Serological diagnosis of autoimmune dermatoses:  
Dermatology Mosaic 9 (Order number: FA 1501-9)

Bullous pemphigoid (anti-epid. basement membrane pos.)

Pemphigus foliaceus (anti-desmoglein 1 pos.)
Introduction

Pemphigus vulgaris (PV) and pemphigus foliaceus (PF) are autoimmune intraepidermal blistering skin diseases characterized by autoantibodies to desmoglein 1 (Dsg1) and desmoglein 3 (Dsg3), respectively. Antigen-specific assays using the immunogenic extracellular Dsg domains can be applied for serological distinction between PV and PF. In this study, two ELISA based on human cell-expressed Dsg1 and Dsg3 were assessed for the first time with respect to their diagnostic performance and their suitability for therapy monitoring.

Methods

Ectodomains of Dsg1 and Dsg3 were expressed in HEK293 cells, purified from cell culture supernatant, and used as solid phase in ELISA for autoantibody determination of circulating IgG. Assay evaluation was performed with a panel of sera from 121 clinically characterized patients with pemphigus and 48 with bullous pemphigoid (BP), 21 with linear IgA dermatosis (LAD) and 401 healthy blood donors (HBD).

Results

Autoantibodies to Dsg1 were detected in 48 (96%) PF sera and in 0.9% of control subjects (1 BP, 0 LAD, 3 HBD). Reactivity to Dsg3 was found in all (100%) PV sera and in 0.4% of the control cohort (1 BP, 0 LAD, 1 HBD). Thus, the applied test systems revealed a sensitivity of 96% and 100% for anti-Dsg1 and anti-Dsg3, respectively, at a specificity of more than 99%. Levels of circulating autoantibodies, which were measured in 12 patients, correlated with the clinical severity in all cases.

Conclusion

ELISA based on human cell-expressed ectodomains of Dsg1 or Dsg3 represent highly sensitive and specific test systems, suitable for both, the serological diagnosis of patients with pemphigus and the monitoring of the disease activity.

Panel | n | anti-Dsg1 positive | anti-Dsg3 positive
--- | --- | --- | ---
Pemphigus vulgaris | 71 | 33 (47%) | 71 (100%)
Pemphigus foliaceus | 50 | 48 (96%) | 0
Linear IgA dermatosis | 21 | 0 | 0
Bullous pemphigoid | 48 | 1 (2%) | 1 (2%)
Blood donors | 401 | 3 (1%) | 1 (0.2%)
Specificity | 470 | 99.1% | 99.6%

Scientific presentation at the 9th Dresden Symposium on Autoantibodies (Dresden, Germany, September 2009)
Sensitivity and specific detection of pemphigoid autoantibodies by an Enzyme-linked immunosorbent assay using multimers of the NC16A domain of BP180 as antigen

C. Probst¹, C. Daehnrich¹, L. Komorowski¹, A. Rosemann¹, E. Schmidt², W. Schlumberger¹, C. Sitaru³C. Rose³, W. Stoecker¹, and D. Zillikens³

¹Institute of Experimental Immunology, affiliated to EUROIMMUN, Luebeck, Germany
²Department of Dermatology, University of Wuerzburg, Germany
³Department of Dermatology, University of Luebeck, Germany

Introduction

Bullous pemphigoid (BP) and pemphigoid gestationis (PG) are acquired autoimmune subepidermal blistering diseases characterized by autoantibodies against the hemidesmosomal proteins BP180/type XVII collagen and BP230. The vast majority of BP and PG patients demonstrate autoantibody binding to epitopes clustered within the 16th noncollagenous domain NC16A of BP180 (a).

Methods

In order to achieve efficient protein expression in E. coli, four copies of the NC16A domain were fused to a carboxyterminal polyhistidine tag (b). This strategy provides a high yield by simple purification of the target protein under denaturing conditions, without the need to cleave off a heterologous fusion partner. The protein, purified by metal chelate affinity chromatography (c), migrated consistent to its calculated mass of 36.6 kDa when separated by SDS-PAGE (lane 2). A monoclonal antibody specific for hexahistidin (lane 3) and serum from a patient with BP (lane 4) but not from a healthy blood donor (lane 5) recognized this recombinant form of NC16A by immunoblot analysis. Based on this tetrameric protein as antigenic substrate an ELISA for the detection of autoantibodies against BP180 was developed and evaluated.

Results

Autoantibodies against BP180 were found in 106 (89.8%) of 118 randomly selected BP sera and in all of 20 (100%) randomly selected PG sera, whereas only 2.1% of a large cohort of control subjects were positive in this assay, including patients with rheumatoid arthritis (RA; 2 of 107), progressive systemic sclerosis (PSS; 2 of 50), systemic lupus erythematosus (SLE; 1 of 72), and healthy blood donors (not shown, 10 of 494). Levels of circulating autoantibodies against BP180 (see columns) paralleled disease activity (see line graph) in pemphigoid patients.

<table>
<thead>
<tr>
<th>Panel</th>
<th>n</th>
<th>Tetrameric NC16A ELISA</th>
<th>GST-NC16A ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP</td>
<td>118</td>
<td>106 (89.8%)</td>
<td>105 (89.0%)</td>
</tr>
<tr>
<td>PG</td>
<td>20</td>
<td>20 (100.0%)</td>
<td>20 (100.0%)</td>
</tr>
<tr>
<td>RA</td>
<td>107</td>
<td>2 (1.9%)</td>
<td>5 (4.7%)</td>
</tr>
<tr>
<td>PSS</td>
<td>50</td>
<td>2 (4.0%)</td>
<td>4 (8.0%)</td>
</tr>
<tr>
<td>SLE</td>
<td>72</td>
<td>1 (1.4%)</td>
<td>3 (4.3%)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>118</td>
<td>89.8%</td>
<td>89.0%</td>
</tr>
<tr>
<td>Specificity</td>
<td>229</td>
<td>97.8%</td>
<td>94.8%</td>
</tr>
</tbody>
</table>

Discussion

The new ELISA showed better performance than formerly described test systems. We propose that the tetramer form of the autoantigen not only increases immunoreactivity, but also accessibility of the target epitope which facilitates binding of autoantibodies. A second advantage of using the recombinant BP180 as target antigen is the clear characterisation of the autoantibody specificity. In conclusion, the use of tetrameric NC16A in ELISA significantly improves making the diagnosis and monitoring disease activity of patients with BP and PG.

Scientific presentation at the 8th Dresden Symposium on Autoantibodies (Dresden, Germany, September 2007)
Autoantibodies against desmoglein 1 and 3 are markers for pemphigus. It is characterised by IgA antibodies against desmoglein 3 which always affects the mucous membranes. The majority of patients initially develop lesions in the mucous membranes which are then followed by skin involvement. Patients with pemphigus vulgaris who show damage exclusively to the skin mucosa exhibit IgG antibodies only against desmoglein 1, whereas patients with lesions of the skin and mucosa produce antibodies against desmoglein 1 and 3. In patients with pemphigus foliaceus, blisters are rarely found due to a very superficial cleft formation in the stratum granulosum. The disease is rather characterised by scaly crusts, especially in the seborrhoeic areas. The mucosa is never affected. Congruently, pemphigus foliaceus is only associated with desmoglein 1.

**Clinical significance:** Autoantibodies against desmoglein 1 and 3 are markers for pemphigus diseases, which can be clinically and immunopathologically subdivided into 4 different forms: pemphigus vulgaris, pemphigus foliaceus, paraneoplastic pemphigus and IgA pemphigus.

Pemphigus vulgaris always affects the mucous membranes. The majority of patients initially only develop lesions in the mucous of the mouth. In the course of the disease some patients show flaccid blisters at the integument, particularly on parts of the body which are exposed to pressure and friction. Patients with pemphigus vulgaris who show damage exclusively to the mouth mucosa exhibit IgG antibodies only against desmoglein 1, whereas patients with lesions of the skin and mucosa produce antibodies against desmoglein 1 and 3. In patients with pemphigus foliaceus, blisters are rarely found due to a very superficial cleft formation in the stratum granulosum. The disease is rather characterised by scaly crusts, especially in the seborrhoeic areas. The mucosa is never affected. Congruently, pemphigus foliaceus is only associated with desmoglein 1.

**Paraneoplastic pemphigus** is always associated with neoplasia. The resulting immune response is not only directed against desmoglein 3 but also against other proteins of desmosomal plaques such as envoplakin, periplakin, desmoplakin and plektin.

IgA pemphigus is characterised by IgA antibodies against desmoglein 1 and desmocollin 1. Desmoglein 1 and 3 are cadherins, which are calcium-dependent transmembrane glycoproteins of epidermal desmosomes. They are components of the maculae adherentes and permit the cell-to-cell contact in the epidermis and the surface mucosa via homophilic and heterophilic extracellular binding. The pathogenetic relevance of autoantibodies against desmoglein 1 and 3 is well proven. For example, the injection of recombinant desmoglein 1 into mice leads to blister formation. The exact mechanisms which cause the blister formation, however, are unknown.

**Clinical sensitivity and specificity:** Sera from 50 patients with pemphigus foliaceus, 71 patients with pemphigus vulgaris, a control panel of 69 patients with other autoimmune diseases and 401 healthy blood donors were investigated using the EUROIMMUN Anti-Desmoglein 1 ELISA. The sensitivity of the ELISA for pemphigus foliaceus was 96.0%, with a specificity of 99.1%. In the pemphigus vulgaris panel 46.5% of patients were found positive.

**Application of the Anti-Desmoglein 1 ELISA:** In the diagnosis of pemphigus, determination of circulating autoantibodies using indirect immunofluorescence (on primate oesophagus as sensitive substrate) has proven successful. However, it does not allow differentiation between antibodies against desmoglein 1 and desmoglein 3. ELISA using desmoglein 1 and 3 offers the same sensitivity and specificity as IIFT. In most cases the Anti-Desmoglein 1 ELISA and Anti-Desmoglein 3 ELISA are sufficient to diagnose pemphigus. In suspected pemphigus cases with a negative ELISA result IIFT should be carried out in addition. The Anti-Desmoglein 3 ELISA is of particular importance in lesions of the mouth mucosa to differentiate pemphigus vulgaris from Lichen ruber mucosae, benign aphtha, Behcet’s disease and Steven-Johnson syndrome.

The Anti-Desmoglein 1 ELISA and Anti-Desmoglein 3 ELISA are highly sensitive and specific test systems for the diagnosis of pemphigus diseases. Untreated patients with a positive result in the Anti-Desmoglein 3 ELISA alone suggests the presence of pemphigus vulgaris with only mucosa involvement. If both the Anti-Desmoglein 3 ELISA and the Anti-Desmoglein 1 ELISA are positive, this indicates pemphigus vulgaris with mucosa and skin involvement. A positive Anti-Desmoglein 1 ELISA result alone is indicative of pemphigus foliaceus. The antibody levels of desmoglein 1 and 3 in the serum generally correlate with the severity and activity of the disease and the therapy success.
Test characteristics

**Anti-Desmoglein 1 ELISA (IgG)**

**Linearity:** The linearity of the ELISA was determined by assaying 4 serial dilutions of 6 serum samples. The linear regression was calculated, R2 amounting to >0.95 in all samples. The Anti-Desmoglein 1 ELISA (IgG) is linear in at least the tested concentration range (9-197 RU/ml).

**Reproducibility:** The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation using 3 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

**Reference range:** Levels of anti-desmoglein 1 antibodies were determined in 401 sera from healthy blood donors of between 18 and 68 years of age (151 women, 250 men) using the EUROIMMUN ELISA. The mean concentration of antibodies against desmoglein 1 was 1.8 RU/ml and the values ranged from 0.1 to 39.0 RU/ml. With a cut-off of 20 RU/ml, 0.7% of the blood donors were anti-desmoglein 1 positive.

**ROC analysis:** In an analysis of 50 samples from patients with pemphigus foliaceus and 470 control samples the following results were achieved:

**Correlation of the EUROIMMUN and MBL Anti-Desmoglein 1 ELISAs:** The antibody concentration was determined in 50 sera from patients with pemphigus foliaceus using the Anti-Desmoglein 1 ELISAs from EUROIMMUN and MBL. The qualitative results of the ELISAs were 94% in agreement.

The antibody concentration was measured in 69 patients with other autoimmune diseases (bullous pemphigoid, linear IgA dermatosis) using the Anti-Desmoglein 1 ELISAs from EUROIMMUN and MBL. The qualitative results of the ELISAs were 97% in agreement.

**Technical data:**

**Antigen:** Recombinant, expressed in mammalian cells, extracellular domain of desmoglein 1 (5 subdomains).

**Calibration:** Quantitative, in relative units per milliliter (RU/ml).

**Measurement:** 450 nm. Reference wavelength between 620 nm and 650 nm.

**Test procedure:** 30 min / 30 min / 15 min. Room temperature. Fully automatable.

**Order no.:** EA 1495-4801 G
Sera from 71 patients with pemphigus vulgaris, 50 patients with pemphigus foliaceus, paraneoplastic pemphigus and IgA pemphigus was 100%, with a specificity of 99.6%. In 401 healthy blood donors were investigated using the EUROIMMUN Anti-Desmoglein 3 ELISA. Autoantibodies against desmoglein 1 and 3 are markers for pemphigus, which can be clinically and immunopathologically subdivided into four different forms: pemphigus vulgaris, pemphigus foliaceus, paraneoplastic pemphigus and IgA pemphigus.

Pemphigus vulgaris always affects the mucous membranes. The majority of patients initially only develop lesions in the mucosa of the mouth. In the course of the disease some patients show flaccid blisters at the integument, particularly on parts of the body which are exposed to pressure and friction. Patients with pemphigus vulgaris who show damage exclusively to the mouth mucosa exhibit IgG antibodies only against desmoglein 3, whereas patients with lesions of the skin and mucosa produce antibodies against desmoglein 3 and 1. In patients with pemphigus foliaceus, blisters are rarely found due to a very superficial cleft formation in the stratum granulosum. The disease is rather characterised by scaly crusts, especially in the seborrhoeic areas. The mucosa is never affected. Congruently, pemphigus foliaceus is only associated with desmoglein 1. Paraneoplastic pemphigus is always associated with neoplasia. The resulting immune response is not only directed against desmoglein 3 but also against other proteins of desmosomal plaques such as envoplakin, periplakin, desmoplakin and plektn. IgA pemphigus is characterised by IgA antibodies against desmoglein 1 and desmocollin 1. Desmoglein 1 and 3 are cadherins, which are calcium-dependent transmembrane glycoproteins of epidermal desmosomes. They are components of the maculae adherentes and permit the cell-to-cell contact in the epidermis and the surface mucosa via homophilic and heterophilic extracellular binding. The pathogenetic relevance of autoantibodies against desmoglein 1 and 3 is well proven. For example, the injection of serum from pemphigus patients into neonatal mice leads to blister formation. The exact mechanisms which cause the blister formation, however, are unknown.

Clinical sensitivity and specificity: Sera from 71 patients with pemphigus vulgaris, 50 patients with pemphigus foliaceus, a control panel of 69 patients with other autoimmune diseases and 401 healthy blood donors were investigated using the EUROIMMUN Anti-Desmoglein 3 ELISA. The sensitivity of the ELISA for pemphigus vulgaris was 100%, with a specificity of 99.6%. In the pemphigus foliaceus panel all patients were found negative.

Application of the Anti-Desmoglein 3 ELISA: In the diagnosis of pemphigus, determination of circulating autoantibodies using indirect immunofluorescence (on primate oesophagus as sensitive substrate) has proven successful. However, it does not allow differentiation between antibodies against desmoglein 1 and desmoglein 3. ELISA using desmoglein 1 and 3 offers the same sensitivity and specificity as IIFT. In most cases the Anti-Desmoglein 3 ELISA is sufficient to diagnose pemphigus. In suspected pemphigus cases with a negative ELISA result IIFT should be carried out in addition. The Anti-Desmoglein 3 ELISA is of particular importance in lesions of the mouth mucosa to differentiate pemphigus vulgaris from Lichen ruber mucosae, benign aphtha, Behçet’s disease and Steven-Johnson syndrome.

The Anti-Desmoglein 3 ELISA and Anti-Desmoglein 3 ELISA are highly sensitive and specific test systems for the diagnosis of pemphigus diseases. In untreated patients a positive result in the Anti-Desmoglein 3 ELISA alone suggests the presence of pemphigus vulgaris with only mucosa involvement. If both the Anti-Desmoglein 3 ELISA and the Anti-Desmoglein 1 ELISA are positive, this indicates pemphigus vulgaris with mucosa and skin involvement. A positive Anti-Desmoglein 1 ELISA result alone is indicative of pemphigus foliaceus. The antibody levels of desmoglein 1 and 3 in the serum generally correlate with the severity and activity of the disease and the therapy success.
Test characteristics

Anti-Desmoglein 3 ELISA (IgG)

Linearity: The linearity of the ELISA was determined by assaying 4 serial dilutions of 6 serum samples. The linear regression was calculated, R2 amounting to > 0.95 in all samples. The Anti-Desmoglein 3 ELISA (IgG) is linear in at least the tested concentration range (14-195 RU/ml).

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation using 3 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Reference range: Levels of anti-desmoglein 3 antibodies were determined in 401 sera from healthy blood donors of between 18 and 68 years of age (151 women, 250 men) using the EUROIMMUN ELISA. The mean concentration of antibodies against desmoglein 3 was 1.8 RU/ml and the values ranged from 0.4 to 25.3 RU/ml. With a cut-off of 20 RU/ml, 0.2% of the blood donors were anti-desmoglein 3 positive.

ROC analysis: In an analysis of 71 samples from patients with pemphigus vulgaris and 470 control samples the following results were achieved:

Correlation of the EUROIMMUN and MBL Anti-Desmoglein 3 ELISAs: The antibody concentration was determined in 71 sera from patients with pemphigus vulgaris using the Anti-Desmoglein 3 ELISAs from EUROIMMUN and MBL. The qualitative results of the ELISAs were 99% in agreement.

The antibody concentration was measured in 69 patients with other autoimmune diseases (bullous pemphigoid, linear IgA dermatosis) using the Anti-Desmoglein 3 ELISAs from EUROIMMUN and MBL. The qualitative results of the ELISAs were 97% in agreement.

Technical data:

Antigen: Recombinant, expression in mammalian cells, extracellular domain of desmoglein 3 (5 subdomains).

Calibration: Quantitative, in relative units per millilitre (RU/ml).

Measurement: 450 nm. Reference wavelength between 620 nm and 650 nm.

Test procedure: 30 min / 30 min / 15 min. Room temperature. Fully automatizable.

Test kit format: 48 break-off wells. Kit includes all necessary reagents.

Order no.: EA 1496-4801 G

EUROIMMUN AG · 23560 Luebeck (Germany) · Seekamp 31 · Telephone +49 451 58550 · Fax 5855991 · E-mail euroimmun@euroimmun.de
Indications: Test system for the in vitro determination of antibodies against BP180 in human serum or plasma for the diagnosis of the following diseases: bullous pemphigoid, pemphigoid gestationis, mucous membrane pemphigoid and lichen ruber pemphigoid.

Clinical significance: Bullous autoimmune dermatoses belong to organ-specific autoimmune diseases. They are characterised by the formation of autoantibodies against structure proteins of the skin. These structural proteins establish the cell-to-cell contact in keratinocytes within the epidermis and the adhesion of the epidermis to the dermis. Bullous autoimmune dermatoses are divided in 4 main groups by means of their target antigens and the localisation of the blisters: pemphigoid and pemphigus diseases, epidermolysis bullosa acquisita and Duhring’s dermatitis herpetiformis. In pemphigus diseases the blisters are formed intraepidermally, whereas they occur in all other bullous autoimmune dermatoses subepidermally.

With 0.7 to 1.8 new cases per year per 100,000 inhabitants, the bullous pemphigoid (BP) is the most frequent subepidermal blister-forming autoimmune dermatosis. The disease mainly affects elderly people. The manifestation of the BP is bulging blisters at the integument. However, the BP may proceed without blisters for weeks or months. Therefore, elderly patients with irritant skin disorders persisting for long periods should be tested for BP in differential diagnosis. Various immunological methods are used for differential diagnosis of the disease. The presence of circulating autoantibodies is significant in 90% of BP patients. These autoantibodies are mainly directed against 2 hemidesmosomal proteins. The BP antigens have molecular masses of 180 kDa (BP180) and 230 kDa (BP230), respectively. BP230 is localised intracellularly in the hemidesmosomal plaque. BP180 is a transmembrane glycoprotein with an intracellularly localised C-terminus and an extracellular N-terminus. The ectodomain consists of 15 collagenous and 16 non-collagenous domains. The 16th non-collagenous domain (NC16A) directly linking the keratinocyte membrane presents the immunogenic epitope. The majority of BP patients have autoantibodies against BP180. Direct and indirect immunofluorescence is used for the determination of autoantibodies. Tissue-bound autoantibodies and/or complement deposits in biopsies of perilesional skin can be determined using direct immunofluorescence. Circulating autoantibodies in the patient serum can be found by means of indirect immunofluorescence (substrate: oesophagus, primate and human skin). Autoantibodies against basement membrane show a fine linear staining between the stratum basale and the connective tissue. Autoantibody specificity can be characterised using monospecific ELISA or immunoblots.

Application of the Anti-BP180-NC16A-4X ELISA: The detection of autoantibodies in the skin and/or in the serum of patients is decisive in BP diagnosis. BP patients mainly have autoantibodies against BP180. These autoantibodies can be found in the skin using direct immunofluorescence. Autoantibodies circulating in the serum can be detected by means of indirect immunofluorescence using organ tissues. The Anti-BP180-NC16A-4X ELISA uses a tetramer of the immunogenic NC16A domain and is a reliable alternative for the indirect immunofluorescence test. The advantage of the ELISA is the clear characterisation of the autoantibody specificity when using the recombinant BP180 and the resulting differentiation of other bullous autoimmune dermatoses such as pemphigus diseases, epidermolysis bullosa acquisita and Duhring’s dermatitis herpetiformis. The multimer form of the autoantigen increases the immunoreactivity, thus improving the efficiency of the autoantibody test. The serum level of autoantibodies against BP180 correlates with the BP activity.

1 German Patent Application No. 10 2006 059 574.2
Test Characteristics

Anti-BP180-NC16A-4X ELISA (IgG)

Linearity: The linearity of the ELISA was determined by assaying serial dilutions of 6 serum samples. The linear regression was calculated, R² amounting to > 0.95 in all samples. The Anti-BP180-NC16A-4X ELISA (IgG) is linear at least in the range of 10 to 199 RU/ml.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation using 4 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Clinical sensitivity and specificity: Sera from 118 BP patients, a control panel of 229 patients with other autoimmune diseases and 494 healthy blood donors were investigated using the EUROIMMUN Anti-BP180-NC16A-4X ELISA. The sensitivity of the ELISA for BP was 90%, with a specificity of 98%. Additionally, 101 patients (>70 years of age) with non-inflammatory skin diseases were tested, resulting in a specificity of the ELISA of 99%.

Reference range: Levels of Anti-BP180 antibodies were determined in 494 sera from healthy blood donors of between 18 and 68 years of age (185 women, 309 men) using the EUROIMMUN ELISA. The mean concentration of antibodies against BP180 was 4.5 RU/ml and the results ranged from 0.01 to 168.0 RU/ml. With a cut-off of 20 RU/ml, 2.0% of blood donors were Anti-BP180 positive.

ROC analysis: In an analysis of 118 BP samples and 723 control sera the following results were found:

Correlation of the EUROIMMUN and MBL Anti-BP180 ELISAS:
The antibody concentration was determined in 118 sera from BP patients and a control panel of 229 sera from patients with other autoimmune diseases (RA, PSS, SLE) using the EUROIMMUN and MBL Anti-BP180-ELISA. The qualitative results of the ELISAS correlated in 99% (BP panel) and 98% (control panel).

Technical data:

Antigen: Tetramer of the immunogenic NC16A domain (BP180), based on human cDNA, expressed in E.coli.

Calibration:
Quantitative, in relative units per millilitre (RU/ml).
Calibration serum 1: 200 RU/ml
Calibration serum 2: 20 RU/ml; cut-off
Calibration serum 3: 2 RU/ml

Sample dilution:
Serum or plasma; 1: 101 in sample buffer.

Reagents:
Ready for use. Exception: wash buffer (10x). Colour-coded solutions, largely exchangeable with those of other EUROIMMUN-ELISA.

Test procedure:
30 min / 30 min / 15 min. Room temperature. Fully automatable.

Measurement:
450 nm. Reference wavelength between 620 nm and 650 nm.

Kit format:
48 single break-off wells, incl. all necessary reagents.

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