigens**
BP180-NC16A-4X	Bullous pemphigoid	EA 1502-4801-2 G	Tetramer of the immunodominant 16th non-collagenous domain of BP180
BP230-CF	Bullous pemphigoid	EA 1502-4801-1 G	C-terminal fragment of BP230
Desmoglein 1	Pemphigus diseases	EA 1495-4801 G	Extracellular domain of desmoglein 1
Desmoglein 3	Pemphigus diseases	EA 1496-4801 G	Extracellular domain of desmoglein 3
Envoplakin	Paraneoplastic pemphigus	EA 1491-4801 G	N-terminal fragment of envoplakin
Collagen type VII	Epidermolysis bullosa acquisita	EA 1947-4801 G	Collagen type VII
Dermatology Profile	Autoimmune bullous dermatoses	EA 1490-1208-1 G	BP180 NC16A-4X, BP230-CF, desmoglein 1, desmoglein 3, envoplakin, collagen type VII