Eur J Dermatol 2016; 26(1): 56-63

Orsolya N. HORVÁTH[1,2]
Rita VARGA[1]
Makoto KANEDA[3]
Enno SCHMIDT[4]
Thomas RUZICKA[1]
Miklós SÁRDY[1]

[1] Department of Dermatology, Allergology, Ludwig Maximilian University, Frauenlobstr. 9-11, 80337 Munich, Germany
[2] Institute of Behavioural Sciences, Semmelweis University, Budapest, Hungary
[3] Medical and Biological Laboratories Company, Ltd., Nagoya, Japan
[4] Department of Dermatology, University of Lübeck, Lübeck, Germany

Reprints: O. N. Horváth <orsolya.horvath@med.uni-muenchen.de>

Article accepted on 09/10/2015

Diagnostic performance of the "MESACUP anti-Skin profile TEST"

Background: The "MESACUP anti-Skin profile TEST" is a new, commercially available ELISA kit to detect circulating IgG autoantibodies against desmoglein 1, desmoglein 3, BP180, BP230, and type VII collagen, both simultaneously and more rapidly than previous assays. *Objectives:* The aim of this study was to evaluate the diagnostic accuracy of this kit for the diagnosis of pemphigus foliaceus, pemphigus vulgaris, bullous pemphigoid and epidermolysis bullosa acquisita. *Materials & Methods:* Dual-centre retrospective study in which 138 patients with autoimmune blistering diseases were compared to 40 controls *Results:* Using the MESACUP anti-Skin profile TEST, both sensitivities and specificities for desmoglein 1, desmoglein 3, BP180, BP230, and type VII collagen autoantibodies were similar to those obtained using previous, specific ELISA systems and 88% of the results were concordant without any significant difference. *Conclusion:* The MESACUP anti-Skin profile TEST had a similar performance to previously produced ELISA systems. The novel kit can be used for rapid diagnosis of most common autoimmune blistering diseases and is especially suitable for identifying overlapping disorders.

Key words: bullous pemphigoid, diagnostics, ELISA, epidermolysis bullosa acquisita, pemphigus foliaceus, pemphigus vulgaris

The pemphigus and pemphigoid groups of autoimmune blistering skin diseases form a heterogeneous group in which specific autoantibodies attack certain adhesion proteins within the connecting structures of keratinocytes. Several methods can be used to detect these autoantibodies for diagnostic purposes. *In vivo,* bound autoantibodies can be detected by direct immunofluorescence (DIF) microscopy in a perilesional biopsy specimen [1]. Indirect immunofluorescence (IIF) is also commonly used, although the evaluation is subjective and the standardization is difficult. Enzyme-linked immunosorbent assay (ELISA) is a highly specific serological diagnostic tool, which can be performed in an objective, quantitative, standardized and automated way [2].

The MBL Company (Medical & Biological Laboratories Co., Ltd., Nagoya, Japan) has produced several ELISA kits for the routine diagnostic detection of autoantibodies in blistering skin diseases. All kits use human recombinant autoantigens. The BP180 ELISA is coated with the NC16A domain [3]. Another kit is in use for detection of autoantibodies directed against the N- and C-terminal parts of BP230 [4]. There are two other plates coated with desmoglein 1 (dsg1) and desmoglein 3 (dsg3) to detect pemphigus autoantibodies [5]. To diagnose epidermolysis bullosa acquisita (EBA), autoantibodies against type VII collagen (col7) are assayed using the NC1 and NC2 domains in the same ELISA plate [6-8].

In a number of clinical situations, quick and precise diagnostics of autoimmune blistering diseases is warranted because of the need for immediate adequate treatment. For this purpose, simultaneous performance of all ELISAs would be ideal. The use of several ELISA kits at the same time is however laborious and expensive. Recently, an IIF test based on the BIOCHIP® was established, allowing the simultaneous analysis of autoantibodies against dsg1, dsg3, BP180 NC16A, BP230, as well as salt-split human skin and monkey oesophagus [9]. However, in this test system, quantification of serum autoantibodies is difficult. To overcome these shortcomings, a new ELISA was created by MBL for the more rapid detection of IgG antibodies to dsg1, dsg3, BP180, BP230, and col7, the "MESACUP anti-Skin profile TEST" (ASPT). The autoantibodies can be detected

Abbreviations:

Ab	antibody
ASPT	MESACUP anti-Skin profile TEST
BP	bullous pemphigoid
95% CIs	95% confidence intervals
col7	type VII collagen
dsg1	desmoglein 1
dsg3	desmoglein 3
DIF	direct immunofluorescence
EBA	epidermolysis bullosa acquisita
IIF	indirect immunofluorescence
MBL	Medical & Biological Laboratories Co., Ltd., Nagoya, Japan
PF	pemphigus foliaceus
PV	pemphigus vulgaris

doi:10.1684/ejd.2015.2692

To cite this article: Horváth ON, Varga R, Kaneda M, Schmidt E, Ruzicka T, Sárdy M. Diagnostic performance of the "MESACUP anti-Skin profile TEST". *Eur J Dermatol* 2016; 26(1): 56-63 doi:10.1684/ejd.2015.2692

simultaneously in a single assay, because the wells are separately coated with the five different recombinant antigens. According to the instructions given by the manufacturer, the incubation times are half those of the previous ELISA systems and one single serum sample can be tested economically [10]. Our goal was to reveal the performance of this new kit in a clinical setting.

Methods

We conducted a retrospective analysis of serum samples from 178 patients. 120 patients were diagnosed with autoimmune blistering diseases between November 2008 and April 2014 at the Department of Dermatology and Allergology, Ludwig Maximilian University, Munich, Germany. Furthermore, 17 EBA serum samples were included from the University of Lübeck, Germany and 1 from the Philipps University Marburg, Germany. In every case the diagnosis was based on the compatible clinical picture, traditional histology and confirmatory results in at least two of three distinct diagnostic methods, including DIF microscopy, IIF microscopy (on monkey oesophagus, rabbit oesophagus and salt-split skin) and specific ELISAs (BP180, BP230, dsg1, dsg3, or col7, as appropriate). Each of these ELISA systems was used according to the manufacturer's (MBL) instructions. The cut-off value was chosen as determined by MBL. It was 6 U/mL for the col7 ELISA test, and 9 U/mL for the BP180 and BP230 tests. For desmogleins, a grey zone was defined between 14 and 20 U/mL for dsg1, as well as between 7 and 20 U/mL for dsg3; results in the grey zone were regarded as negative in this study. All sera having false positivity or negativity were double or triple checked. DIF and IIF microscopy were performed using standard laboratory methods [11]. DIF was performed in 54% of the patients; IIF microscopy and a specific ELISA test were performed in every case as part of the routine diagnostics, so we could compare the previous data with the data of the ASPT. We will refer to the distinct MBL ELISAs performed as routine diagnostics on our sera as 'previous ELISAs' throughout this article, because these assays were performed before the ASPT was done. The three specific diagnostic methods, i.e. the DIF microscopy, the IIF microscopy, and the ELISAs have a high specificity of >90%; therefore, positivity of a sample in two of these assays had a positive predictive value of >99%. This means that a wrong diagnosis may have occurred in 0-2 of 138 patients, which does not significantly change the results of the study.

The ASPT was also performed according to the instructions of MBL. The cut-off was 15 U/mL for every sub assay in the ASPT [10]. A total of 25 PF, 40 PV, 52 BP, 21 EBA, and 40 control serum samples were tested in the study.

All the sera from patients having PF, PV or EBA, obtained from patients examined in the study period at the Department of Dermatology in Munich, were included. In addition, some EBA serum samples were selected randomly from serum pools of the other study centres to increase the number of samples. The number of BP sera had to be reduced, thus from 313 available BP sera which had been pre-tested using individual ELISA kits (MBL) for detection of BP180 and BP230 autoantibodies, 52 serum samples were selected so that the specificities and sensitivities of the results approximately corresponded to those obtained in a previous study performed on these 313 sera [11], but we also deliberately included some samples with borderline results so that differences in the performance were better detected. Control sera were chosen randomly from patients in whom bullous autoimmune diseases had been clearly excluded.

Mann-Whitney's nonparametric, unpaired, two-tailed test was performed for statistical comparison of autoantibody titres. Sensitivities and specificities were calculated with 95% CIs. For comparison of (positive and negative) test results, Fisher's exact test was performed. GraphPad Prism version 4.03 for Windows, GraphPad Software, San Diego, California, USA was used for calculations, SPSS version 21.0, SPSS Inc., Chicago, IL, USA for ROC analysis.

This type of retrospective, non-interventional study on sera does not require ethical approval in Germany.

Results

The ASPT could be performed significantly more rapidly than previous ELISA systems, because the incubation times were only 50% of those required in previous specific MBL kits, and all five tests could be performed simultaneously in one single strip of assay. Thus the time needed between pipetting the serum into the wells until the printing of the results was approximately 1.8 hours. The ASPT could be performed economically even with one single serum as each strip of assay contained eight ELISA wells; five for the autoantigens and three for positive and negative controls. Therefore, one strip of assay was used for each single serum sample, and any number of strips could be used at the same time.

Comparison of data obtained from the distinct, previously performed MBL ELISAs with those from the ASPT showed that results from the ASPT were concordant with those from previous ELISAs in 88.2%. Sensitivity and specificity values of the ASPT are illustrated in *figure 1*. The autoantibody

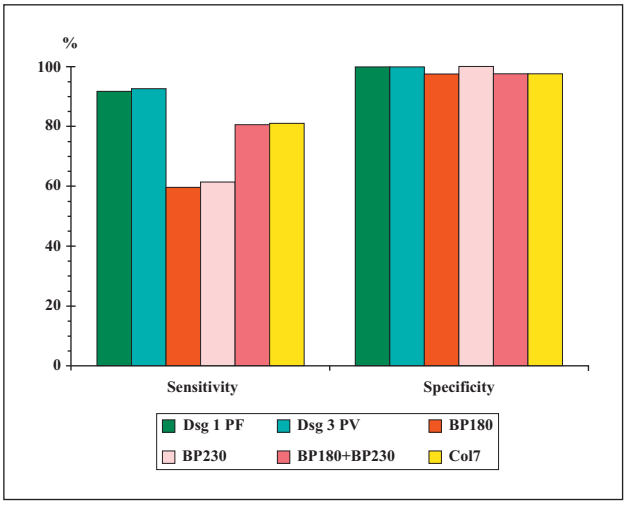

Figure 1. Sensitivity and specificity values of the "MESACUP anti-Skin profile TEST". BP, bullous pemphigoid; Col7, type VII collagen; dsg1, desmoglein 1; dsg3, desmoglein 3; EBA, epidermolysis bullosa acquisita; PF, pemphigus foliaceus; PV, pemphigus vulgaris.

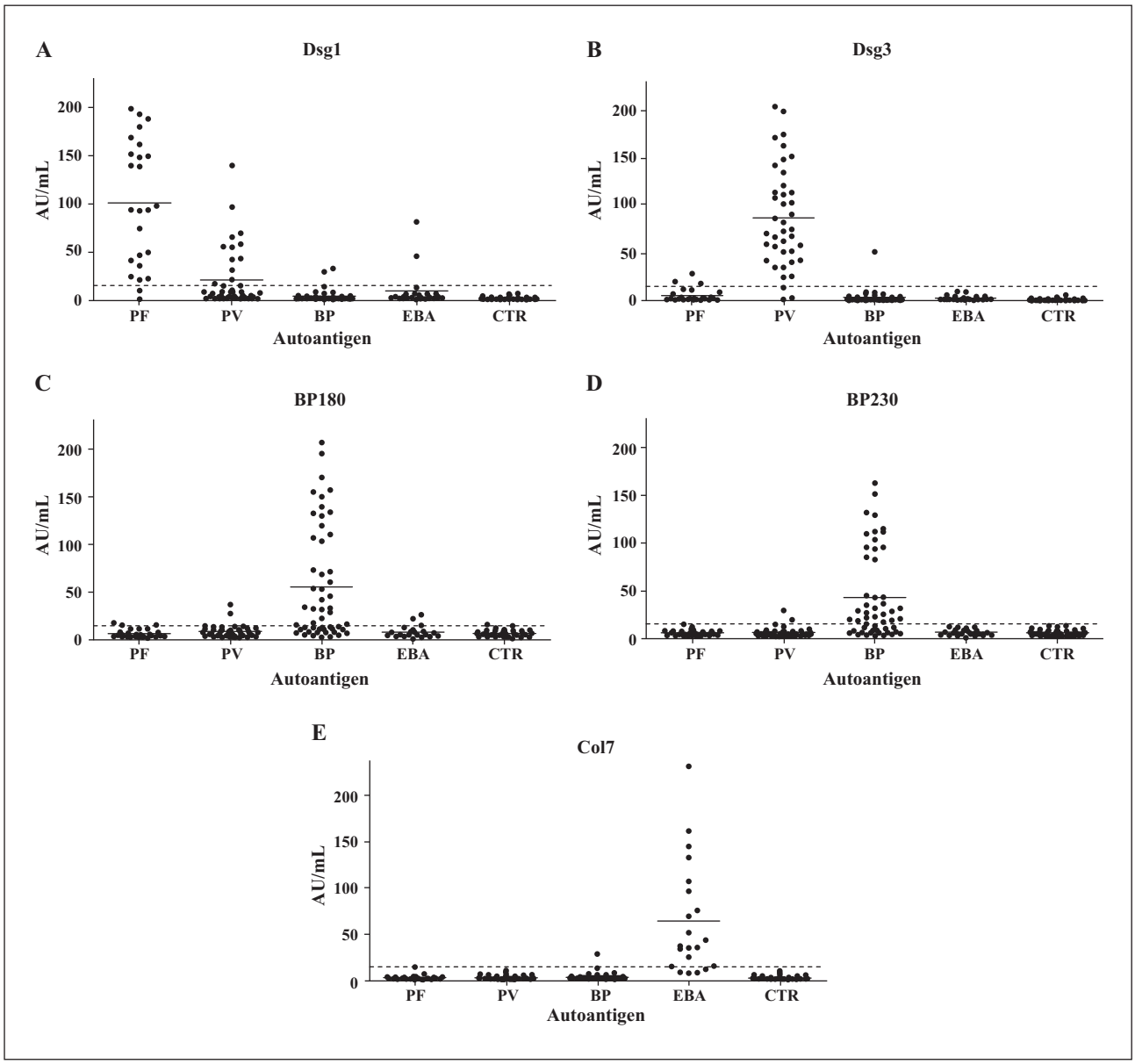

Figure 2. Dot diagrams showing dsg1, dsg3, BP180, BP230 and col7 autoantibody levels in PF, PV, BP, EBA and control sera. The cut-off line is drawn with a dashed line as recommended by the manufacturer (15 AU/mL in all assays in the ASPT). CTR, control sera. Other abbreviations see *figure 1*.

titres in sera from PF, PV, BP, EBA patients and controls are shown in *figure 2*. The results were significantly different between each disease compared to the controls. The results of the ASPT and the results obtained from previous ELISAs did not differ significantly when comparing dsg1, dsg3, BP180, BP230, and col7 tests (P = 0.49, 0.62, 1.0, 0.32, and 0.34, respectively).

The specificities of ASPT were 100% for dsg1 in PF, dsg3 in PV, BP230 in BP, and col7 in EBA. The specificity of ASPT for BP180 in BP was 97.5%. There were two PF sera with false positive BP180 values in the ASPT, but these results could be not reproduced in either a repeated ASPT or a BP180 ELISA. Specificities were not calculated for our previous ELISAs because of no scientific relevance, since literature data shows a nearly 100% specificity value *(tables 2-6).*

In the ASPT, the sensitivities were 92.5% for dsg3 in PV, 92% for dsg1 in PF, 59.62% for BP180 and 61.54% for BP230 antibodies, and 80.95% for col7 in EBA. Using the previous MBL ELISAs, the sensitivities for dsg1, dsg3, BP180, BP230 and col7 ELISAs were 100%, 97.5%, 59.62%, 50.0% and 95.24%, respectively. The sensitivities of BP180 and BP230 tests evaluated simultaneously in the ASPT and in previous MBL ELISAs were 80.77% and 75%, respectively (specificities: 97.5% and 100%, respectively). The performance of the ASPT could be further optimised by ROC curve analysis. Data are shown in *figure 3*. The sensitivity for dsg1 antibodies in PF could be increased from 92% to 96% by decreasing the cut-off from 15 to 8.8 U/mL, while the specificity remained 100%. The area under the curve (AUC) was 0.962 (95%CI, 0.889-1.0). The dsg3 test performed even better than the dsg1. Keeping

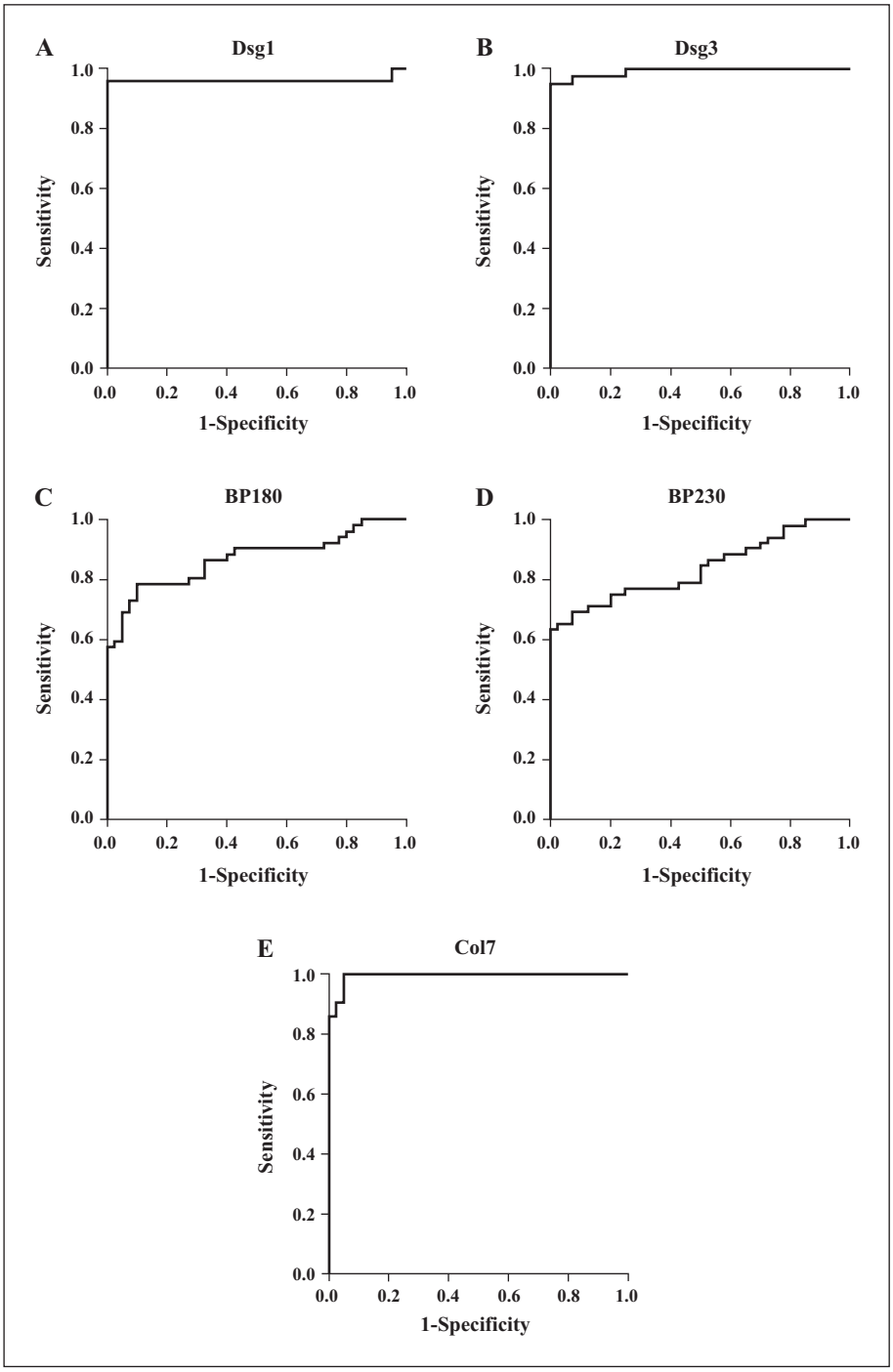

Figure 3. ROC analysis of dsg1, dsg3, BP180, BP230 and col7 values. Abbreviations: see *figure 1*.

100% specificity, the sensitivity could be increased up to 95% by decreasing the cut-off value from 15 to 10.2 U/mL; the AUC was 0.992 (95% CI, 0.978-1.0). The cut-off value of 15 U/mL was optimal for both the BP180 and the BP230 tests in the ASPT. The AUC values for BP180 and BP230 antibodies were 0.870 (95% CI, 0.796-0.943) and 0.838 (95% CI, 0.756-0.919), respectively. The performance of col7 ASPT could also be improved by setting the cut-off to 11.6 U/mL, which resulted in an increase of sensitivity to 85.7% without affecting the specificity; the AUC was 0.994 (95% CI, 0.983-1.0). By decreasing the cut-off further to 9 U/mL, the sensitivity could be increased to 90.5% (specificity: 97.5%).

The specificities given above were calculated in relation to the control sera. However, some sera from patients having bullous autoimmune disease showed false positivity in a number of the ASPT assays *(Table 1)*. In PF, BP180 was falsely positive in three cases; nevertheless, all of them were borderline positive (15.2, 15.4, 17.8 U/mL), and both the IIF and the clinical picture were specific for PF. Three PV patients' sera were falsely positive: for BP180 alone, BP230 alone, and both BP180 and BP230, respectively.

Table 1. The number of false positive results with each individual test in the "MESACUP anti-Skin profile TEST". BP, bullous pemphigoid; Col7, type VII collagen; dsg1, desmoglein 1; dsg3, desmoglein 3; EBA, epidermolysis bullosa acquisita; PF, pemphigus foliaceus; PV, pemphigus vulgaris.

Diagnosis (n)	dsg1 (%)	dsg3 (%)	BP180 (%)	BP230 (%)	col7 (%)
PF (25)	-	-	3 (12)	0	0
PV (40)	-	-	2 (5)	2 (5)	0
BP (52)	2 (3.85)	1 (1.92)	-	-	1 (1.92)
EBA (21)	2 (9.52)	0	3 (14.29)	0	-
Controls (40)	0	0	1	0	0

Table 2. Sensitivity and specificity values in the literature for desmoglein 1 ELISAs in pemphigus foliaceus.

Author	n	Sensitivity (%)	Specificity (%)	Manufacturer
Amagai *et al.* [12]	48	97.9	98.9	MBL
Huang *et al.* [13]	9	100	97.4	MBL
Schmidt *et al.* [14]	50	96	99.1	home-made
Schmidt *et al.* [14]	50	100	95.7	MBL
Ishii *et al.* [5]	49	96	96	home-made

In all ELISA systems, the recombinant purified ectodomain of desmoglein 1 was used.

Table 3. Sensitivity and specificity values in the literature for desmoglein 3 ELISAs in pemphigus vulgaris.

Author	n	Sensitivity (%)	Specificity (%)	Manufacturer
Amagai *et al.* [12]	81	97.5	97.8	MBL
Daneshpazhooh *et al.* [15]	73	94.5	-	MBL
Hallaji *et al.* [16]	50	94	-	MBL
Huang *et al.* [13]	20	85.0	99.1	MBL
Ishii *et al.* [5]	46	94	96	home-made
Schmidt *et al.* [14]	71	100.0	99.6	home-made
Schmidt *et al.* [14]	71	100.0	95.7	MBL
Sharma *et al.* [17]	27	85.2	100.0	MBL

In all ELISA systems, the recombinant purified ectodomain of desmoglein 3 was used.

According to the clinical picture, the DIF and the IIF microscopy, two of the three cases had only PV without any sign for an overlap syndrome. The third case with BP180 and BP230 positivity had only PV, diagnosed four years previously. The diagnosis was confirmed with IIF, dsg3 ELISA, DIF and histology, so the sample was selected for examination with the ASPT as a PV follow-up serum. With the ASPT, both desmoglein results remained negative, but the BP180 and BP230 were clearly positive (with titres around 30 U/mL), which was also confirmed by the previous ELISA kits. The patient had an oral relapse under immunosuppressive treatment at the time of sample taking despite negative desmoglein ELISAs. Additionally, the serum was analysed by immunoblot, confirming reactivity with both BP180 and BP230. Without the ASPT, this epitope spreading would not have been recognised.

There were four false-positive sera in patients having BP; two for dsg1, one for dsg3 and one for col7. However, there were no other clinical or laboratory signs for overlap syndromes. The patient's serum that was falsely positive for col7 in the ASPT was repeatedly negative using the previous col7 ELISA (28.5 U/mL versus 2.38 and 2.44 U/mL, respectively), without any signs for an EBA. Two EBA sera were falsely positive for dsg1 and three for BP180; however, we had only limited access to the clinical data, thus we were unable to verify the possibility of overlap syndromes. Among the negative controls, one borderline false-positive case of BP180 was detected (16.0 U/mL with the ASPT) which was negative using the previous ELISA or IIF.

Discussion

The sensitivities and specificities of different desmoglein ELISA tests varied in diverse studies, even when the same kit of the same manufacturer was used [12-15]. Literature data for sensitivities and specificities of dsg1 ELISA for PF lie between 96%-100% and 95.7%-99.1%, respectively. The numbers are similar for PV, the sensitivities and the specificities of dsg3 ELISAs vary in the range of 85.2%-100% (in most studies, however, 94%-100%) and 95.7%-100%, respectively *(tables 2, 3)*.

All dsg1 and dsg3 kits have outstanding performances in clinical practice, including the ASPT. Our results did not differ significantly from the sensitivity and specificity

Table 4. Sensitivity and specificity values in the literature for BP180 ELISAs.

Author	n	Sensitivity (%)	Specificity (%)	Manufacturer
Barnadas *et al.* [18]	24	92.0	96.0	MBL
Charneux *et al.* [19]	138	86.0	-	MBL
Kobayashi *et al.* [3]	64	84.4	98.9	home-made
Damoiseaux *et al.* [20]	60	80.0	98.0	MBL
Damoiseaux *et al.* [20]	60	86.7	93.0	EUROIMMUN
Hofmann *et al.* [21]	116	93.1	98.0	home-made
Mariotti *et al.* [22]	78	82.0	100.0	home-made
Roussel *et al.* [23]	190	79.0	90.0	MBL
Sakuma-Oyama *et al.* [24]	102	89.0	98.0	MBL
Sárdy *et al.* [11]	313	72.0	94.1	MBL
Sitaru *et al.* [25]	118	89.8	97.8	EUROIMMUN
Sitaru *et al.* [25]	118	89.0	94.8	MBL
Tampoia *et al.* [26]	20	90.0	98.8	MBL
Thoma-Uszynski *et al.* [27]	127	95.3	94.0	home-made
Yoshida *et al.* [28]	239	69.9	98.8	MBL
Zillikens *et al.* [29]	50	94.0	99.9	home-made

Used antigens: MBL, Kobayashi et al., [12] Zillikens et al., [29] recombinant, purified NC16A domain of BP180 protein. Euroimmun: tetramer of the recombinant NC16A domain. Hofmann et al., [21] recombinant NC16A domain and the collagenous Col15 domain+NC3-NC1 domains of BP180 protein. Mariotti et al., [22] NC16A domain+the mid-portion and the C-terminus of BP180. Thoma-Uszynski et al., [27] entire extracellular domain of BP180.

Table 5. Sensitivity and specificity values in the literature for BP230 ELISAs.

Author	n	Sensitivity (%)	Specificity (%)	Manufacturer
Blöcker *et al.* [30]	118	56.8	97.6	EUROIMMUN
Charneux *et al.* [19]	138	59.0	-	MBL
Damoiseaux *et al.* [20]	60	58.3	93.0	MBL
Damoiseaux *et al.* [20]	60	60.0	82.5	EUROIMMUN
Roussel *et al.* [23]	190	61.0	96.0	MBL
Sárdy *et al.* [11]	313	59.0	99.2	MBL
Tampoia *et al.* [26]	20	60.0	98.8	MBL
Thoma-Uszynski *et al.* [27]	127	81.5	64.8	home-made
Yoshida *et al.* [28]	239	72.4	99.5	home-made

Used antigens: MBL, Yoshida et al.,. [28] recombinant N-terminal and C-terminal domains of BP230. Thoma-Uszynski et al.,. [27] one N-terminal and two C-terminal constructs of BP230.

Table 6. Sensitivity and specificity values in the literature for type VII collagen ELISAs.

Author	n	Sensitivity (%)	Specificity (%)	Manufacturer
Chen *et al.* [32]	24	100	100	home-made
Kim *et al.* [33]	30	96.7	98.1	MBL
Komorowski *et al.* [34]	73	94.5	98.7	home-made
Marzano *et al.* [35]	14	86	98.6	MBL
Saleh *et al.* [8]	49	93.8	98.1	MBL
Terra *et al.* [36]	28	54	97.8	MBL

Used antigens: Chen et al.,. [32] and Komorowski et al.,. [34] entire NC1 domain of human collagen VII.

values of the specific previous kits. A few BP sera showed false positive results for desmogleins in the ASPT. This observation was previously reported about specific ELISA kits 15 years ago [5, 12-14]. Although we cannot completely exclude the possibility of overlap syndromes, the diagnosis remained BP in each case based on DIF microscopy and clinical data.

Specificity and sensitivity values for BP180 and BP230 are shown in supplementary *Tables 3 and 4*. Since the first BP180 ELISA, the kits have been through a huge

development. The sensitivity was only 53% with 100% specificity in 1994 [31], but in later studies, 69.9% sensitivity was the worst result [28]. In our study, the sensitivities were lower than in previous studies because we selected sera with a special focus on borderline results to challenge the diagnostic performance of the ASPT. This means sensitivities for MBL BP180 and BP230 ELISAs in our previous study (n = 313) were 72% and 59% respectively [11]; those using the current selected sera (n = 52) were 60% and 50% respectively (with 100% specificity). The sensitivity for BP180 using the ASPT was similar to the previous tests but the sensitivity of BP230 in the ASPT was notably better, the difference being 11.5%. In addition, when the BP180 and BP230 results were evaluated together, the ASPT had a higher sensitivity in comparison with the previous MBL ELISAs (81% *versus* 75%).

Chen *et al*. [32] developed the first col7 ELISA, which was a sensitive and specific in-house assay *(table 6)*. Our ASPT assay had 81% sensitivity and 100% specificity. The sensitivity values are very variable in the literature; the data are limited by the low number of studies and included patients *(table 6)*. Recently, an IIF assay was established based on the recombinant NC1 domain of col7 expressed on the cell surface of HEK293 cells revealing a sensitivity and specificity of 94.5% and 98.7%, respectively [34].

Our study was somewhat limited by its retrospective nature and the possible bias when selecting sera. However, we could evaluate the ASPT in a real clinical setting by comparing its performance to our previous results. Determination of precise sensitivity and specificity was not our primary goal because many studies of this kind have already been performed. We primarily intended to compare the diagnostic performance (especially in borderline cases of BP) and the laboratory usability.

In conclusion, the ASPT has a similar clinical performance to each previous, specific ELISA alone, so it is suitable for routine diagnostics. The BP230 test of the ASPT has been considerably improved compared to the previous BP230 ELISA. The incubation time of the ASPT is just half that of the previous specific tests and the setup of the assay makes the testing of one single serum sample economic. In addition, the ASPT is an excellent tool to examine problematic sera, overlap syndromes, and it can also indicate the appearance of epitope spreading during follow-up. ■

Disclosure. *Acknowledgments. We are grateful to Mr. Ivica Anić, Ms. Andrea Hess, and Ms. Karin Woischwill (Department of Dermatology and Allergology, Ludwig Maximilian University, Munich, Germany) for technical assistance. We thank Dr. Rüdiger Eming and Dr. Melika Behzad (Department of Dermatology and Allergology, Philipps University, Marburg, Germany) for providing one EBA serum sample. We are indebted to Dr. Rupa Sivakumaran for linguistic review of the manuscript.*

Financial support: MBL Co., donation of the ASPT kits.

Conflicts of interest: Dr. Makoto Kaneda is a global program manager of the Medical and Biological Laboratories Company, Ltd., Nagano, Japan (MBL); however, he did not have any influence on the selection of sera or the evaluation of results. Dr. Miklós Sárdy was recipient of a registration fee for the ESDR 2014 conference donated by MBL.

References

1. Schmidt E, Zillikens D. Modern diagnosis of autoimmune blistering skin diseases. *Autoimmun Rev* 2010; 10: 84-9.

2. Tampoia M, Giavarina D, Di Giorgio C, *et al*. Diagnostic accuracy of enzyme-linked immunosorbent assays (ELISA) to detect anti-skin autoantibodies in autoimmune blistering skin diseases: a systematic review and meta-analysis. *Autoimmun Rev* 2012; 12: 121-6.

3. Kobayashi M, Amagai M, Kuroda-Kinoshita K, *et al*. BP180 ELISA using bacterial recombinant NC16a protein as a diagnostic and monitoring tool for bullous pemphigoid. *J Dermatol Sci* 2002; 30: 224-32.

4. Tampoia M, Lattanzi V, Zucano A, *et al*. Evaluation of a new ELISA assay for detection of BP230 autoantibodies in bullous pemphigoid. *Ann N Y Acad Sci* 2009; 1173: 15-20.

5. Ishii K, Amagai M, Hall RP, *et al*. Characterization of autoantibodies in pemphigus using antigen-specific enzyme-linked immunosorbent assays with baculovirus-expressed recombinant desmogleins. *J Immunol* 1997; 159: 2010-7.

6. Chen M, Chan LS, Cai X, *et al*. Development of an ELISA for rapid detection of anti-type VII collagen autoantibodies in epidermolysis bullosa acquisita. *J Invest Dermatol* 1997; 108: 68-72.

7. Chen M, Keene DR, Costa FK, *et al*. The carboxyl terminus of type VII collagen mediates antiparallel dimer formation and constitutes a new antigenic epitope for epidermolysis bullosa acquisita autoantibodies. *J Biol Chem* 2001; 276: 21649-55.

8. Saleh MA, Ishii K, Kim YJ, *et al*. Development of NC1 and NC2 domains of type VII collagen ELISA for the diagnosis and analysis of the time course of epidermolysis bullosa acquisita patients. *J Dermatol Sci* 2011; 62: 169-75.

9. van Beek N, Rentzsch K, Probst C, *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. *Orphanet J Rare Dis* 2012; 7: 49.

10. http://www.mblintl.com/products/datasheets/7115EC-D_anti-Skin_profile.pdf. (accessed on September 3, 2014).

11. Sárdy M, Kostaki D, Varga R, *et al*. Comparative study of direct and indirect immunofluorescence and of bullous pemphigoid 180 and 230 enzyme-linked immunosorbent assays for diagnosis of bullous pemphigoid. *J Am Acad Dermatol* 2013; 69: 748-53.

12. Amagai M, Komai A, Hashimoto T, *et al*. Usefulness of enzyme-linked immunosorbent assay using recombinant desmogleins 1 and 3 for serodiagnosis of pemphigus. *Br J Dermatol* 1999; 140: 351-7.

13. Huang CH, Chen CC, Wang CJ, *et al*. Using desmoglein 1 and 3 enzyme-linked immunosorbent assay as an adjunct diagnostic tool for pemphigus. *J Chin Med Assoc* 2007; 70: 65-70.

14. Schmidt E, Dähnrich C, Rosemann A, *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* 2010; 19: 458-63.

15. Daneshpazhooh M, Chams-Davatchi C, Khamesipour A, Mansoori P, Taheri A, Firooz A, Mortazavi H, Esmaili N, *et al*. Desmoglein 1 and 3 enzyme-linked immunosorbent assay in Iranian patients with pemphigus vulgaris: correlation with phenotype, severity, and disease activity. *J Eur Acad Dermatol Venereol* 2007; 21: 1319-24.

16. Hallaji Z, Mortazavi H, Lajevardi V, *et al*. Serum and salivary desmoglein 1 and 3 enzyme-linked immunosorbent assay in pemphigus vulgaris: correlation with phenotype and severity. *J Eur Acad Dermatol Venereol* 2010; 24: 275-80.

17. Sharma VK, Prasad HR, Khandpur S, *et al*. Evaluation of desmoglein enzyme-linked immunosorbent assay (ELISA) in Indian patients with pemphigus vulgaris. *Int J Dermatol* 2006; 45: 518-22.

18. Barnadas MA, Rubiales MV, González MJ, *et al*. Enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence testing in a bullous pemphigoid and pemphigoid gestationis. *Int J Dermatol* 2008; 47: 1245-9.

19. Charneux J, Lorin J, Vitry F, *et al*. Usefulness of BP230 and BP180-NC16a enzyme-linked immunosorbent assays in the initial diagnosis

of bullous pemphigoid: a retrospective study of 138 patients. *Arch Dermatol* 2011; 147: 286-91.

20. Damoiseaux J, van Rijsingen M, Warnemünde N, *et al*. Autoantibody detection in bullous pemphigoid: clinical evaluation of the EUROPLUS™ Dermatology Mosaic. *J Immunol Methods* 2012; 382: 76-80.

21. Hofmann S, Thoma-Uszynski S, Hunziker T, *et al*. Severity and phenotype of bullous pemphigoid relate to autoantibody profile against the NH2- and COOH-terminal regions of the BP180 ectodomain. *J Invest Dermatol* 2002; 119: 1065-73.

22. Mariotti F, Grosso F, Terracina M, *et al*. Development of a novel ELISA system for detection of anti-BP180 IgG and characterization of autoantibody profile in bullous pemphigoid patients. *Br J Dermatol* 2004; 151: 1004-10.

23. Roussel A, Benichou J, Randriamanantany ZA, *et al*. Enzyme-linked immunosorbent assay for the combination of bullous pemphigoid antigens 1 and 2 in the diagnosis of bullous pemphigoid. *Arch Dermatol* 2011; 147: 293-8.

24. Sakuma-Oyama Y, Powell AM, Oyama N, *et al*. Evaluation of a BP180-NC16a enzyme-linked immunosorbent assay in the initial diagnosis of bullous pemphigoid. *Br J Dermatol* 2004; 151: 126-31.

25. Sitaru C, Dähnrich C, Probst 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* 2007; 16: 770-7.

26. Tampoia M, Lattanzi V, Zucano A, *et al*. Evaluation of a new ELISA assay for detection of BP230 autoantibodies in bullous pemphigoid. *Ann N Y Acad Sci* 2009; 1173: 15-20.

27. Thoma-Uszynski S, Uter W, Schwietzke S, *et al*. BP230- and BP180-specific auto-antibodies in bullous pemphigoid. *J Invest Dermatol* 2004; 122: 1413-22.

28. Yoshida M, Hamada T, Amagai M, *et al*. Enzyme-linked immunosorbent assay using bacterial recombinant proteins of human BP230 as a diagnostic tool for bullous pemphigoid. *J Dermatol Sci* 2006; 41: 21-30.

29. Zillikens D, Mascaro JM, Rose PA, *et al*. A highly sensitive enzyme-linked immunosorbent assay for the detection of circulating anti-BP180 autoantibodies in patients with bullous pemphigoid. *J Invest Dermatol* 1997; 109: 679-83.

30. Blöcker IM, Dähnrich C, Probst C, *et al*. Epitope mapping of BP230 leading to a novel enzyme-linked immunosorbent assay for autoantibodies in bullous pemphigoid. *Br J Dermatol* 2012; 166: 964-70.

31. Giudice GJ, Wilske KC, Anhalt GJ, *et al*. Development of an ELISA to detect anti-BP180 autoantibodies in bullous pemphigoid and herpes gestationis. *J Invest Dermatol* 1994; 102: 878-81.

32. Chen M, Chan LS, Cai X, *et al*. Development of an ELISA for rapid detection of anti-type VII collagen autoantibodies in epidermolysis bullosa acquisita. *J Invest Dermatol* 1997; 108: 68-72.

33. Kim JH, Kim YH, Kim S, *et al*. Serum levels of anti-type VII collagen antibodies detected by enzyme-linked immunosorbent assay in patients with epidermolysis bullosa acquisita are correlated with the severity of skin lesions. *J Eur Acad Dermatol Venereol* 2013; 27: e224-30.

34. Komorowski L, Müller R, Vorobyev A, *et al*. Sensitive and specific assays for routine serological diagnosis of epidermolysis bullosa acquisita. *J Am Acad Dermatol* 2013; 68: e89-95.

35. Marzano AV, Cozzani E, Fanoni D, *et al*. Diagnosis and disease severity assessment of epidermolysis bullosa acquisita by ELISA for anti-type VII collagen autoantibodies: an Italian multicentre study. *Br J Dermatol* 2013; 168: 80-4.

36. Terra JB, Jonkman MF, Diercks GF, *et al*. Low sensitivity of type VII collagen enzyme-linked immunosorbent assay in epidermolysis bullosa acquisita: serration pattern analysis on skin biopsy is required for diagnosis. *Br J Dermatol* 2013; 169: 164-7.