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Title:

Comparison of the EUROIMMUN Dermatology Profile ELISA to the novel BIOCHIP Mosaic 7 for the diagnosis of immunobullous skin disease

Date:

2021-08-01

Citation:

Simpson, K., Scardamaglia, L., Kok, Y., Vu, M., Kidd, D., Yap, T., Tran, Y. & Kern, J. S. (2021). Comparison of the EUROIMMUN Dermatology Profile ELISA to the novel BIOCHIP Mosaic 7 for the diagnosis of immunobullous skin disease. *Australasian Journal of Dermatology*, 62 (3), pp.314-322. <https://doi.org/10.1111/ajd.13611>.

Persistent Link:

<https://hdl.handle.net/11343/298591>

Informative title: Comparison of the EUROIMMUN Dermatology Profile ELISA to the novel BIOCHIP Mosaic 7 for the diagnosis of immunobullous skin disease

Short running title: BIOCHIP and ELISA immunobullous disease

Key Words: Indirect immunofluorescence, direct immunofluorescence, screening test, pemphigus, pemphigoid, skin blistering disease, Australian cohort

Word Count: 2999

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This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/AJD.13611](https://doi.org/10.1111/AJD.13611)

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Acknowledgements: Nil

Ethics approval statement:

This study was conducted according to Declaration of Helsinki principles and in accordance with local regulations (The Royal Melbourne Hospital HREC/17/MH/334).

Conflict of Interest Statement:

BIOCHIP Mosaic 7 tests were provided by EUROIMMUN, Lübeck, Germany, free of charge. EUROIMMUN had no role in the study design, data collection, data analysis, interpretation of data, writing of the manuscript or publication decisions

Funding statement:

The authors received no financial support for the research, authorship, and/or publication of this article.

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Article type : Original Research

Title: Comparison of the EUROIMMUN Dermatology Profile ELISA to the novel BIOCHIP Mosaic 7 in immunobullous disease

Abstract:

Background: The BIOCHIP is an indirect immunofluorescence diagnostic investigation which identifies multiple autoantibodies with a mosaic panel of target antigen-specific substrates in a single incubation field. The EUROIMMUN Dermatology Profile ELISA allows simultaneous investigation of the six most important autoantibodies in bullous autoimmune dermatoses. Evaluation of the BIOCHIP Mosaic 7, compared to that of the EUROIMMUN Dermatology Profile ELISA, when used as a diagnostic investigation in pemphigus and pemphigoid, was undertaken in an Australian cohort.

Methods: The serum of 27 patients was analysed including patients with pemphigus vulgaris (n=10), pemphigus foliaceus (n=4), bullous pemphigoid (n=8), mucous membrane pemphigoid (n=3) and negative controls (n=2). Results of the BIOCHIP were compared with the EUROIMMUN Dermatology Profile ELISA, as well as with histology, direct immunofluorescence and indirect immunofluorescence.

Results: In pemphigus vulgaris, sensitivity & specificity for the BIOCHIP Mosaic 7 were 100% and 94.1%, comparable to that of the EUROIMMUN Dermatology Profile ELISA with 80% sensitivity and 100% specificity. In bullous pemphigoid, sensitivity of the BIOCHIP was 87.5% and sensitivity of the EUROIMMUN Dermatology ELISA profile was 75%, whilst specificities for both diagnostic methods were 100% in our limited cohort. There was substantial or

almost perfect concordance between the BIOCHIP Mosaic 7 and EUROIMMUN Dermatology Profile ELISA for pemphigus vulgaris and bullous pemphigoid.

Conclusion: The BIOCHIP Mosaic 7 is a rapid, reliable diagnostic investigation in pemphigus and bullous pemphigoid. Results indicate it is comparable to the EUROIMMUN Dermatology Profile ELISA, whilst also providing additional testing with salt split skin, on one field.

Word count: 249

Introduction

Immunobullous diseases are characterised by the production of autoantibodies against specific target antigens in the skin and mucous membranes.

In bullous pemphigoid the target are the hemidesmosomes that connect the basal layer of the epidermis to the dermis via the basal lamina (1). Specifically, the components of the hemidesmosomes targeted are BP180, a transmembrane protein, and or BP230, a cytoplasmic protein (2). When the connections of the hemidesmosomes are disrupted, this clinically presents as tense bullae (3) and histologically appears as subepidermal cleft formation with an eosinophilic infiltrate (1).

In pemphigus the desmosomes that form connections between the keratinocytes of the epidermis are targeted, desmoglein 1 (DSG1) and or desmoglein 3 (DSG3) (4).

Autoantibodies against DSG3 are characteristic of mucosal pemphigus vulgaris, whilst antibodies against both DSG1 and DSG3 are seen in mucocutaneous forms of the disease (4). In pemphigus foliaceus, the antibodies produced are against DSG1 only (4). Pemphigus vulgaris has mucous membrane involvement and may or may not have erosions and blisters of the skin, depending on the presence of DSG1 antibodies. In comparison pemphigus foliaceus classically presents with shallow erosions or flaccid bulla of the skin without mucosal involvement (4).

The diagnostic methods routinely available in Australia for autoimmune blistering diseases include histology of Formalin-Fixed Paraffin-Embedded patient skin tissue, direct

immunofluorescence of perilesional skin cryosections, indirect immunofluorescence on monkey oesophagus substrate, and enzyme-linked immunosorbent assay (ELISA). Direct immunofluorescence on perilesional skin biopsy is considered the gold standard in diagnostic testing (5).

For autoimmune immunobullous testing, ELISA is available commercially as either EUROIMMUN or MBL kits. The EUROIMMUN Dermatology Profile ELISA (IgG) allows detection of the six most common autoantibodies in immunobullous conditions in one test run; BP180, BP230, Desmoglein 1, Desmoglein 3, envoplakin, collagen type VII. The EUROIMMUN ELISA provides a quantitative measurement of antibody levels for DSG1 and DSG3. Salt split skin is difficult to access within routine pathology services in Australia.

The BIOCHIP is a more recent indirect immunofluorescence diagnostic method for autoimmune blistering conditions (EUROIMMUN, Lübeck, Germany). One BIOCHIP is made up of several smaller mosaics, and includes a combination of tissue sections, transfected cell substrates and microdrops of highly purified antigen (6, 7). It provides the results of multiple individual tests on a single field.

Whilst studies on the validation of the BIOCHIP in different immunobullous conditions have been conducted (5, 8-12), to the best of our knowledge there is no literature evaluating the EUROIMMUN Dermatology Profile ELISA compared to the BIOCHIP Mosaic 7 in an Australian cohort when used as a diagnostic screening method.

Materials and Methods

Patients

The serum of 27 treatment naïve patients was collected who had been given a diagnosis of an autoimmune blistering condition based on standard of care testing outlined in consensus guidelines (13). Eight patients had a diagnosis of bullous pemphigoid. Ten and four patients had diagnoses of pemphigus vulgaris and pemphigus foliaceus respectively. Three patients had a diagnosis of mucous membrane pemphigoid. Two patients were initially suspected to have an immunobullous condition clinically, however, standard of care testing, EUROIMMUN Dermatology Profile ELISA, and BIOCHIP diagnostics were negative and on further review alternate diagnoses were made. Consent was obtained from patients prior to serum collection and all samples were de-identified. Scientists and pathologists who processed and interpreted the EUROIMMUN Dermatology Profile ELISA or the BIOCHIP Mosaic 7 were blinded with regards to patient identifying details and suspected clinical diagnosis. This study was conducted according to Declaration of Helsinki principles and in accordance with local regulations (The Royal Melbourne Hospital HREC/17/MH/334).

BIOCHIP Mosaic 7

Serum from all patients was tested at The Royal Melbourne Hospital Pathology Department using the BIOCHIP Mosaic 7 (EUROIMMUN) provided by EUROIMMUN, Lübeck, Germany, according to the manufacturer's instructions. On the BIOCHIP Mosaic 7 the substrates include tissue section of monkey oesophagus, salt split skin, human embryonic kidney (HEK293) cells transfected with DSG1 protein ectodomain, HEK293 cells transfected with DSG3 protein ectodomain, microdrops of BP180 free antigen and HEK293 cells transfected with C-terminal globular domain of the BP230 domain (6).

The substrates were coated on thin glass slides and then attached to microscopy slides (11). As per manufacturer instruction, in the first step the substrates were incubated with diluted 1:10 patient serum samples. In the second incubation step, the antibodies were stained with fluorescein-labelled anti-human antibodies. Results were then evaluated by fluorescence microscopy (figure 1, 2).

For the purposes of this study a diagnosis of pemphigus vulgaris was given based on the BIOCHIP when there was reactivity with DSG3, or both DSG3 and DSG1, and intercellular substance staining on monkey oesophagus was visualised. A diagnosis of pemphigus foliaceus was made when there was reactivity of DSG1, no reactivity of DSG3, and intercellular substance staining with monkey oesophagus. Bullous pemphigoid was diagnosed based on basement membrane zone staining on monkey oesophagus and or salt split skin reactivity on the epidermal roof side and or reactivity with BP180 and or BP230.

EUROIMMUN Dermatology Profile ELISA

All EUROIMMUN Dermatology profile ELISA samples were processed by St Vincent's Pathology laboratory, Victoria. Testing was undertaken as per manufacturer instructions. This study defined pemphigus vulgaris when there was reactivity with DSG3, or both DSG3 and DSG1. A diagnosis of pemphigus foliaceus was made when there was reactivity with

DSG1, and no reactivity with DSG3. Reactivity with either BP180 or BP230 was considered supportive of a diagnosis of bullous pemphigoid.

Standard of care testing

In this study standard of care testing referred to histology, direct immunofluorescence and indirect immunofluorescence on monkey oesophagus, based on the wider availability of these tests in Australia and consensus diagnosis guidelines (13). Participants who did not previously have any element of standard of care testing completed, had these tests performed from serum collected for BIOCHIP testing. EUROIMMUN ELISA quantitative testing for DSG1 and DSG3 (Immunology Laboratory at the Institute for Clinical Pathology and Medical Research (ICPMR), Westmead Hospital, NSW Health Pathology) was also performed where clinically relevant.

Statistical analysis

Statistical analysis was performed using Minitab and MedCalc statistical software. The cohort was analysed as four datasets of 27 participants with the reference true positive determined as per diagnosis based on clinical presentation and overall interpretation of standard of care testing. For example, for pemphigus vulgaris, there were 10 positive, and 17 negative cases. Sensitivity and specificity for each diagnostic method in the different conditions were analysed with 95% confidence intervals. As no control group was available, specificity was calculated from the ability of the test to identify the remainder of the cohort from the disease of interest as negative. Cohen's Kappa (k) was calculated for concordance between the EUROIMMUN Dermatology Profile ELISA and the BIOCHIP Mosaic 7 for each condition, as well as compared to the diagnosis reached by standard of care methods. There was considered to be almost perfect concordance when Cohen's kappa was >0.81 , substantial concordance when between $0.61 - 0.80$, and moderate concordance when the value was $0.41 - 0.60$ (14). $P < 0.05$ was considered statistically significant.

Results

Pemphigus vulgaris

Eight out of ten patients with a diagnosis of pemphigus vulgaris were correctly identified using the EUROIMMUN Dermatology Profile ELISA with a positive DSG3 result. Of these patients, six were also positive for DSG1, consistent with mucocutaneous pemphigus vulgaris. In comparison, ten out of ten patients were correctly diagnosed with pemphigus vulgaris using the BIOCHIP Mosaic 7 (table 1). Patient 4, whose diagnosis of pemphigus vulgaris was not identified by the EUROIMMUN Dermatology profile, with a lack of positive DSG3, had positive intercellular substance staining and DSG3 on BIOCHIP testing (table 1). Patient 8 had positive DSG1 on EUROIMMUN Dermatology profile testing only, consistent with pemphigus foliaceus, whilst both DSG1 and DSG3 were identified on the BIOCHIP (table 1). Sensitivity was 80% (95% CI 44.39-97.48) and 100% (95% CI 58.72-99.77) for the EUROIMMUN Dermatology Profile ELISA and BIOCHIP Mosaic 7 respectively (table 2). Specificity was 100% (95% CI 80.49-100.00) and 94.1% (95% CI 79.4-100.00) (table 2). Cohen's kappa was 0.75 between the EUROIMMUN Dermatology Profile ELISA and BIOCHIP Mosaic 7 for pemphigus vulgaris suggesting substantial concordance. Concordance between the EUROIMMUN Dermatology Profile ELISA and standard of care testing was 0.83, compared to 0.92 between the BIOCHIP and standard of care, both representing almost perfect concordance.

Pemphigus foliaceus

Of those with a diagnosis of pemphigus foliaceus, the EUROIMMUN Dermatology Profile ELISA results were consistent with this diagnosis in 4 out of 4 patients, with a DSG1 positive result only, resulting in a sensitivity of 100% (95%CI 28.36-99.49) (table 2). There were two patients who had diagnoses of pemphigus foliaceus based on clinical and histological findings as well as per the EUROIMMUN Dermatology profile ELISA and EUROIMMUN ELISA quantitative testing, in whom this diagnosis was not supported by the BIOCHIP (table 1) resulting in a sensitivity of 50% (95%CI 6.79-93.24) (table 2). This included patient 25 in whom both DSG1 and DSG 3 were positive on the BIOCHIP, more consistent with pemphigus vulgaris, and patient 11 in whom all aspects of the BIOCHIP were negative. The

EUROIMMUN Dermatology Profile ELISA and standard of care testing had almost perfect concordance for pemphigus foliaceus, with a cohen's kappa value of 0.86. The BIOCHIP Mosaic 7 and standard of care testing had only moderate concordance with a value of 0.63.

Bullous Pemphigoid

In bullous pemphigoid, the EUROIMMUN Dermatology Profile ELISA identified 6 out of 8 diagnoses, in comparison to the BIOCHIP which identified 7 out of 8 (table 1). This resulted in a sensitivity of 75% (95%CI 34.91-96.81) for the EUROIMMUN Dermatology profile ELISA and 87.5% (95%CI 47.35-99.68) for the BIOCHIP, with specificities of 100% (95%CI 34.91-96.81, 82.35-100.00) for both (table 2). In those correctly identified by the BIOCHIP, three patients had basement membrane zone staining on monkey oesophagus substrate, epidermal stained salt split skin and positivity for BP180 and or BP230 (table 1). Patient 19 (table 1) had epidermal stained salt split skin and was positive for BP180 and BP230, but had an intercellular substance staining pattern, whilst patient 24 was BP180 positive and had epidermal salt split skin staining, but no staining on monkey oesophagus (table 1). Patient 23 had a positive basement membrane zone staining pattern along with epidermal staining on salt split skin on the BIOCHIP, but was not positive for BP180 or BP230 (table 1). There was almost perfect concordance ($k=0.89$) between the two methods. There was substantial concordance between the EUROIMMUN Dermatology Profile ELISA and standard of care testing ($k=0.72$) for bullous pemphigoid, and almost perfect concordance between the BIOCHIP and standard of care testing ($k=0.82$).

Mucous Membrane Pemphigoid

Out of the three patients with a diagnosis of mucous membrane pemphigoid, no patients were correctly identified based on serology by the EUROIMMUN Dermatology Profile ELISA. Patient 26 had epidermal salt split skin staining on the BIOCHIP (table 1) which was considered consistent with mucous membrane pemphigoid, in the context of mucosal involvement clinically, and histology and indirect immunofluorescence results consistent with this result.

Standard of care testing

Sensitivity and specificity for standard of care diagnostics including histology, direct immunofluorescence and indirect immunofluorescence can be found in table 2. Sensitivity and specificity of the individual subsets of the EUROIMMUN Dermatology Profile ELISA, EUROIMMUN DSG1 and DSG3 quantitative testing, and individual subsets of the BIOCHIP relevant to each condition were also calculated (table 2).

Discussion

A number of studies have assessed the validity of the BIOCHIP in different immunobullous conditions. The majority assessed the sensitivity and specificity of the individual mosaics of the BIOCHIP, rather than evaluating the BIOCHIP as a whole. In pemphigus vulgaris, sensitivity of the cells transfected with DSG3 on the BIOCHIP has been reported to range from 60.9 to 100% (8, 9, 12). The sensitivity of cells transfected with DSG1 is reported to be much lower, between 13 and 52.3% (8, 9, 12). This reflects the nature of pemphigus vulgaris where autoantibodies against DSG1 are only expected in mucocutaneous forms of the disease (15). The sensitivity of DSG1 transfected cells on the BIOCHIP has been reported to be between 75 and 90% for pemphigus foliaceus (8, 9).

In bullous pemphigoid, the sensitivity of the BP180 mosaic on the BIOCHIP has been found to range more widely between 55.3 and 100% (8-12, 16, 17), with a specificity between 96.5 and 100% (8-12, 16, 17). This is compared to sensitivities of 45.1-66.7% for the BP230 mosaic on the BIOCHIP, and a specificity of 39.0-100% (8-12, 16, 17). The lower sensitivity of the BP230 mosaic compared to the BP180 mosaic is not unexpected given that it is more common to have autoantibodies against BP180, compared to BP230 in bullous pemphigoid.

The BIOCHIP offers the provision of results simultaneously, considerably aiding the diagnostic process. For this reason, our study focussed on the sensitivity and specificity of the BIOCHIP when evaluated as one diagnostic test, following interpretation of all the individual mosaic results. The same analysis principle was applied to the EUROIMMUN Dermatology Profile ELISA, with comparison made between the two diagnostic methods.

Our study found that the BIOCHIP Mosaic 7 had slightly higher sensitivity than the EUROIMMUN Dermatology Profile ELISA for diagnosis of pemphigus vulgaris (100% vs 80%) and bullous pemphigoid (87.5% vs 75%) (table 2). Specificity was the same for both diagnostic methods in bullous pemphigoid (100%), and slightly higher for the EUROIMMUN Dermatology Profile ELISA for pemphigus vulgaris (100% vs 94.1%) (table 2). The BIOCHIP had lower sensitivity for pemphigus foliaceus compared to the EUROIMMUN Dermatology Profile ELISA (50% vs 100%) (table 2), however this was based on a small population of only 4 patients. Sensitivity was low for the EUROIMMUN Dermatology Profile and BIOCHIP in mucous membrane pemphigoid (0% vs 33.3%) (table 2), which not only reflects the small number of patients included in the study with this condition, but is consistent with the evidence and our clinical experience that mucous membrane pemphigoid is notoriously difficult to diagnose based on serology (18).

The use of the BIOCHIP in immunobullous diagnostics in Australia has several advantages, particularly in cases in where there is a high index of suspicion. The high specificity seen across our results has likely been influenced by our patient population which included only patients with a high pre-test probability. Being able to perform all the required serological testing with one serum sample, in one laboratory, on one field, reduces the time to availability of results, minimising time spent sending samples to several different laboratories. The BIOCHIP Mosaic 7 does have a shorter preparation time, however the performance of the EUROIMMUN Dermatology Profile ELISA and skin immunofluorescence is similar, approximately 1.5-2 hours. The BIOCHIP allows multiple results to be available in a concurrent manner for clinicians, aiding management decisions and resulting in more optimal patient outcomes.

The BIOCHIP is a suitable alternative for the testing of specific skin autoantibodies towards DSG1 and DSG3, as well as BP180 and BP230. An advantage of the EUROIMMUN ELISA Profile over the BIOCHIP Mosaic 7 is the inclusion of collagen type VII and envoplakin which can assist in the diagnosis of epidermolysis bullosa acquisita and paraneoplastic pemphigus.

Cost is an important consideration when comparing the two diagnostic methods, although this is complex and difficult to analyse precisely. Variable factors include the number of

samples to be processed, frequency of the testing, slide well configuration, staffing expertise and laboratory quality control. Therefore, it is more efficient per test when there are higher test volumes. We have calculated an estimated cost per test for 8 samples, including 3 hours of scientific labour, for the BIOCHIP Mosaic 7 to be AUD \$54. This is compared to an estimated cost of AUD \$69 for the EUROIMMUN Dermatology profile ELISA. The BIOCHIP Mosaic 7 is listed and approved by the Therapeutic Goods Administration (TGA) and is therefore suitable for introduction to a pathology service in Australia after appropriate verification studies. The Medicare Benefits Schedule rebate for the testing of skin autoantibodies may be insufficient to cover the full cost of the BIOCHIP Mosaic 7 or the EUROIMMUN Dermatology profile ELISA, especially when multiple tests are ordered on the one pathology episode. Therefore, some out-of-pocket costs to the patient or clinical departments may be required in some diagnostic laboratories.

The BIOCHIP is available in a range of different configurations. The use of commercially available BIOCHIP containing only the DSG1 and DSG3 substrate assay would assist in differentiating pemphigus from a non-specific blood group antibody pattern found on standard indirect immunofluorescence on primate oesophageal tissue (19). Commercial neutralising antibodies have been inefficient in removing this non-specific staining, highlighting the importance of specific antibody testing to DSG1 and DSG3 when this staining pattern is noted on standard monkey oesophagus-based assays. The BIOCHIP also makes available the diagnostic test salt split skin which has not been routinely easily accessible in some parts of Australia.

Due to its unique technology utilising hyper-expressing cell cultures, microdrops and tissue substrates, the BIOCHIP may detect autoantibodies directed to antigen epitopes that are different from traditional ELISA based assays. A more extensive Dermatology Mosaic BIOCHIP is available that can be customised to include collagen type VII, transitional epithelia, gliadin and other tissue/cell-based mosaics to support laboratory diagnosis of these and other rare autoimmune dermatological conditions. In future, assays assessing for Laminin 332 and Laminin γ 1 (anti-p200) may become commercially available (20). Further research in this area is required and a larger multinational, multicentre study is currently

being undertaken, evaluating the BIOCHIP with the inclusion of these additional panels, aiming to provide further validation for their use.

Limitations of this study include the small study population and absence of patients with epidermolysis bullosa acquisita. Due to the low incidence of immunobullous disease this is frequently a challenge for research in this field. Secondly, although only treatment naïve patients were included, if standard of care diagnostics had previously been completed and were not required to be repeated, these were not recollected at the same time sera was taken for BIOCHIP processing. Finally, only two patients who did not have a diagnosis of an immunobullous condition were included. A larger control group would be preferable, minimising spectrum bias.

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Tables:

Table 1. Summary of patient results

Patient	Standard of care testing					ELISA		BIOCHIP Mosaic 7					
	Diagnosis	Ethnicity	Histology	DIF	IIF	Dermatology Profile	Quantitative	Monkey oesophagus	Salt split skin	DSG1	DSG3	BP180	BP230
1	PV	Southern Asian	+	+	+	DSG1, DSG3	DSG1, DSG3	ISS	-	+	+	-	-
2	PV	Southern Asian	+	+	+	DSG1, DSG3	DSG1, DSG3	ISS	-	+	+	-	-
3	PV	Southern European	+	+	+	DSG3	DSG3	ISS	-	-	+	-	-
4	PV	Southern European	+	-	+	-	-	ISS	-	-	+	-	-
5	PV	Middle Eastern	-	+	+	DSG1, DSG3	DSG1, DSG3	ISS	-	+	+	-	-
6	BP	North West European	+	+	+	-	N/A	BMZ	-	-	-	-	-
7	BP	North West European	+	+	+	BP180	N/A	BMZ	epidermal	-	-	+	+
8	PV	Southern European	+	+	+	DSG1	DSG1	ISS	-	+	+	-	-
9	PV	Oceanian	+	+	+	DSG3	DSG3	ISS	-	-	+	-	-
10	BP	Southern European	+	+	+	BP180, BP230	N/A	BMZ	epidermal	-	-	+	+
11	PF	North American	+	+	+	DSG1	DSG1	-	-	-	-	-	-
12	Other	Oceanian	+	-	-	-	-	-	-	-	-	-	-
13	PF	Eastern European	+	+	+	DSG1	DSG1	ISS	-	+	-	-	-
14	MMP	Southern European	+	+	-	-	-	-	-	-	-	-	-
15	MMP	Oceanian	+	+	-	-	-	-	-	-	-	-	-
16	BP	Eastern European	+	+	+	-	N/A	BMZ	epidermal	-	-	-	+
17	Other	Southern European	-	-	-	-	-	-	-	-	-	-	-
18	PV	Southern Asian	+	+	+	DSG1, DSG3	DSG1, DSG3	ISS	-	+	+	-	-
19	BP	Oceanian	+	+	+	BP180, BP230	N/A	ISS	epidermal	-	-	+	+

20	PV	Southern European	+	+	+	DSG1, DSG3	DSG1, DSG3	ISS	-	+	+	-	-
21	BP	Eastern European	+	+	+	BP180	N/A	-	epidermal	-	-	+	-
22	PV	South East Asian	+	+	+	DSG1, DSG3	DSG3	ISS	-	+	+	-	-
23	BP	Oceanian	+	+	+	BP180	N/A	BMZ	epidermal	-	-	-	-
24	BP	Southern European	+	+	-	BP180	-	-	epidermal	-	-	+	-
25	PF	Oceanian	+	+	-	DSG1	DSG1	ISS	-	+	+	-	-
26	MMP	Oceanian	+	+	-	-	N/A	-	epidermal	-	-	-	-
27	PF	Oceanian	+	+	+	DSG1	DSG1	ISS	-	+	-	-	-

DIF = direct immunofluorescence, IIF = indirect immunofluorescence, Dermatology Profile = EUROIMMUN ELISA Dermatology profile, Quantitative = EUROIMMUN ELISA titre, DSG 1 = desmoglein 1, DSG3 = desmoglein 3, ISS = Intercellular substance staining, BMZ = basement membrane zone staining, PV = pemphigus vulgaris, PF = pemphigus foliaceus, BP = bullous pemphigoid, MMP = mucous membrane pemphigoid, PV = pemphigus vulgaris, BP = bullous pemphigoid, PF = pemphigus foliaceus, MMP = mucous membrane pemphigoid, ISS = intercellular substance staining, BMZ = basement membrane zone, + = positive, - = negative, N/A = not clinically indicated

Table 2. Standard of care testing, BIOCHIP Mosaic 7 and EUROIMMUN Dermatology Profile ELISA sensitivity and specificity

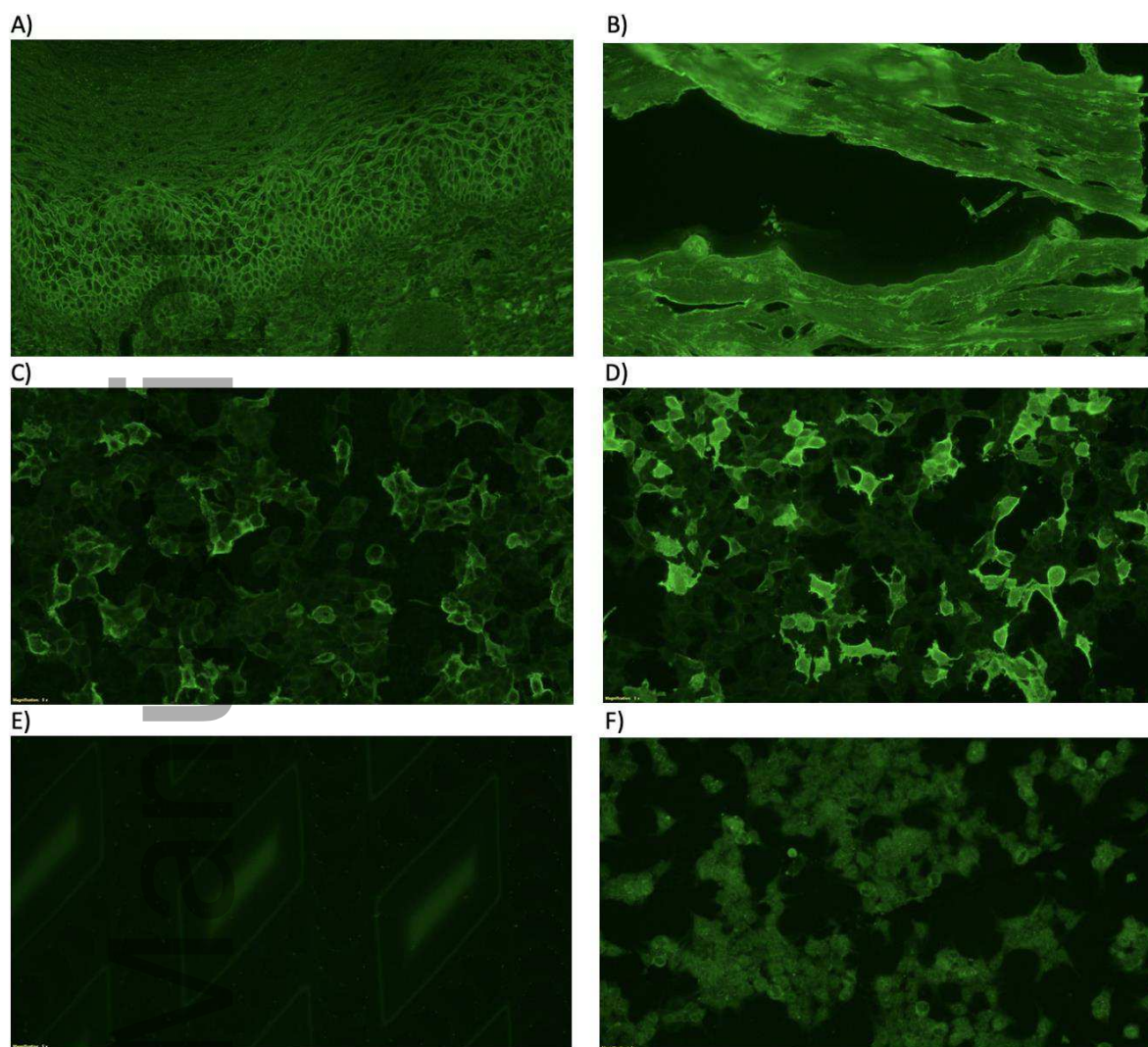
	Sensitivity (%)	Specificity (%)
Pemphigus Vulgaris (n=10)	(% n/10)	(% n/27)
Histology	90.0	88.2
DIF	90.0	76.5
IIF staining	100	70.6
ELISA profile DSG1	70.0	76.5
ELISA profile DSG3	80.0	100
ELISA profile overall	80.0	100
ELISA titre DSG1	60.0	-
ELISA titre DSG3	80.0	-
ELISA titre overall	80.0	-
BIOCHIP oesoph.	100	70.6
BIOCHIP DSG1	70.0	82.4
BIOCHIP DSG3	100	94.1
BIOCHIP overall	100	94.1
Pemphigus Foliaceus (n=4)	(% n/4)	(% n/27)
Histology	100	60.9
DIF	100	60.9
IIF staining	75.0	47.8
ELISA profile DSG1	100	69.6
ELISA profile overall	100	100
ELISA titre DSG1	100	-
ELISA titre overall	100	-
BIOCHIP oesoph.	75.0	47.8
BIOCHIP DSG1	75.0	69.6
BIOCHIP overall	50.0	100
Bullous Pemphigoid (n=8)	(% n/8)	(% n/27)
Histology	100	84.2
DIF	88.9	100
IIF staining	100	100
ELISA BP180	75.0	100
ELISA BP230	25.0	100
ELISA overall	75.0	100
BIOCHIP oesoph.	62.5	100
BIOCHIP SSS	87.5	100
BIOCHIP BP180	62.5	100
BIOCHIP BP230	50.0	100

BIOCHIP overall	87.5	100
MMP (n=3)	(% n/3)	(% n/27)
Histology	100	66.7
DIF	100	70.8
IIF staining	0.0	70.8
ELISA profile BP180	0.0	75.0
ELISA profile envoplakin	0.0	100
ELISA profile collagen VII	0.0	100
ELISA profile overall	0.0	75.0
BIOCHIP oesoph.	0.0	66.7
BIOCHIP SSS	33.3	70.8
BIOCHIP BP180	0.0	79.1
BIOCHIP overall	33.3	66.6

DIF = direct immunofluorescence, IIF = indirect immunofluorescence, ELISA profile = EUROIMMUN dermatology profile ELISA, BIOCHIP oesoph. = BIOCHIP monkey oesophagus mosaic, BIOCHIP SSS = BIOCHIP salt split skin, MMP = mucous membrane pemphigoid

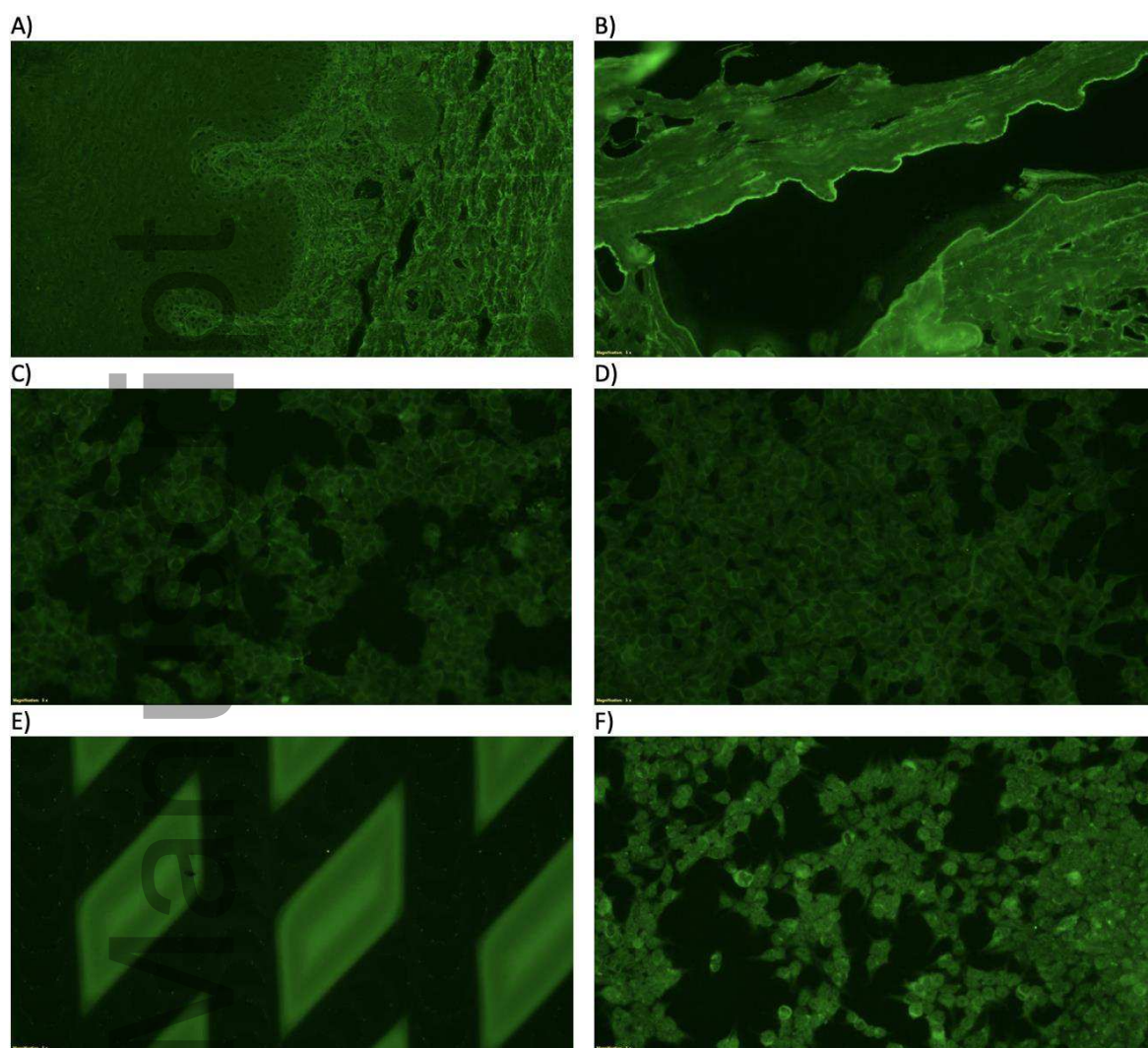
Figure Legends

Figure 1. Patient 20 BIOCHIP results, diagnosis of pemphigus vulgaris

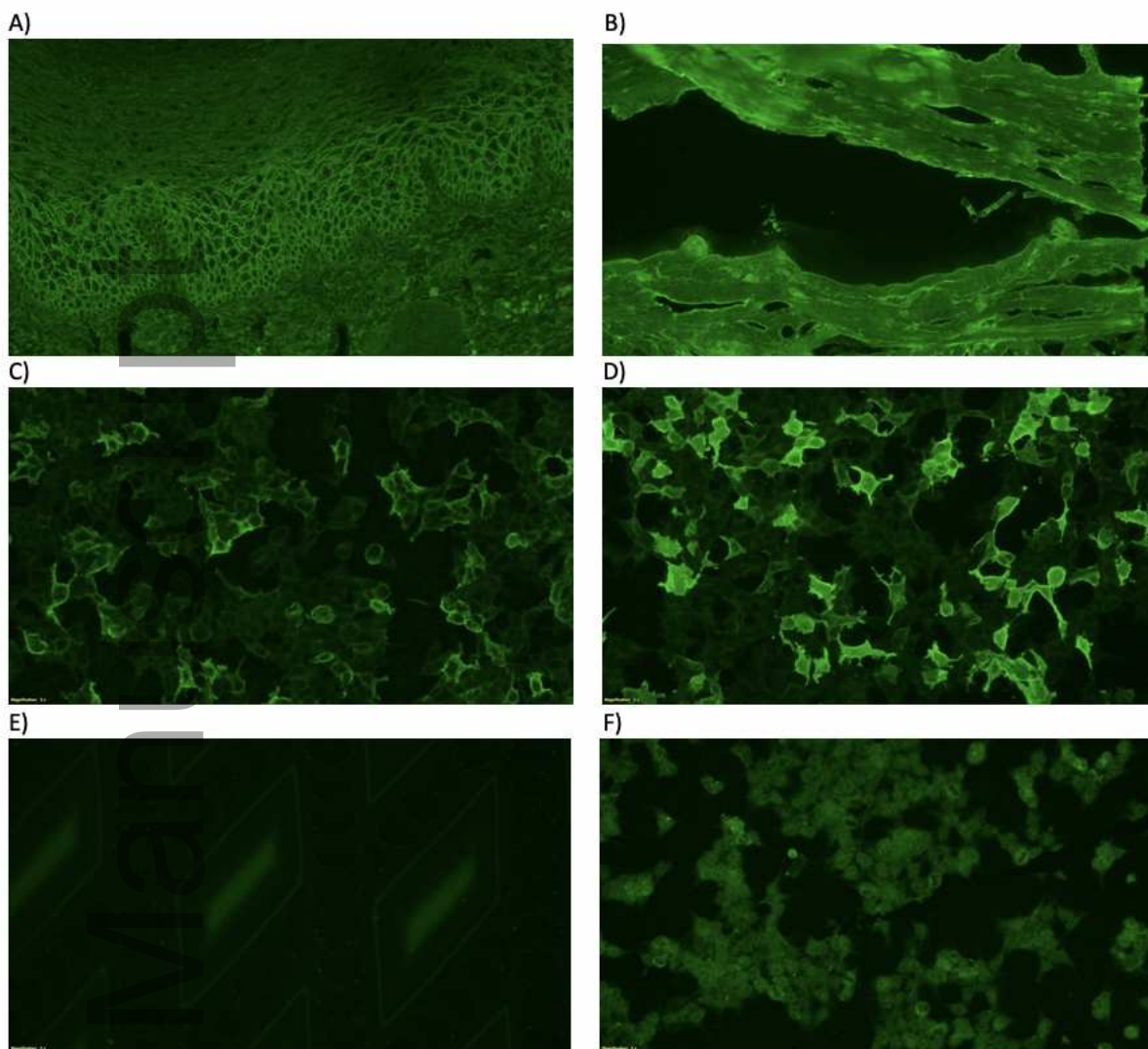


A) monkey oesophagus ISS staining, B) negative salt split skin, C) anti DSG1 positive transfected cells, D) anti DSG3 positive transfected cells, E) anti BP180 negative, F) anti BP230 negative transfected cells.

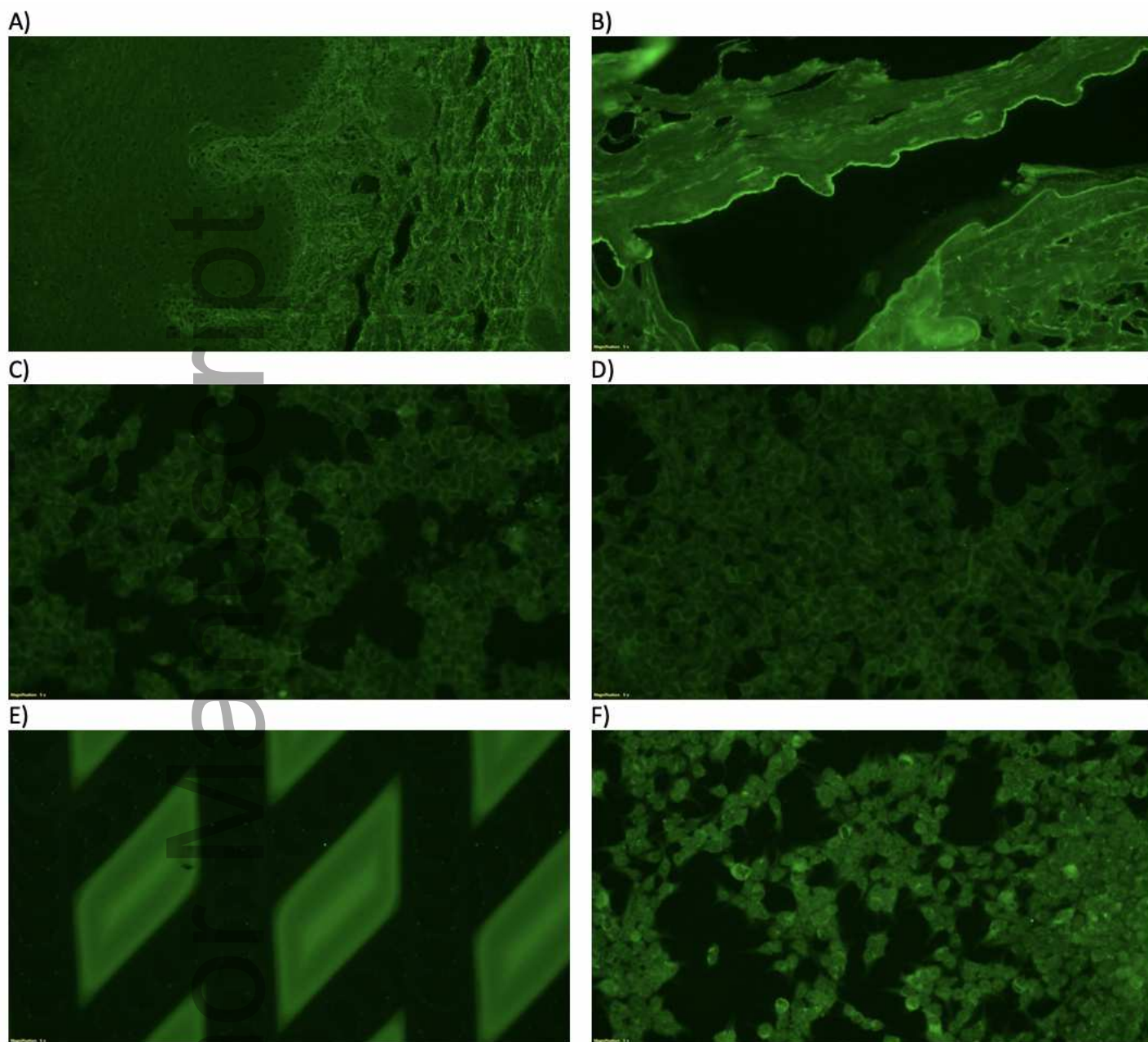
Figure 2. Patient 21 BIOCHIP results, diagnosis of bullous pemphigoid



A) monkey oesophagus staining negative, B) salt split skin with epidermal staining, C) anti DSG1 negative transfected cells, D) anti DSG3 negative transfected cells, E) anti BP180 positive, F) anti BP230 negative transfected cells.



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