EUROIMMUN Medizinische Labordiagnostika AG

Detection of autoantibodies against structural proteins of the skin

Overview of serological test systems

- **Most comprehensive product portfolio worldwide to support the serological diagnostics of blistering autoimmune dermatoses**
- **Detection of all relevant autoantibodies in pemphigus and pemphigoid diseases as well as in epidermolysis bullosa acquisita and dermatitis herpetiformis Duhring using ELISA and IIFT**
- **Effective screening and differentiation tests in only one incubation using highly sensitive BIOCHIP Mosaics**
- **Multiple automation options**

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 (stratum 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 plectin, 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.

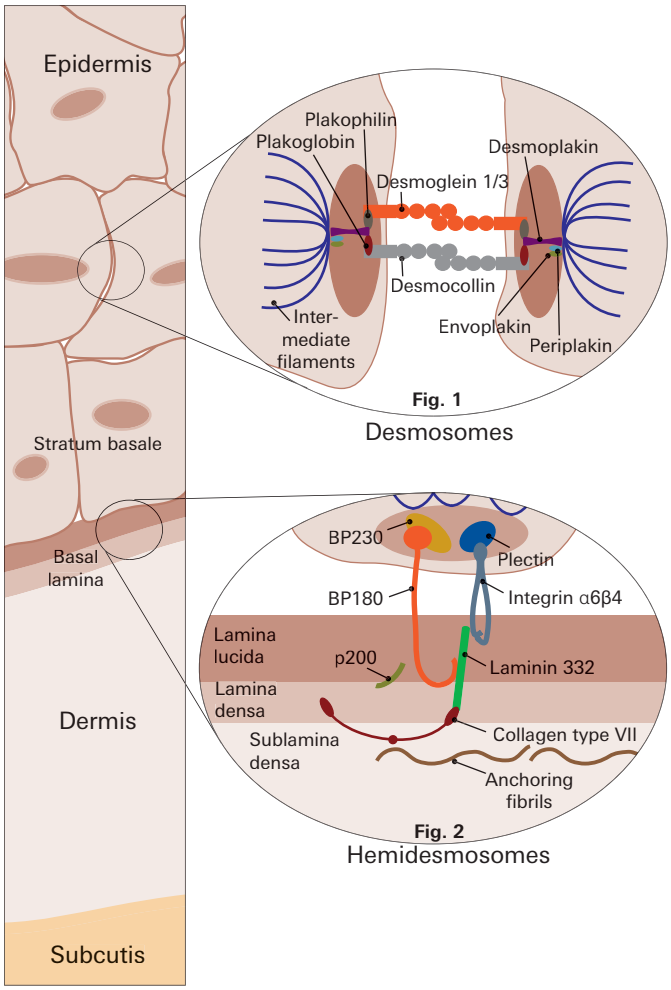


Fig. 1 Desmosomes

Fig. 2 Hemidesmosomes

Autoimmune bullous dermatoses

Bullous dermatoses are rare blistering diseases of the outer skin and neighbouring mucous membranes. These are autoimmune diseases in which the immune system produces antibodies 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).

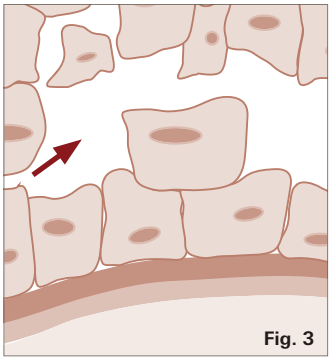


Fig. 3

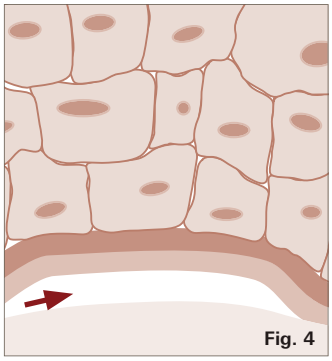


Fig. 4

Classification of autoimmune bullous dermatoses

- Pemphigus diseases
  - Pemphigus vulgaris
  - Pemphigus foliaceus
  - Paraneoplastic pemphigus
  - Further: IgA pemphigus P. vegetans, P. herpetiformis, P. erythematosus, drug-induced pemphigus
- Pemphigoid diseases
  - Bullous pemphigoid
  - Pemphigoid gestationis
  - Mucosal pemphigoid
  - Linear IgA dermatosis
  - Anti-p200 pemphigoid
- Epidermolysis bullosa acquisita
- Dermatitis herpetiformis

Pemphigus diseases

Pemphigus 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. 5). 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.

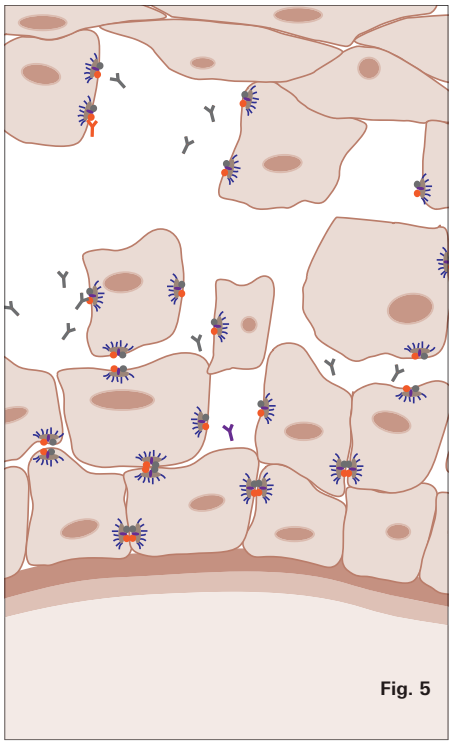


Fig. 5

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, BP230, α-2-macroglobulin-like-1

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. 6). 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 p200, 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.

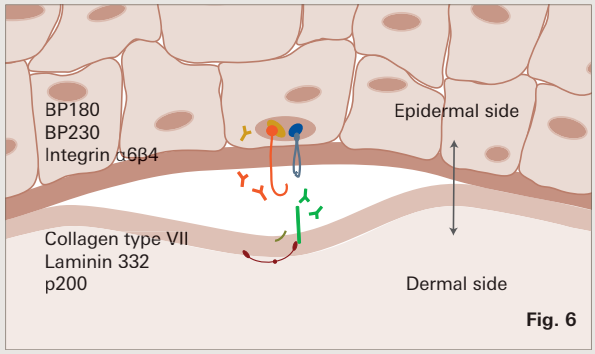


Fig. 6

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 a 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 (IgG, binds to the dermal side of salt-split skin)

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.

Disease	Characteristics	Target antigen (autoantibodies)
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)

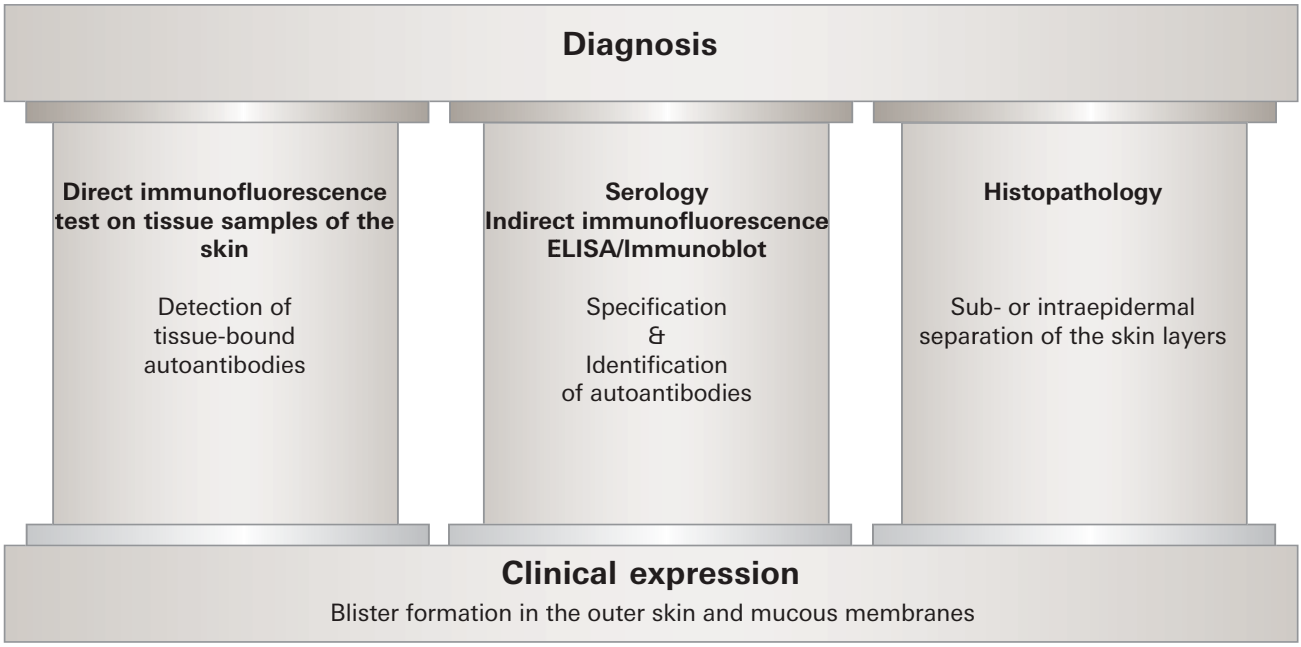
Dermatitis herpetiformis

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

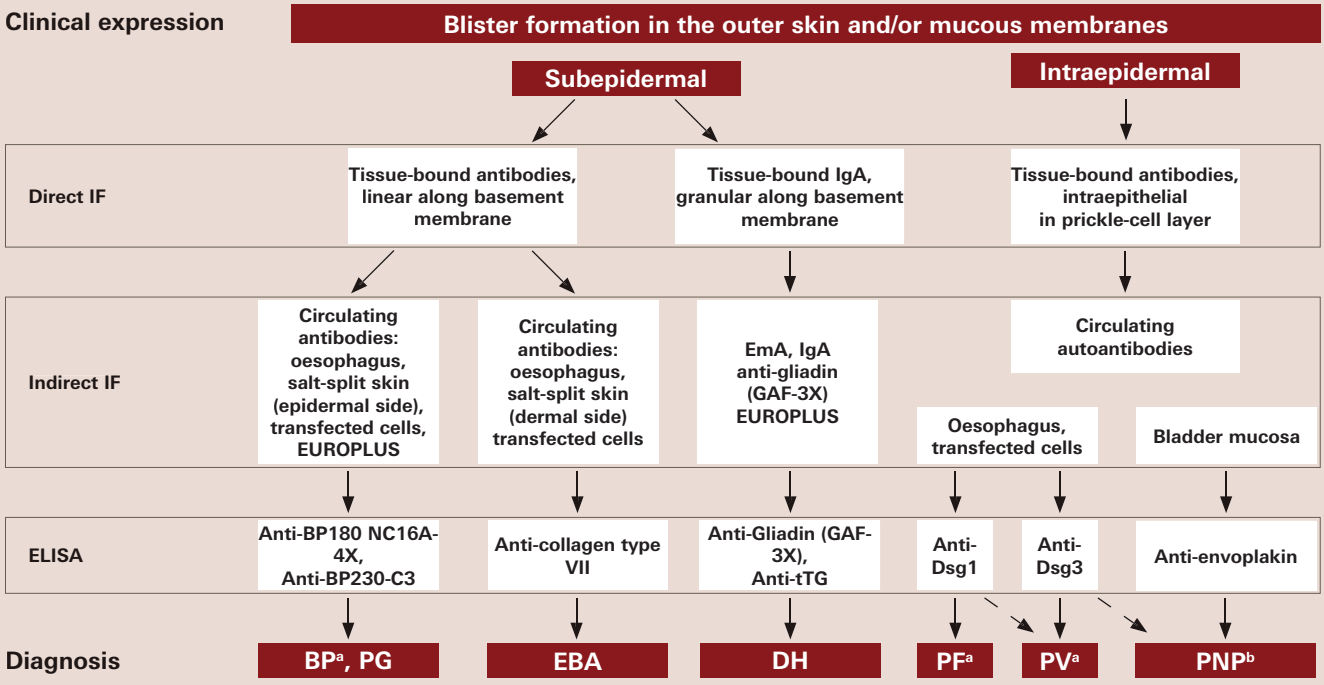
The three pillars of laboratory diagnostics of autoimmune dermatoses

In cases of a suspected clinical diagnosis of bullous autoimmune dermatosis, the current guidelines recommend the combination of three laboratory diagnostic measures to confirm and differentiate the diagnosis.[1-5]



Worm M et al., AWMF-S2k-Leitlinie "Diagnostik und Therapie des Pemphigus vulgaris / foliaceus und des bullösen Pemphigoids" (2019)

Diagnostics in autoimmune bullous dermatoses using EUROIMMUN dermatology test systems



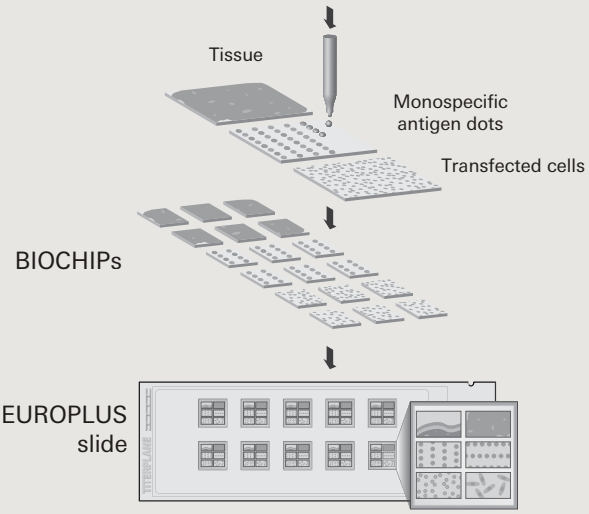
[a] Criteria for securing diagnosis in Worm M et al., AWMF-S2k-Leitlinie "Diagnostik und Therapie des Pemphigus vulgaris / foliaceus und des bullösen Pemphigoids" (2019)
[b] Detection of neoplasia is obligatory for diagnosis.

Schematic illustration according to Otten JV et al., Molecular Diagnosis in Autoimmune Skin Blistering Conditions, Current Molecular Medicine 14: 69-95 (2014)

Indirect immunofluorescence 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

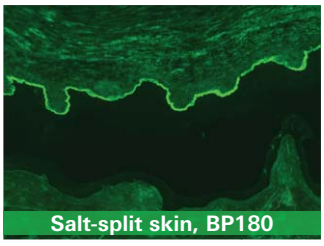
EUROIMMUN offers a wide range of different IIFT substrates as BIOCHIP/EUROPLUS mosaics for the differentiation of the various autoimmune bullous dermatoses: tissues, transfected cells and EUROPLUS substrates.

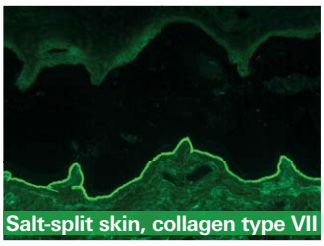
A study from van Beek et al. on the Dermatology Mosaic 7 confirmed the high sensitivity and specificity of the substrates. 98.8% of 42 BP sera reacted with the basement membrane of the tissue substrates (oesophagus, salt-split skin), 100% with the EUROPLUS substrate BP180-NC16A-4X and 55% with BP230 (globular C-terminal domain, gC)-transfected cells.[6] 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%.

Goletz et al. confirmed the excellent diagnostic benefit of laminin 332-transfected cells in the diagnosis of laminin 332 mucous membrane pemphigoid. The sensitivity measured with the BIOCHIP Mosaic was 84%, with a specificity of 99.5%.[7]

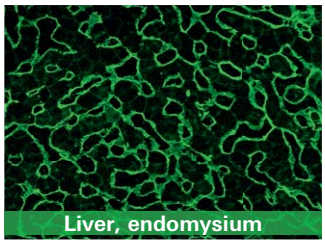
Since then, studies carried out worldwide by independent laboratories have shown dermatology mosaics to be effective screening and differentiation tests for the serological diagnosis of bullous autoimmune dermatoses.[8-12]

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

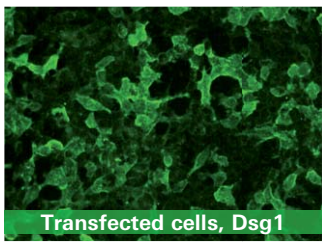


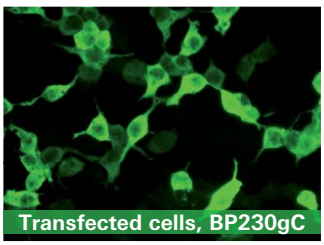


Salt-split skin: differentiation of autoantibodies against antigens of the epidermal (**BP180, BP230**) and dermal (**collagen type VII, laminin 332, p200**) sides of the skin.

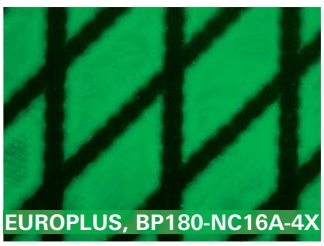


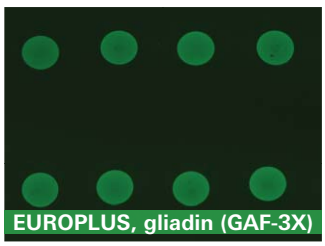
Endomysium: Detection of **EmA**, associated with coeliac disease and DH.





Transfected cells: Monospecific detection of antibodies against **Dsg1**, **Dsg3** (pemphigus), **BP230gC, laminin 332** (pemphigoid) and **collagen type VII** (EBA).

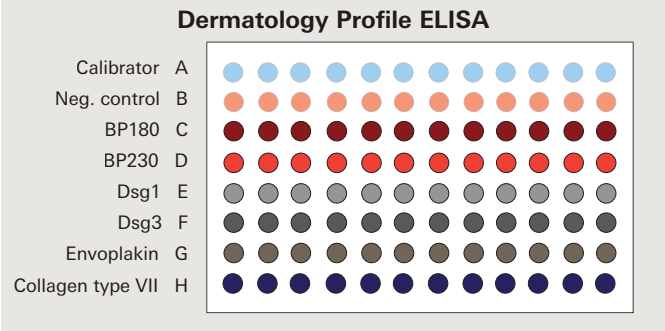




EUROPLUS substrates: Monospecific detection of antibodies against **BP180-NC16A-4X** (pemphigoid) and deamidated **gliadin (GAF-3X)** (coeliac disease, DH).

Enzyme-linked immunosorbent assays (ELISA) for dermatology

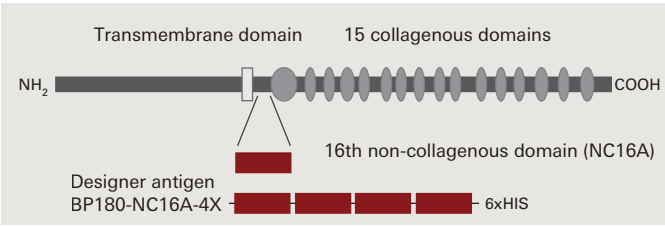
Since the corresponding autoantibodies are highly specific for the autoimmune diseases with which they are associated, monospecific antibody detection with the EUROIMMUN ELISAs helps to establish a reliable diagnosis. These tests also allow quantitative determination of antibody titers. The new Dermatology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



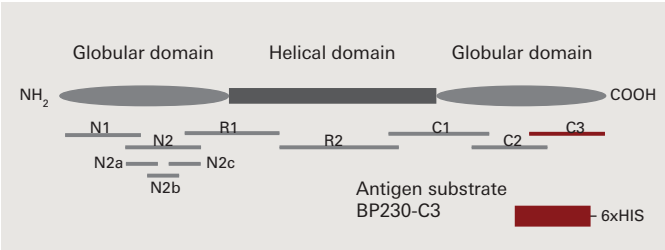
The **Dermatology Profile ELISA** is a convenient and standardised test to support routine diagnostics of bullous autoimmune dermatoses. This was demonstrated in an international study in which it achieved results concordant with those from direct immunofluorescence microscopy (for 94 % of pemphigus patients and 71 % of pemphigoid patients) and those from the conventional multivariate diagnostic procedures (for 91 % of pemphigus patients, 88 % of bullous pemphigoid patients and 93 % of autoantibody-negative sera).[13]

The Dermatology Profile ELISA – Combination of six important antigens

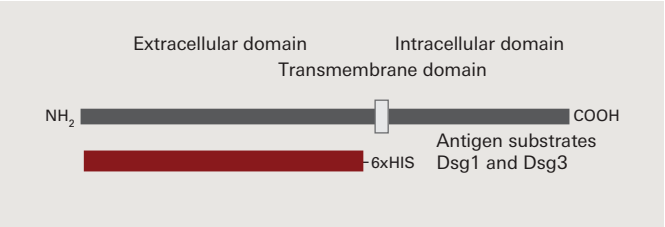
BP180-NC16A-4X: The antigen substrate is a tetramer of the immunodominant extracellular 16th non-collagenous domain (NC16A) of the glycoprotein BP180. Autoantibodies against BP180 are indicative of BP. The monospecific ELISA has a sensitivity (118 BP sera, 20 PG sera) of 89.8 % and specificity (229 control sera, 494 blood donors) of 97.8 %. Antibody titers correlate with disease activity.[14]



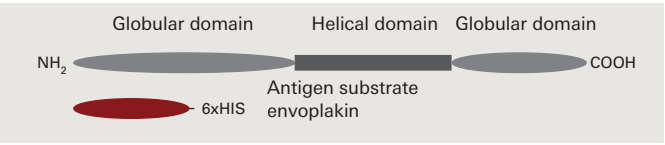
BP230-C3: A C-terminal fragment of BP230 is used as antigen substrate. The detection of autoantibodies against BP230 complements the serological diagnosis of BP. The monospecific ELISA has a sensitivity of 56.8 % (118 BP sera) and a specificity of 97.6 % (276 control sera, 483 blood donors). Parallel determination of anti-BP180 and anti-BP230 autoantibodies increases the diagnostic value for BP patient identification by an additional 4.2 %.[15]



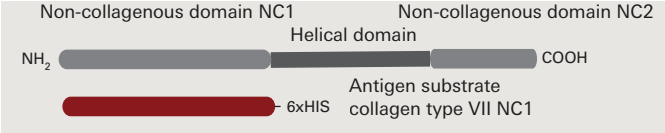
Dsg1 and Dsg3: Ectodomains of Dsg1 and Dsg3 represent the antigen substrates for the diagnosis of PF and PV. The Anti-Dsg1 ELISA has 96 % sensitivity for PF (50 PF sera) and 97.9 % (48 BP sera)/99.3 % (401 blood donors) specificity. The Anti-Dsg3 ELISA has 100 % sensitivity for PV (71 PV sera) and 97.9 % (48 BP sera)/99.8 % (401 blood donors) specificity. Antibody titers correlate with the disease activity of pemphigus.[16]



Envoplakin: The N-terminus of envoplakin is used as antigen substrate, which shows the highest reactivity in PNP sera. The monospecific ELISA showed a sensitivity (31 PNP sera) of 80.6 % and a specificity (30 PV sera, 50 BP sera) of 98.8 %.[17]



Collagen type VII: Antigen substrate is the N-terminal non-collagenous domain NC1. The monospecific ELISA achieved 94.5 % sensitivity (73 EBA sera) and 98.7 % specificity (395 control sera, 254 blood donors).[18]



Order information

Indirect immunofluorescence tests (IIFT)			
Antibodies against	**Disease**	**Order no.**	**Substrates**
Epidermis (prickle-cell desmosomes, basal membrane)	Autoimmune bullous dermatoses (bullous pemphigoid, pemphigus diseases, paraneoplastic pemphigus, epidermolysis bullosa acquisita)	FA 1501	Oesophagus (primate)
Dermatology Mosaic 7		FA 1501-7	Oesophagus (primate), salt-split skin (primate), transfected cells (BP230gC, desmoglein 1, desmoglein 3), EUROPLUS (BP180-NC16A-4X)
Dermatology Mosaic 20		FA 1501-20	Oesophagus (primate), salt-split skin (primate)
Laminin 332	Laminin 332 mucous membrane pemphigoid	FA 150b-50	Transfected cells, control transfection

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	NC1 domain of 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

Tests, for research use only*

Name	Test system	Order no.	Substrates
Dermal Binder Mosaic 1**	IIFT	FA 150b-1005-1	Transfected cells (laminin 332, LAMB4, collagen type VII), control transfection
Anti-Laminin Subunit Beta 4 (LAMB4) IIFT**		FA 150b-1005-51	Transfected cells (LAMB4), control transfection

* not for in vitro diagnostics
** Protected by patent applications EP3655060, CN111072768, SG10201908826U and patents US11208465, JP7326108 and KR10-2637893.

References

[1] Worm M et al. **AWMF-S2k-Guideline "Diagnostik und Therapie des Pemphigus vulgaris / foliaceus und des bullösen Pemphigoids".** (2019) [In German]. [2] Joly P et al. **Updated S2K guidelines on the management of pemphigus vulgaris and foliaceus initiated by the european academy of dermatology and venereology (EADV).** J Eur Acad Dermatol Venereol 34(9):1900-1913 (2020). [3] Rashid H et al. **European guidelines (S3) on diagnosis and management of mucous membrane pemphigoid, initiated by the European Academy of Dermatology and Venereology – Part I.** J Eur Acad Dermatol Venereol. 35(9):1750-1764 (2021). [4] Schmidt E et al. **European Guidelines (S3) on diagnosis and management of mucous membrane pemphigoid, initiated by the European Academy of Dermatology and Venereology – Part II.** J Eur Acad Dermatol Venereol 35(10):1926-1948 (2021). [5] Görög A et al. **S2k guidelines (consensus statement) for diagnosis and therapy of dermatitis herpetiformis initiated by the European Academy of Dermatology and Venereology (EADV).** J Eur Acad Dermatol Venereol. 35(6):1251-1277 (2021). [6] van Beek N et al. **Serological diagnosis of autoimmune bullous skin diseases: Prospective comparison of the BIOCHIP mosaic-based indirect immunofluorescence technique with the conventional multi-step single test strategy.** Orphant J Rare Dis 7:49 (2012). [7] Goletz S et al. **A sensitive and specific assay for the serological diagnosis of antilaminin 332 mucous membrane pemphigoid.** Br J Dermatol. 180(1):149-156 (2019). [8] Adaszewska A et al. **The use of BIOCHIP technique in diagnosis of different types of pemphigus: Vulgaris and foliaceus.** J Immunol Methods 468:35-39 (2019). [9] Özkesici B et al. **The Value of the BIOCHIP Mosaic-based Indirect Immunofluorescence Technique in the Diagnosis of Pemphigus and Bullous Pemphigoid in Turkish Patients.** Acta Dermatovenerol Croat 25(3):202-209 (2017). [10] Wang M et al. **BIOCHIP mosaic for the diagnosis of autoimmune bullous diseases in Chinese patients.** Eur J Dermatol 30(4):338-344 (2020). [11] Zarian H et al. **Biochip technology for the serological diagnosis of bullous pemphigoid.** ISRN Dermatol. 2012:237802 (2012) [12] Adaszewska A et al. **The use of BIOCHIP mosaics in diagnostics of bullous pemphigoid: Evaluation and comparison to conventional multistep procedures.** J Cutan Pathol. 47(2):121-127 (2020). [13] van Beek N et al. **Prospective studies on the routine use of a novel multivariant enzyme-linked immunosorbent assay for the diagnosis of autoimmune bullous diseases.** J Am Acad Dermatol 76(5):889-894 (2017). [14] Sitaru C et al. **Enzyme-linked immunosorbent assay using multimers of the 16th non-collagenous domain of the BP180 antigen for sensitive and specific detection of pemphigoid autoantibodies.** Exp Dermatol 16:770-777 (2007). [15] Blöcker IM et al. **Epitope mapping of BP230 leading to a novel enzyme-linked immunosorbent assay for autoantibodies in bullous pemphigoid.** Br J Dermatol 166:964-970 (2012). [16] Schmidt E et al. **Novel ELISA systems for antibodies to desmoglein 1 and 3: correlation of disease activity with serum autoantibody levels in individual pemphigus patients.** Exp Dermatol 19:458-463 (2010). [17] Probst C et al. **Development of ELISA for the specific determination of autoantibodies against envoplakin and periplakin in paraneoplastic pemphigus.** Clin Chim Acta 410:13-18 (2009). [18] Komorowski L et al. **Sensitive and specific assays for routine serological diagnosis of epidermolysis bullosa acquisita.** J Am Acad Dermatol 68(3):e89-95 (2012)