

New diagnostic techniques in autoimmune blistering diseases

Gilles Diercks, pathologist



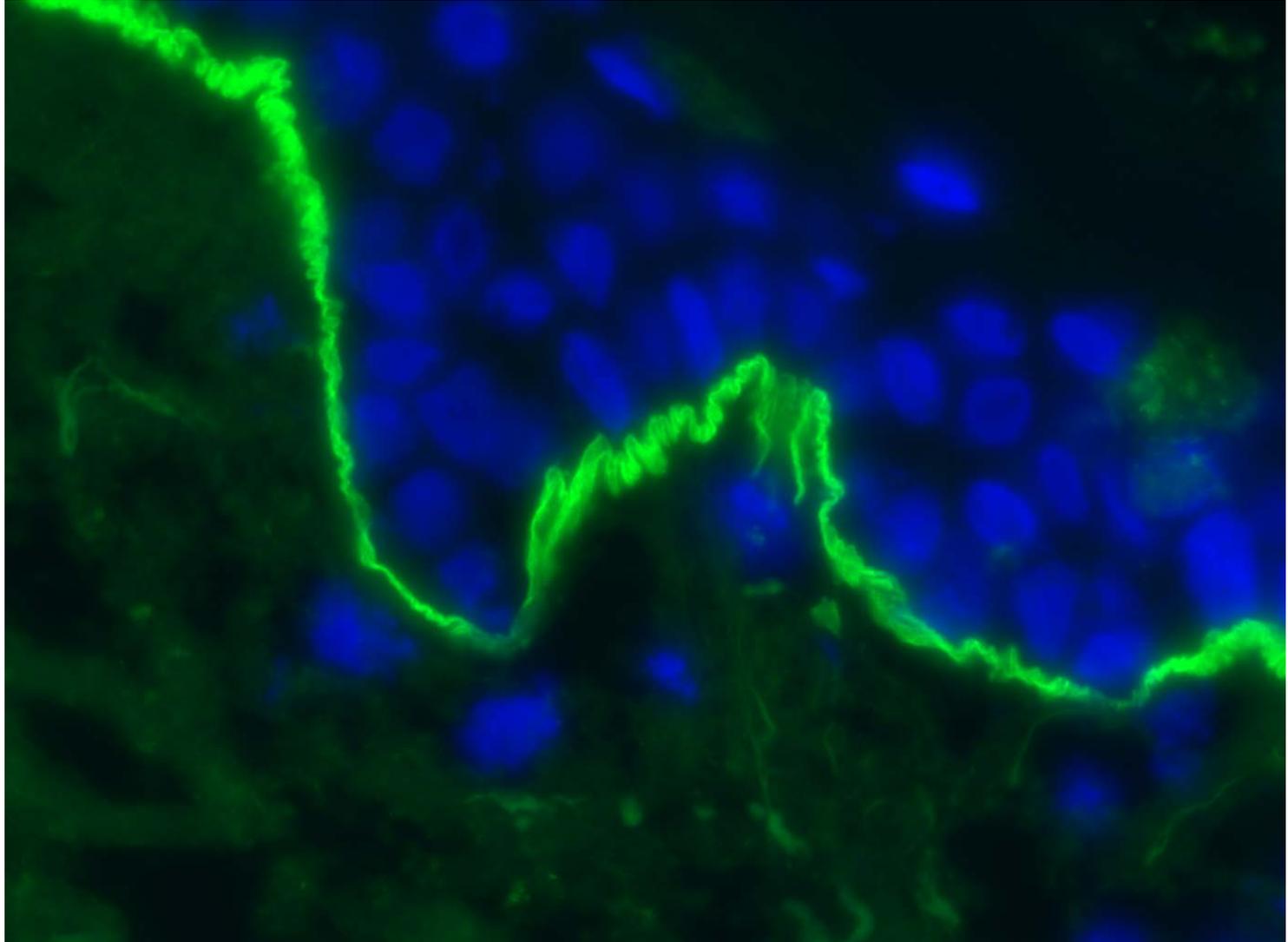
Maastricht, 15-11-2019

Woman, 42 years

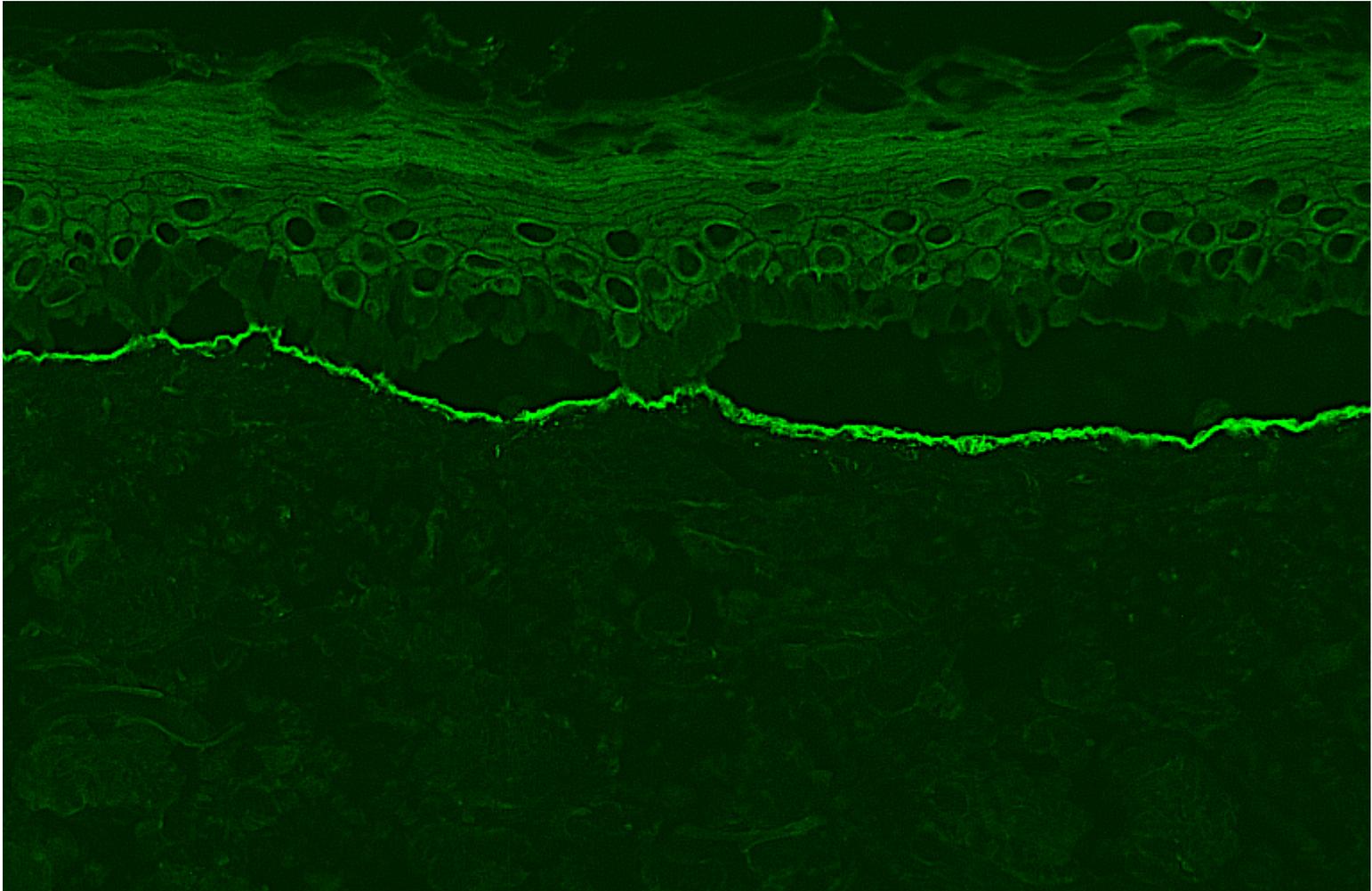
- Since 1 year fatigue, gingivitis and nasal ulceration
- Wegener's disease ruled by internal medicine elsewhere

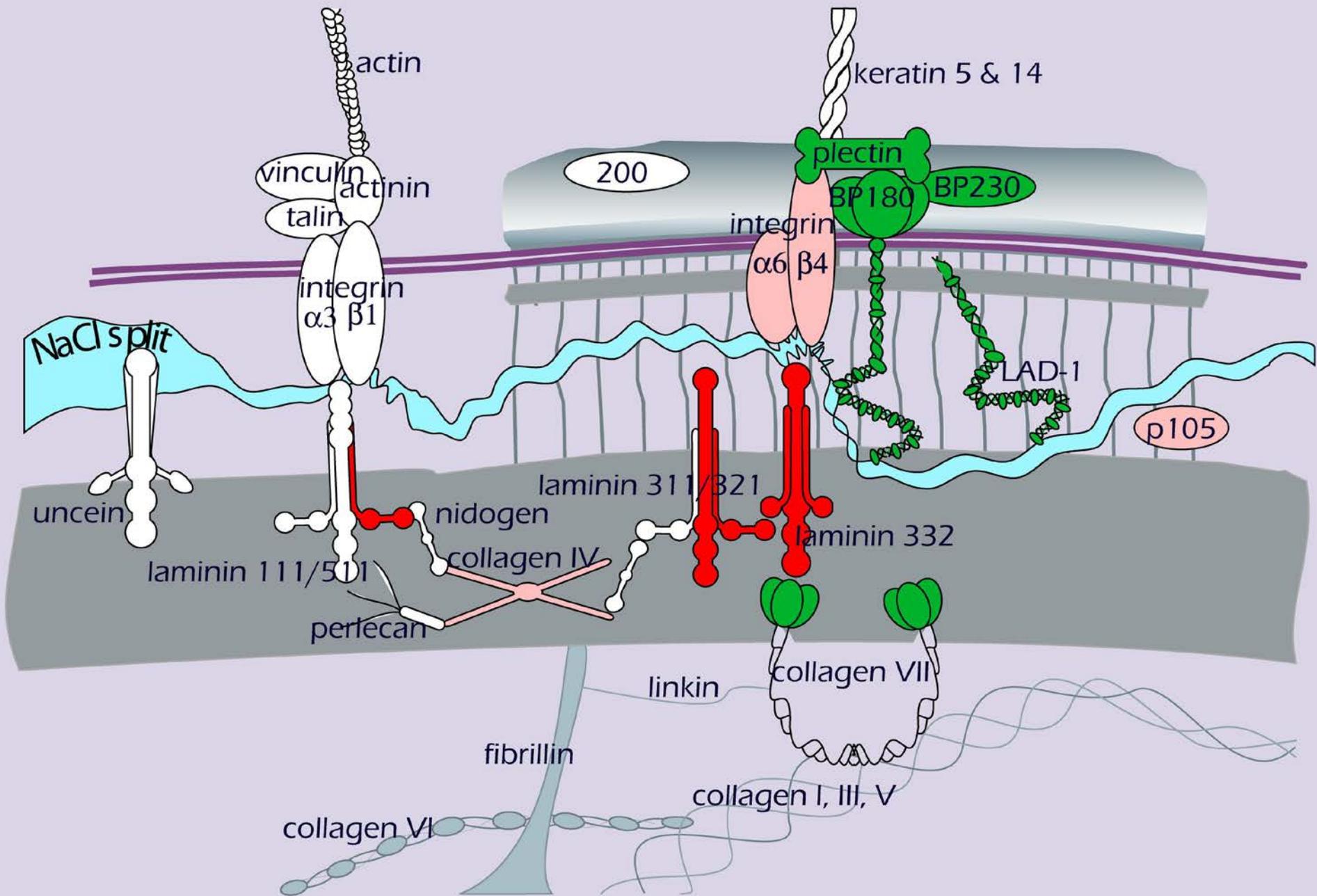


Direct immunofluorescence



Salt split skin





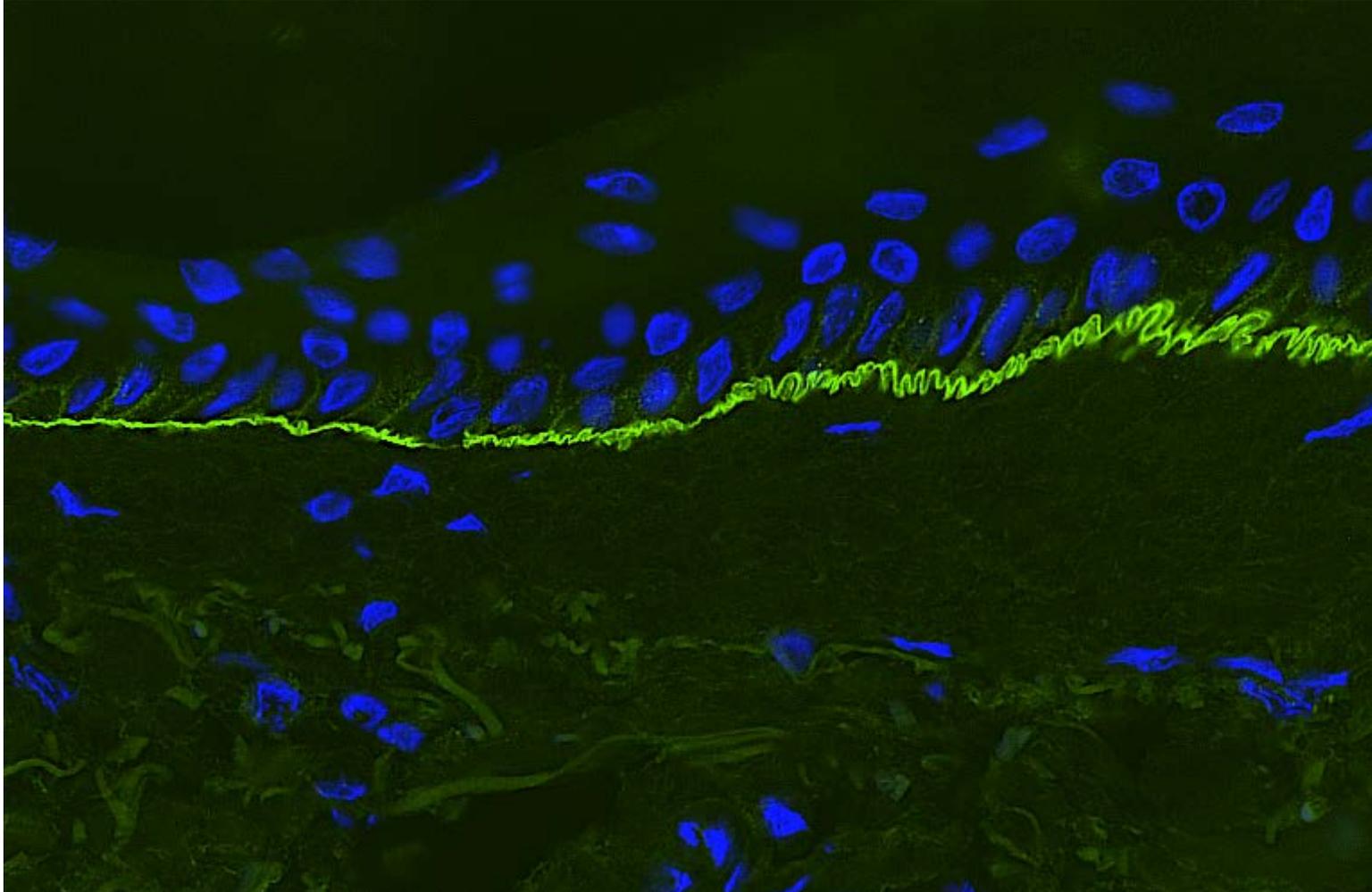
Importance of (sub)classification

- Epidermolysis Bullosa Acquisita:
 - therapy resistant
- P200:
 - better prognosis
- Laminine 332:
 - Severe mucosal involvement
 - Association with malignancies

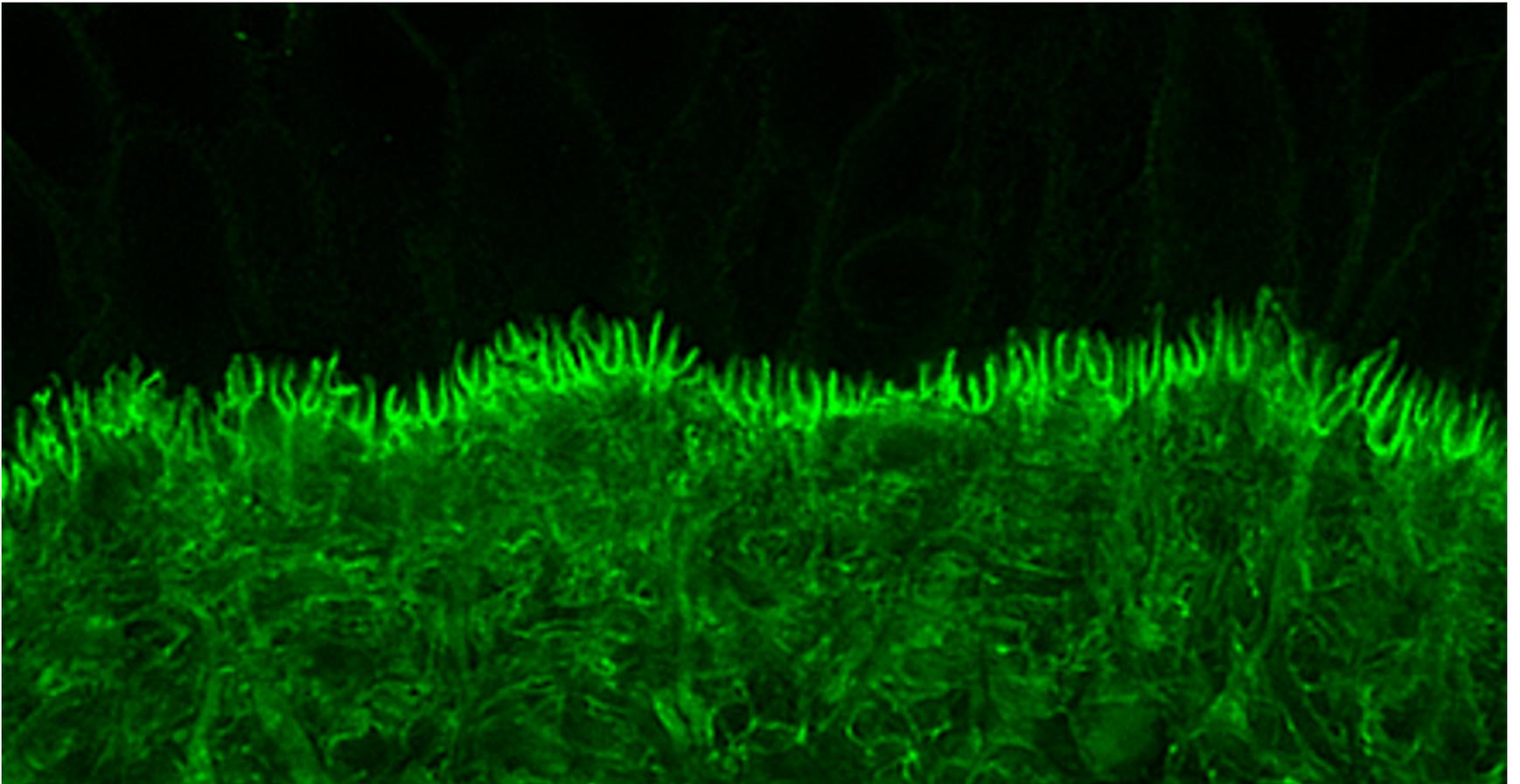
What tests are available

- N versus U serration

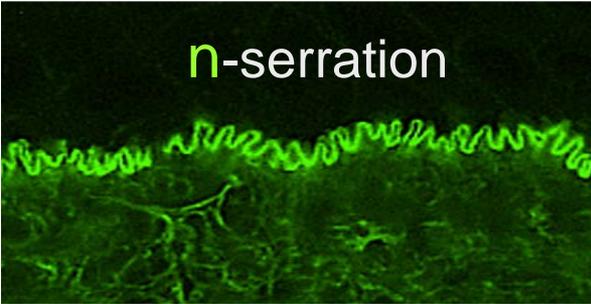
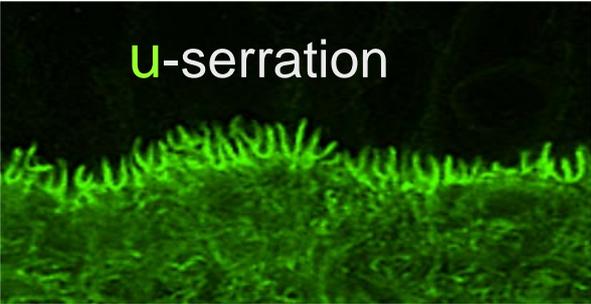
N serration: P200 or Laminin 332



U serration: EBA



Serration pattern and pemphigoid variant

DIF BMZ	 n-serration		 u-serration	
Salt split skin	Epidermal	Dermal	Dermal	Epidermal
Pemphigoid variant	BP MMP/CP LAD	p200 Anti-LN-332	EBA bSLE	?

Vodegel et al: Brit J Dermatol 2004; 151: 112-18

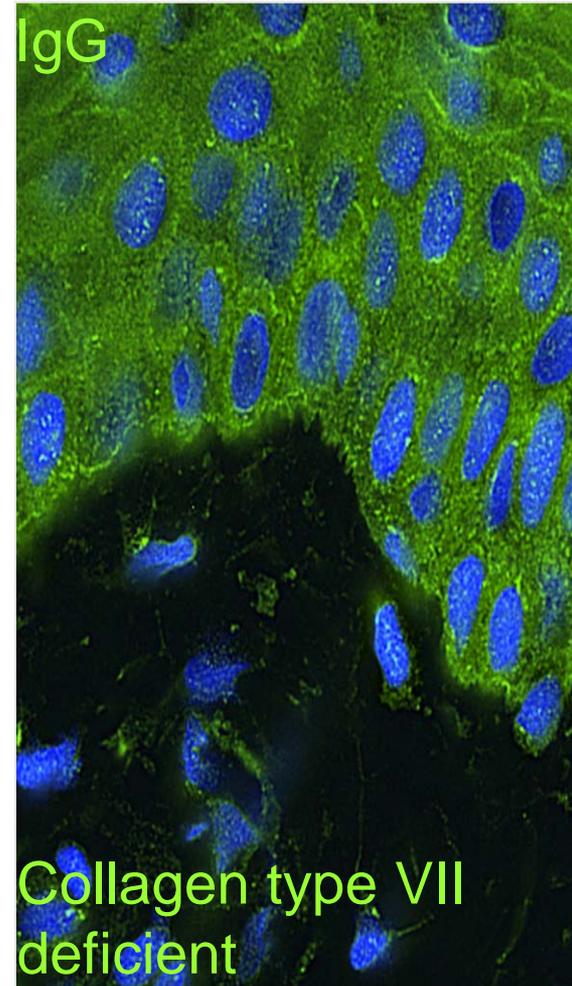
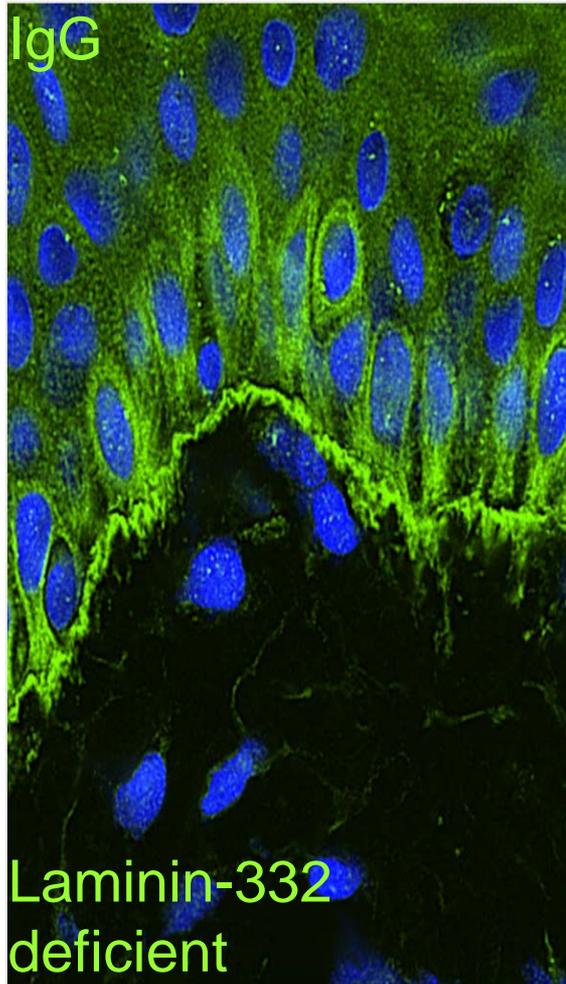
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 - No difference between p200 and laminin 332
 - Serration not always recognizable

What tests are available

- N versus U serration
 - No difference between p200 and laminin 332
 - Serration not always recognizable
- Immunoprecipitation for laminin 332
 - Sensitive and specific
 - Very specialized technique
- ELISA for laminin 332
 - Easy to perform
 - Not commercially available
 - Not specific
- Knock out Epidermolysis Bullosa skin

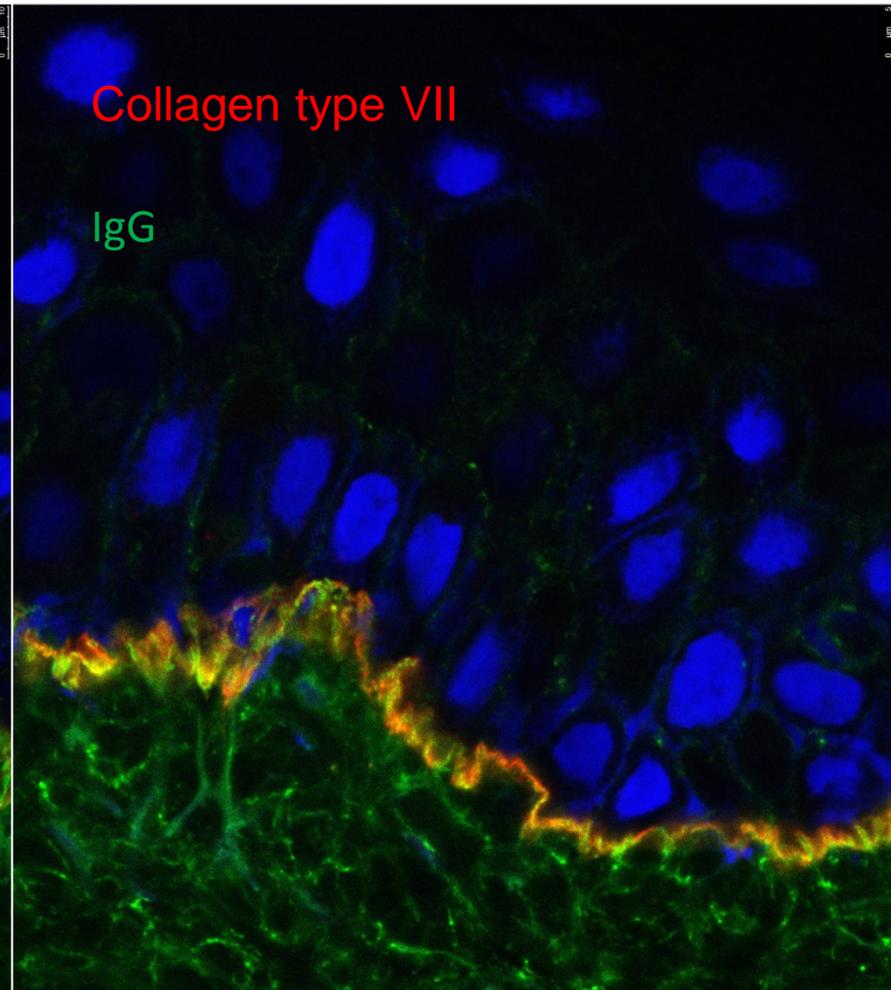
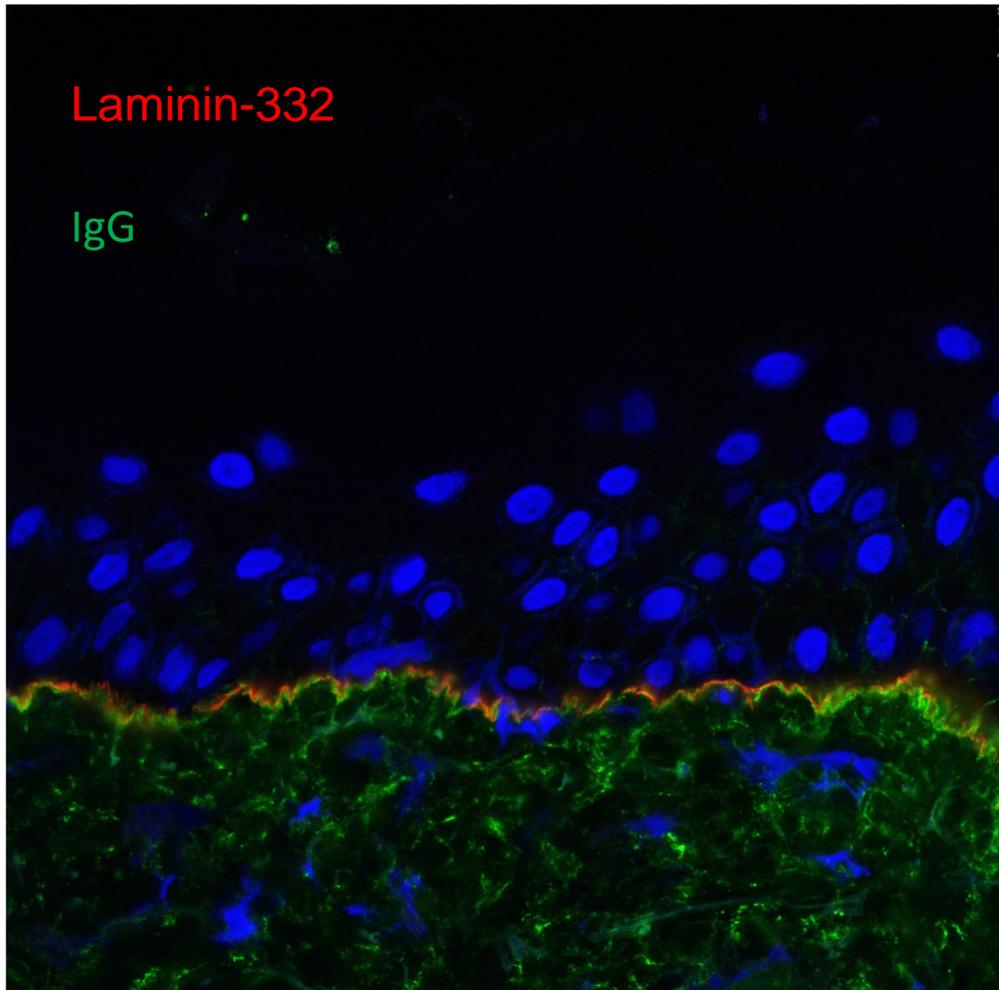
Knock-out skin



What tests are available

- N versus U serration
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- Knock out Epidermolysis Bullosa skin
- Confocal microscopy.

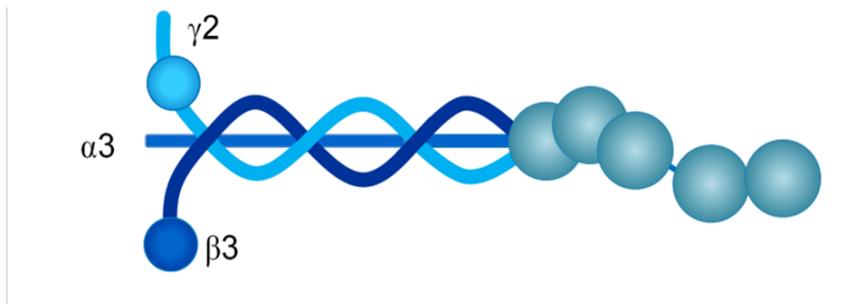
Confocal microscopy



Keratinocyte footprint assay discriminates antilaminin-332 pemphigoid from all other forms of pemphigoid diseases

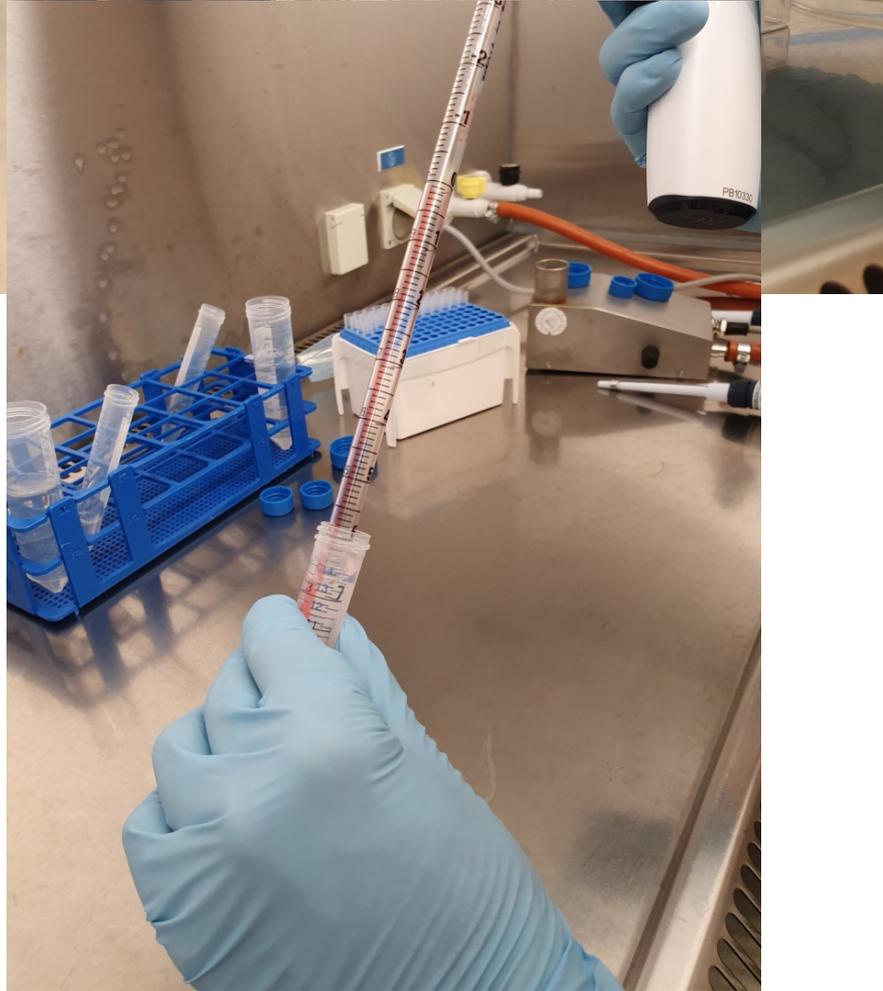
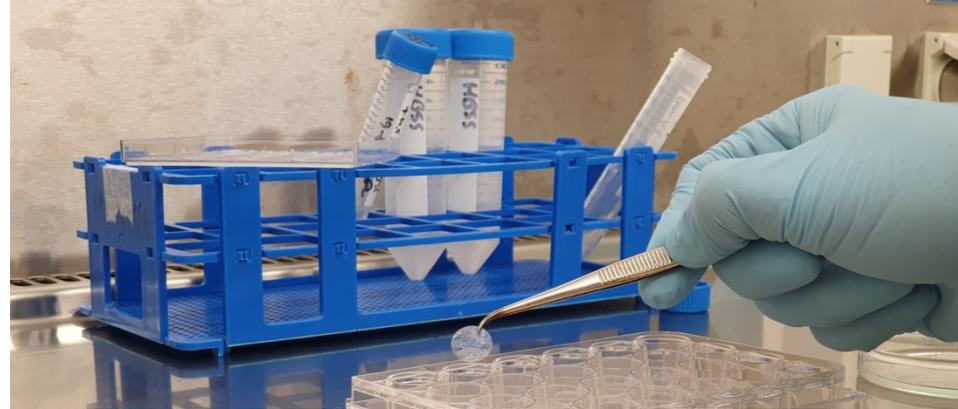
F. Giurdanella , A.M. Nijenhuis, G.F.H. Diercks, M.F. Jonkman  and H.H. Pas 

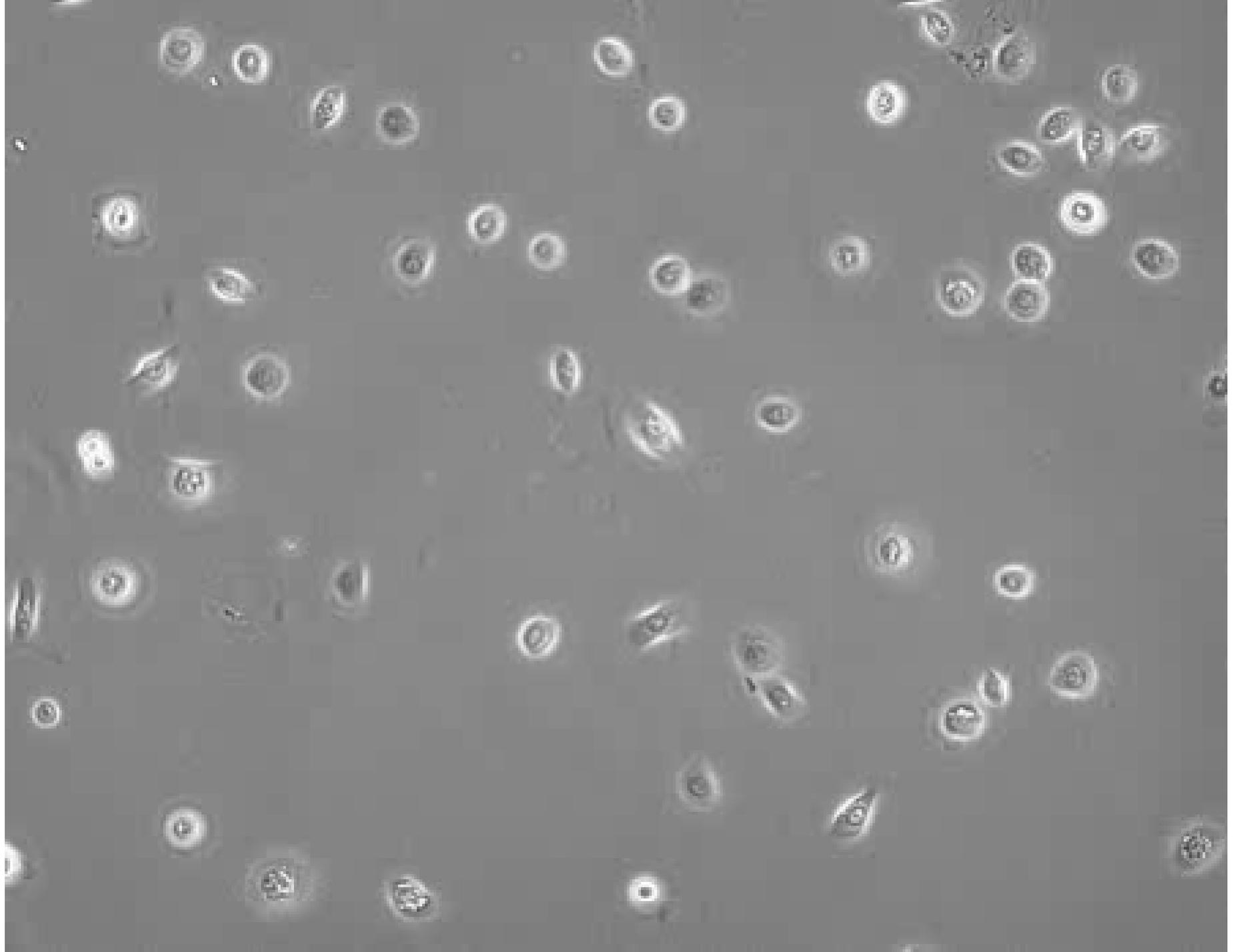
Center for Blistering Diseases, Department of Dermatology, University of Groningen, University Medical Center Groningen, PO Box 30 001, 9700 RB Groningen, the Netherlands

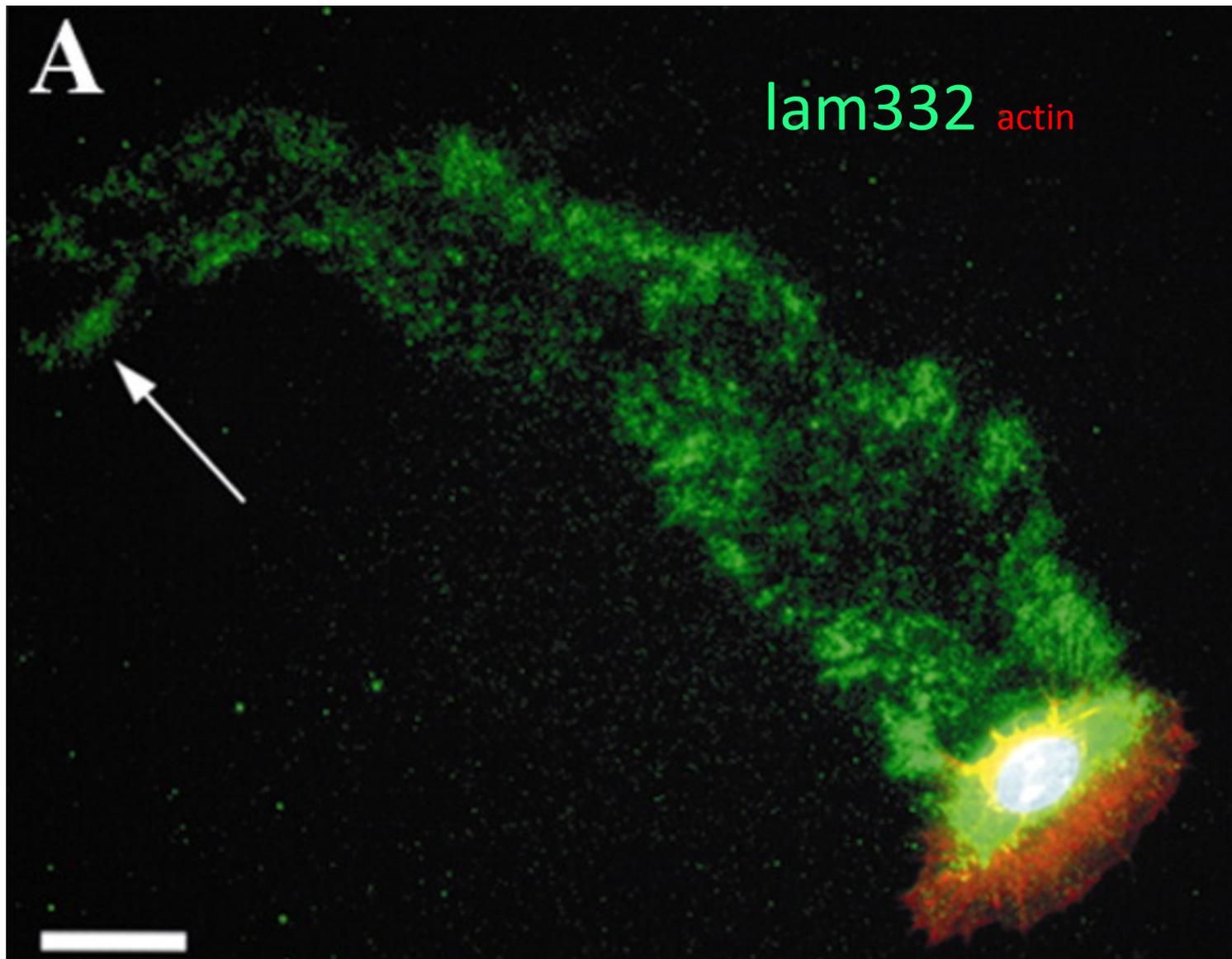


α 3 chain		1724
β 3 chain		1172
γ 2 chain		1111

Figure 2. (A) Schematic diagram of the laminin 332 structure. Laminin 332 is a glycoprotein composed of three individual protein chains (laminin α 3, β 3 and γ 2).

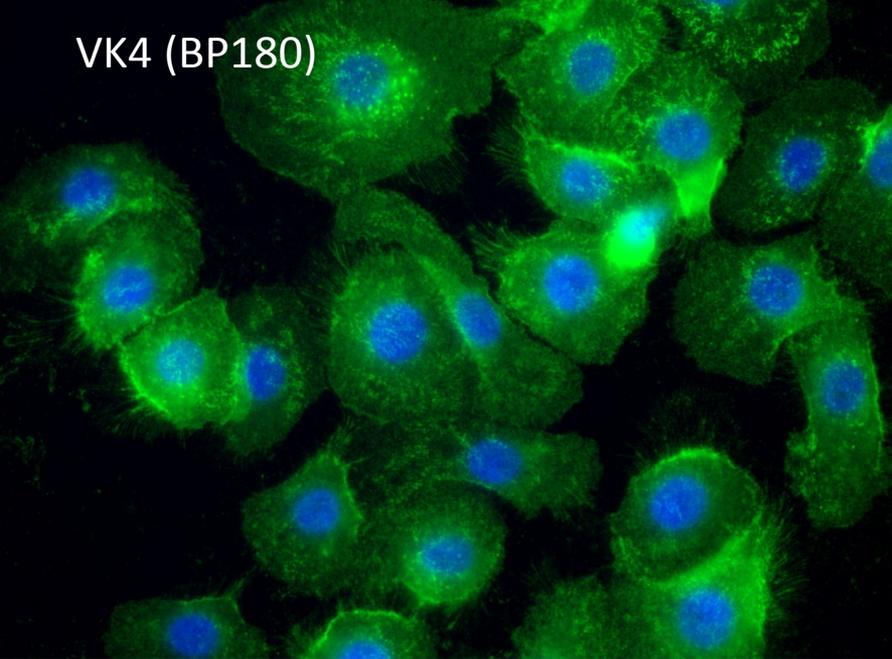




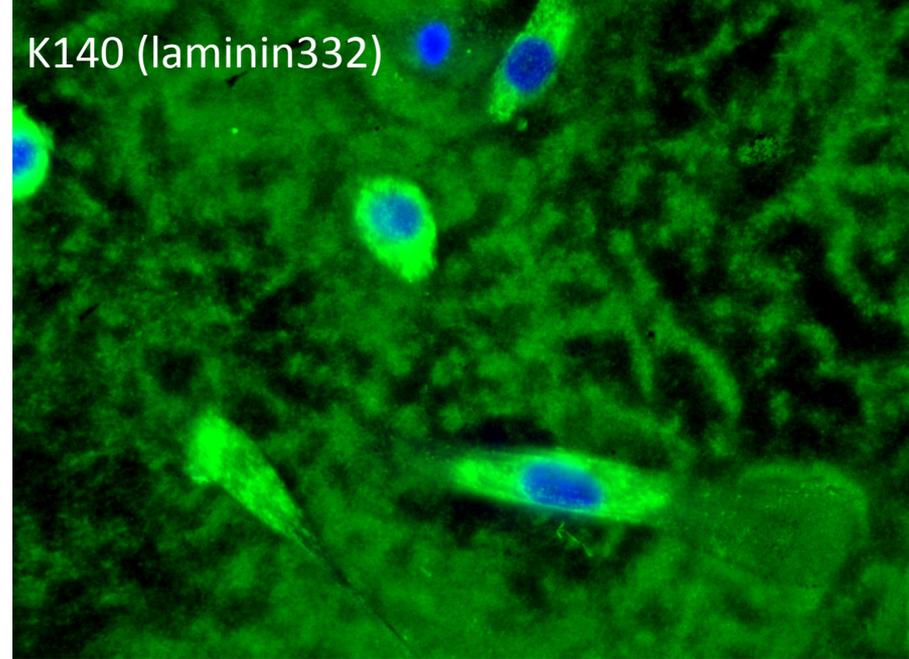


Diane E. Frank, William G. Carter
Journal of Cell Science 2004.

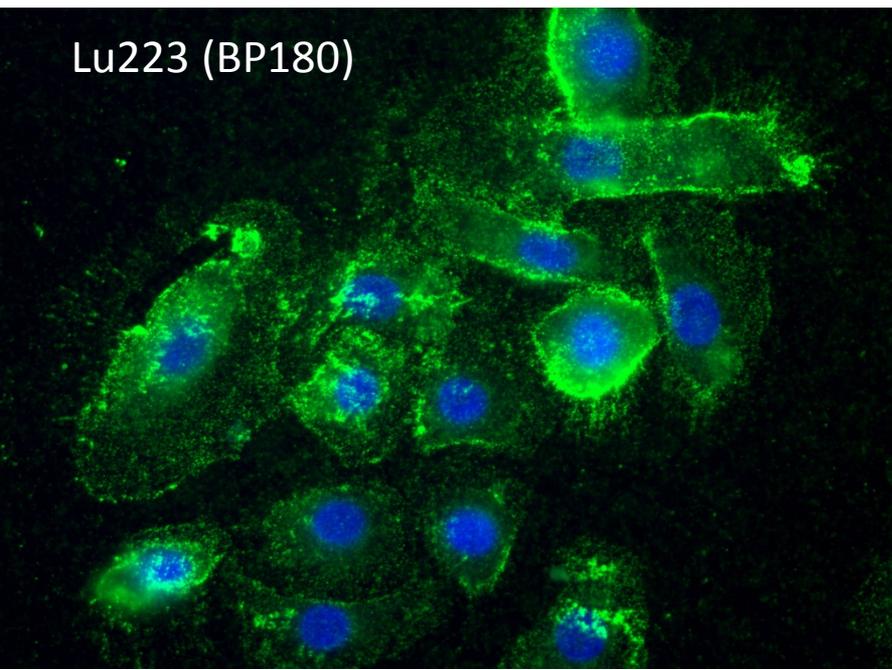
VK4 (BP180)



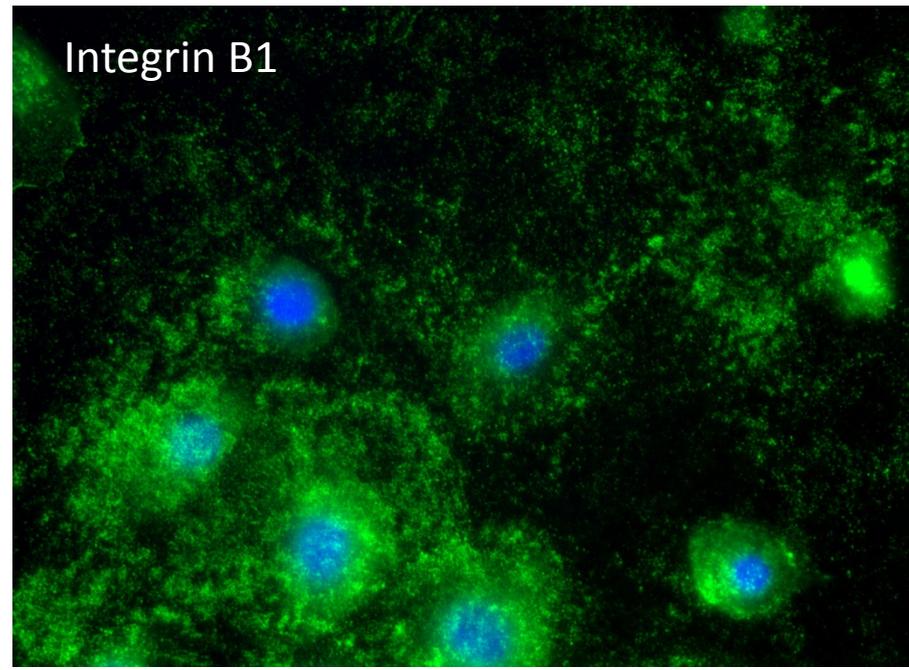
K140 (laminin332)



Lu223 (BP180)

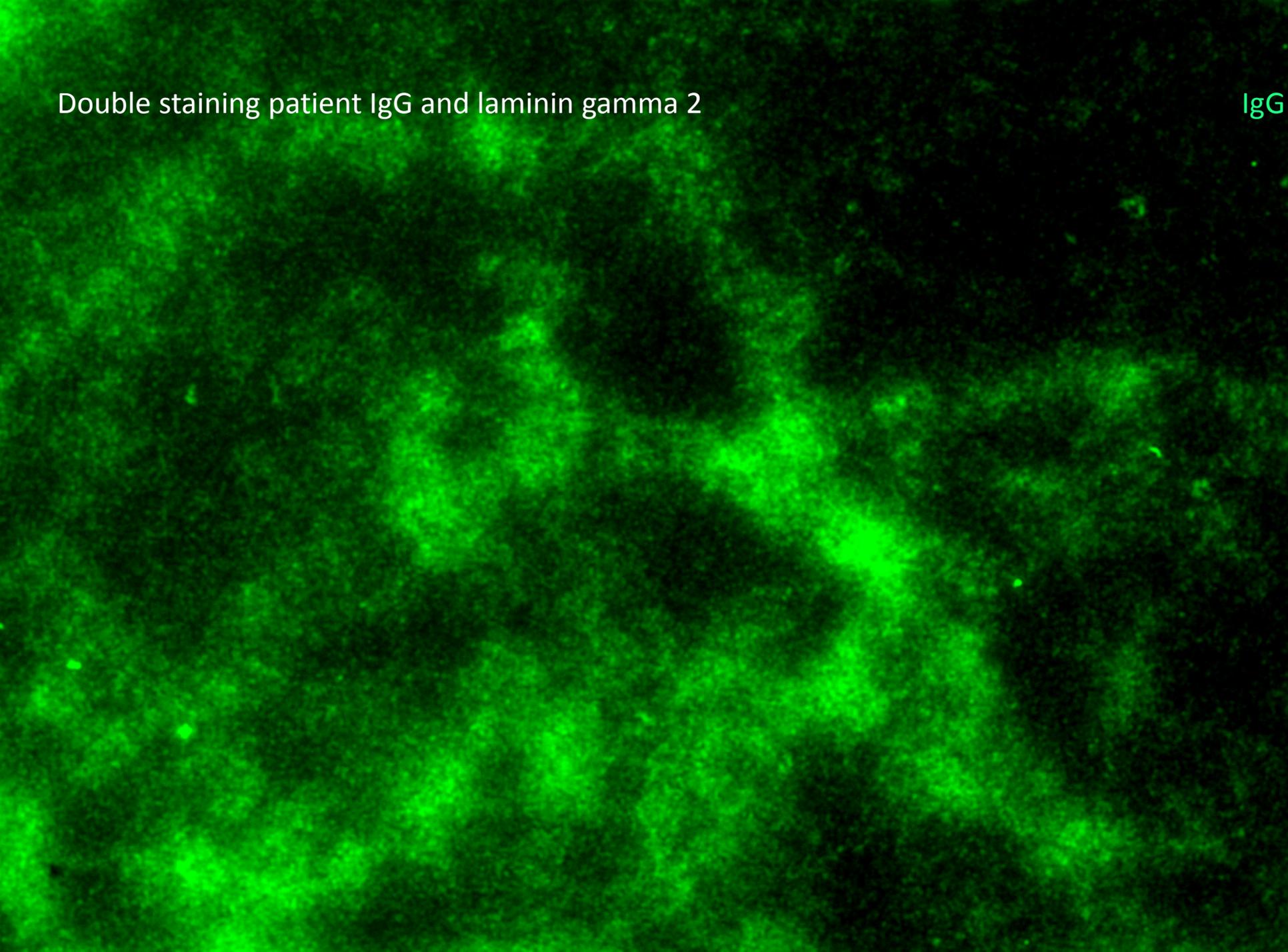


Integrin B1



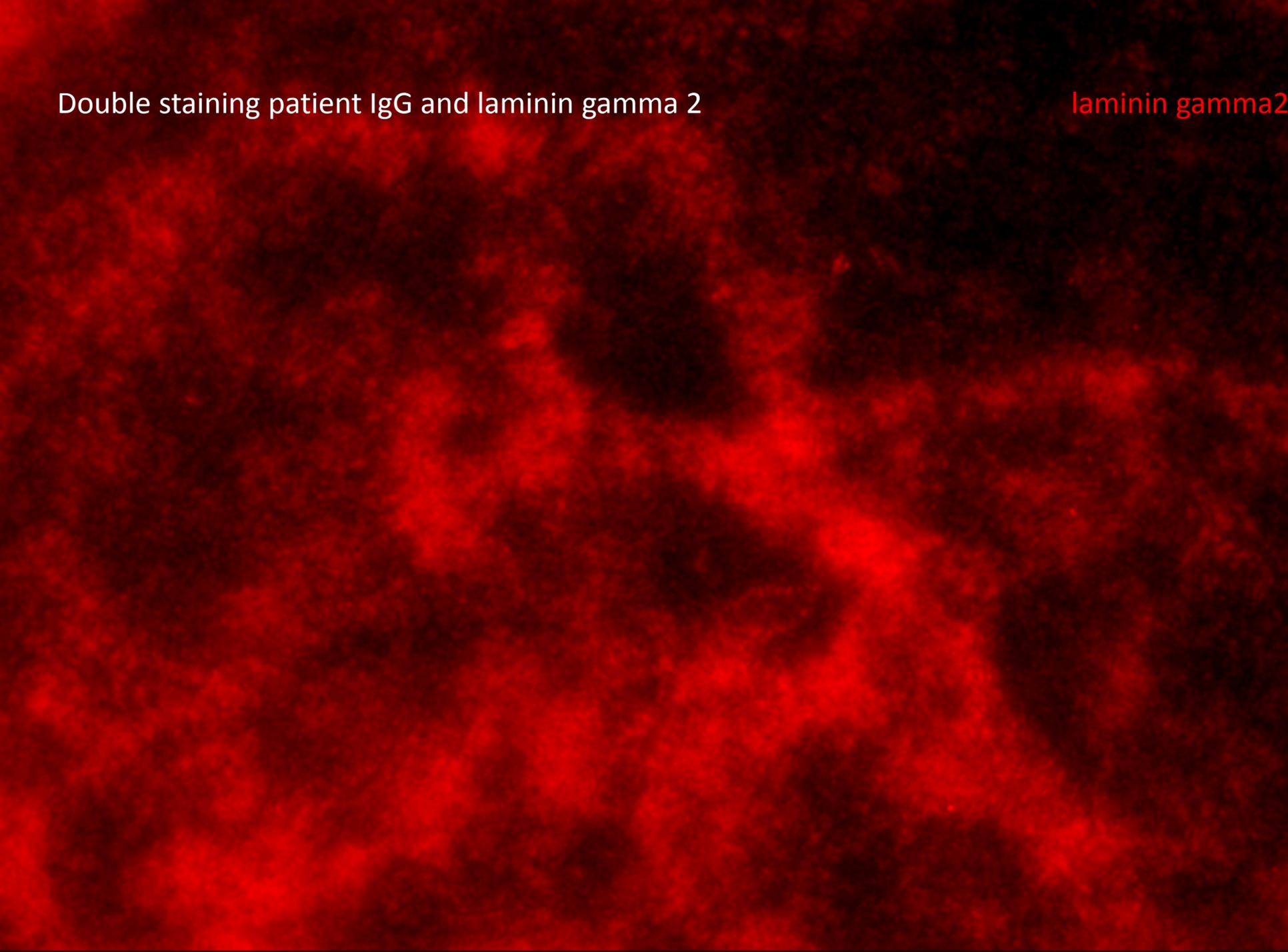
Double staining patient IgG and laminin gamma 2

IgG



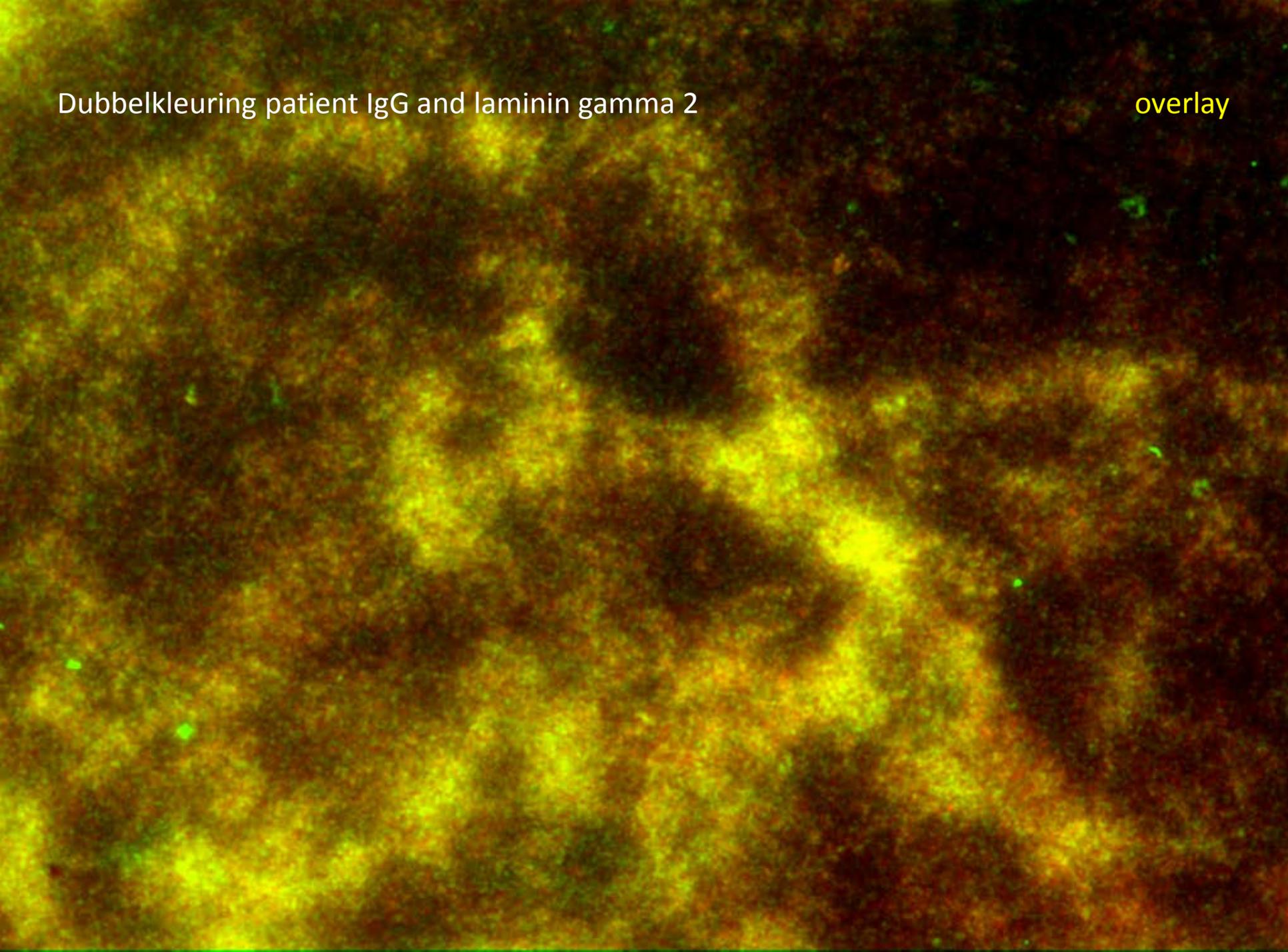
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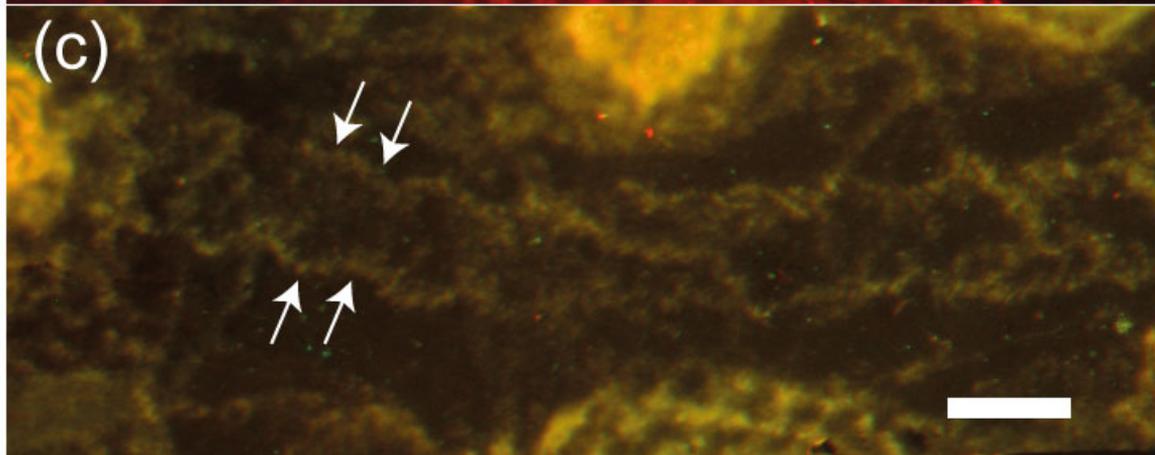
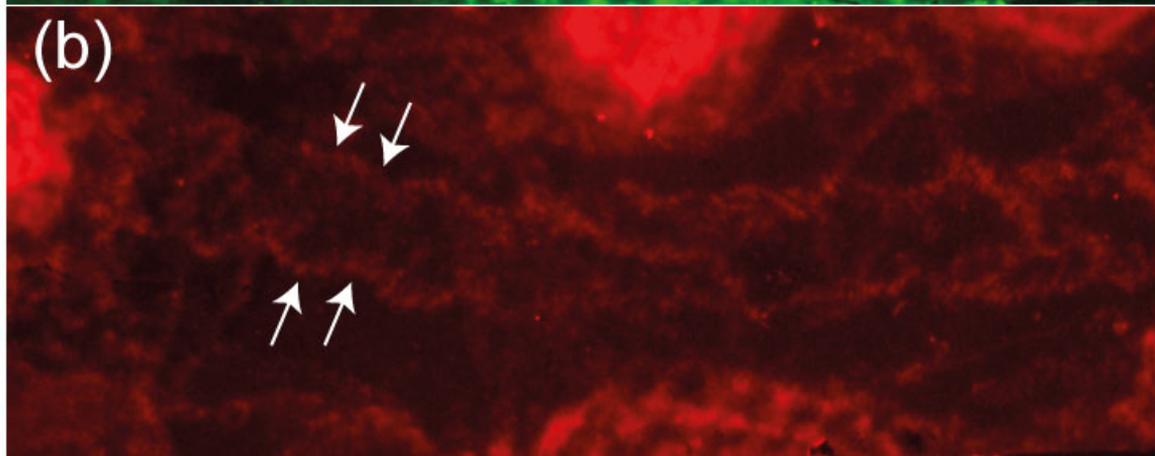
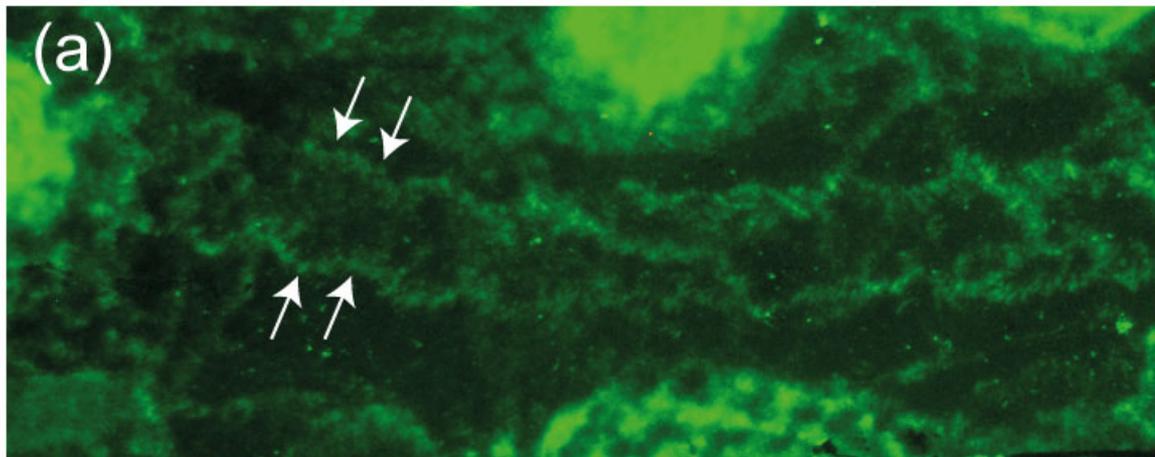
laminin gamma2



Dubbelkleuring patient IgG and laminin gamma 2

overlay





#1 63x These are the laminin332 footprints to look for

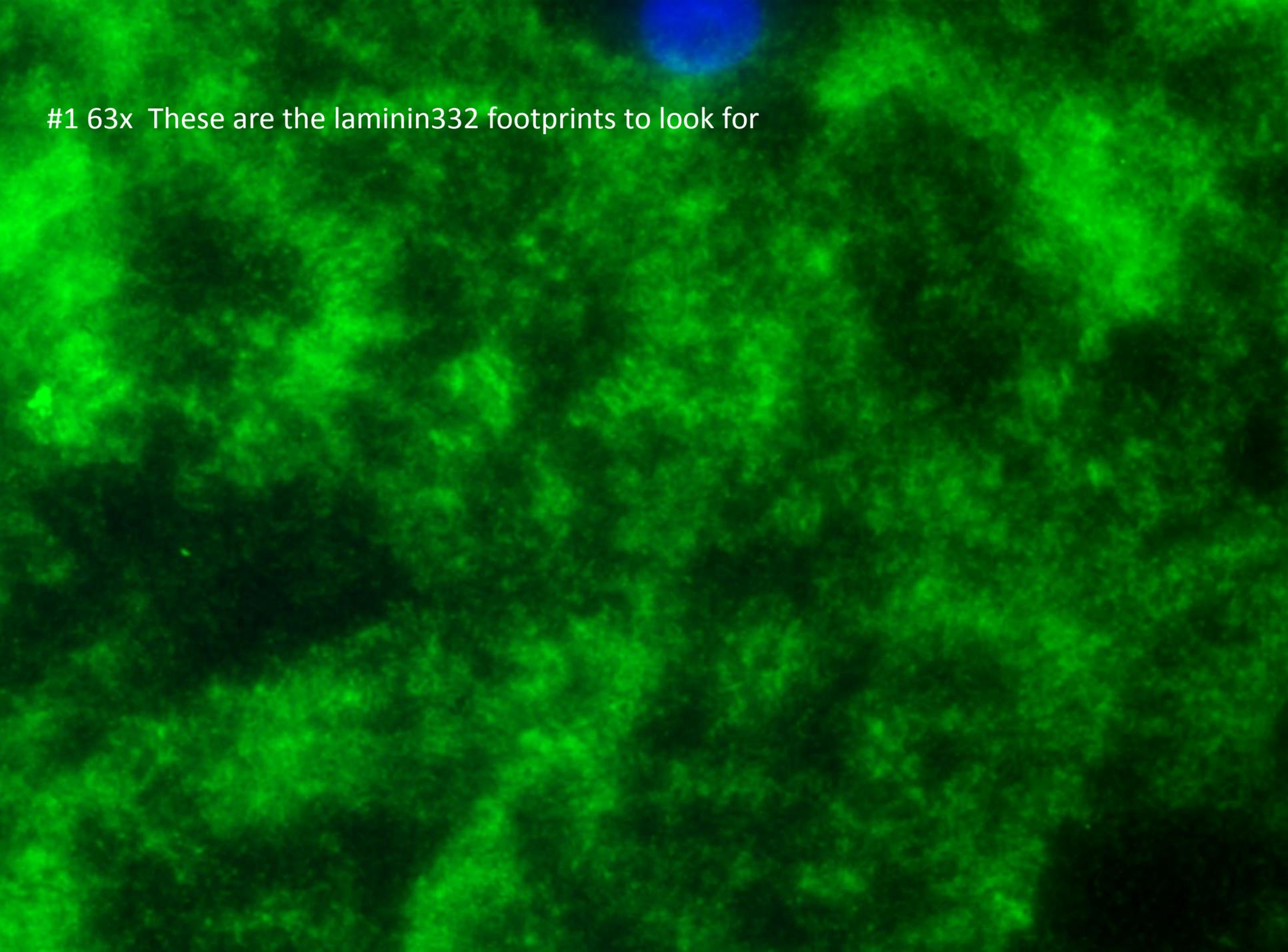
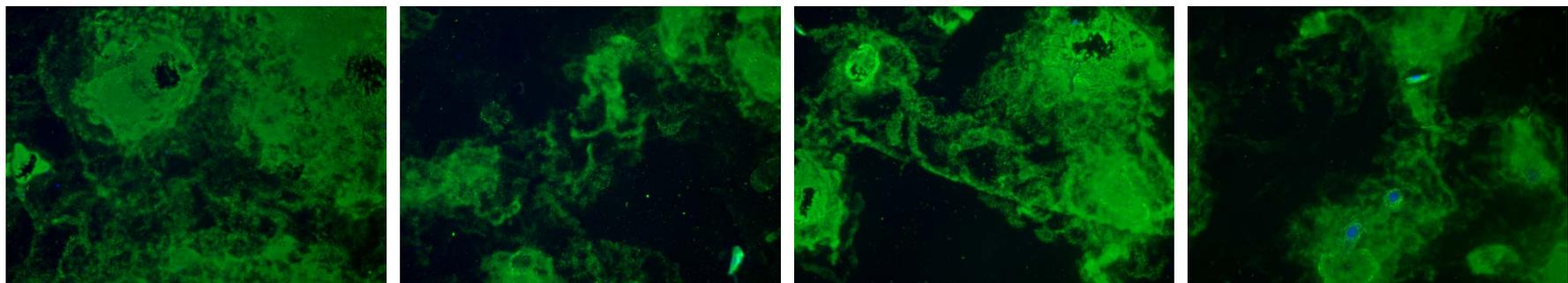
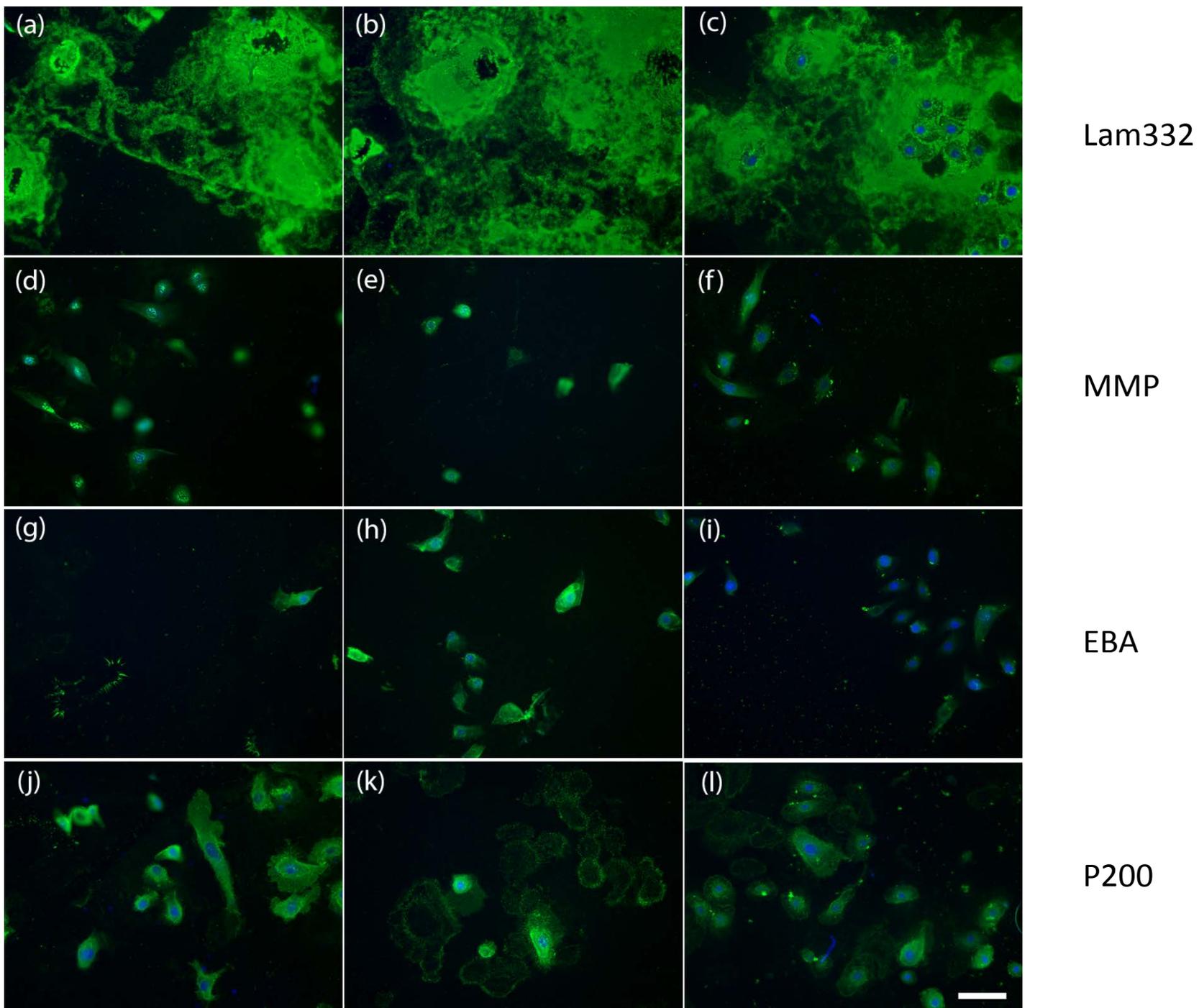


Table 1 Diagnostic findings in 16 patients with antilaminin-332 mucous membrane pemphigoid

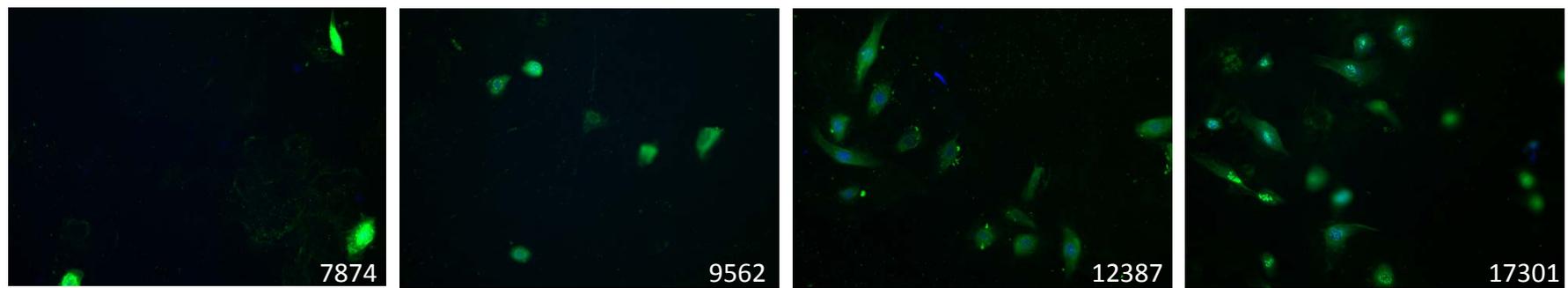
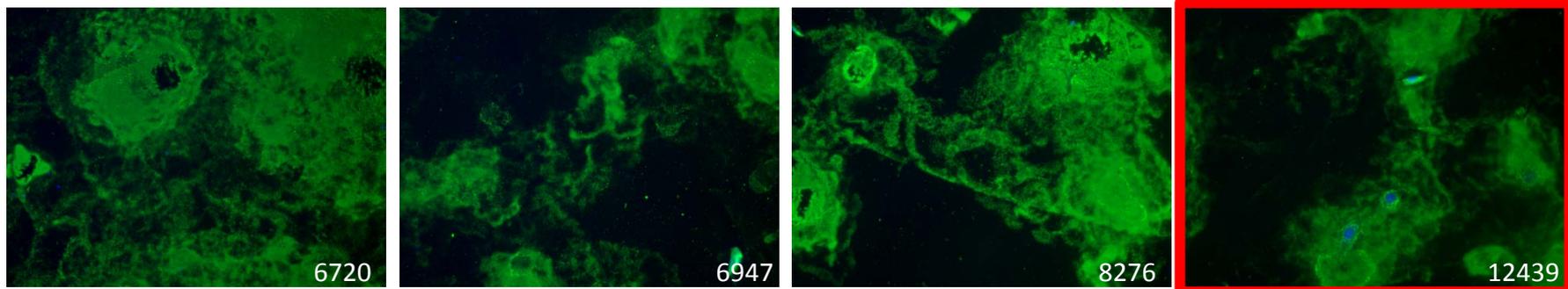
Sample	Sex	Age (years)	DIF (BMZ)	SSS dermal			ELISA		IIF (KO skin)		Cancer
				binding	IB Ln-332	IP Ln-332	Ln-332	Col7	Col7	Ln-332	
1	M	54	IgG 4+, C3c 3+, IgA 2+, IgM 2+	IgG 3+, IgA+	Neg	np	np	—	+	—	Yes
2	F	59	Neg	IgG 2+	$\alpha 3, \beta 3$	np	np	—	+	—	No
3	M	65	IgG 3+, C3c 2+, IgA 1+	IgG 1+	$\alpha 3$	np	np	np	np	np	No
4	F	86	IgG 3+, C3c 3+, IgA 2+, IgM \pm	IgG 1+	$\gamma 2$	np	np	—	+	—	No
5 ^a	M	27	IgG 2+, C3c 3+	IgG 2+	$\alpha 3$	np	+	—	np	np	No
6 ^a	M	25	IgG 3+, C3c 3+	IgG 2+	$\alpha 3$	np	+	—	np	np	No
7 ^a	F	65	IgG 3+, C3c +/2+	IgG 2+	$\alpha 3$	np	+	np	np	np	No
8 ^a	F	42	IgG 3+, C3c 2+	IgG 2+	$\alpha 3$	np	+	—	np	np	No
9 ^a	M	70	IgG 2+	IgG 1+	$\alpha 3$	+	np	np	np	np	Yes
10	M	82	IgG 3+, C3c 3+	IgG 3+	$\alpha 3, \beta 3, \gamma 2$	np	np	—	np	np	No
11	M	77	np	IgG 2+	$\alpha 3, \beta 3$	np	np	—	+	—	na
12 ^b	F	52	IgG 3+, C3c 3+, IgA 1+	IgG 2+	Neg	np	np	—	+	—	No
13	M	81	np	IgG 3+	$\alpha 3$	np	np	np	np	np	na
14 ^a	M	56	IgG 2+, IgA 2+	IgG dubious	$\alpha 3$	np	+	np	np	np	Yes
15	M	63	IgG 3+, C3c 2+	IgG 1+	$\alpha 3$	np	np	np	np	np	na
16 ^{a, c}	F	64	IgG 3+/4+, C3c +/2+	IgG 3+	$\alpha 3$	—	+	np	np	np	No

BMZ, basement membrane zone; Col7, type VII collagen; DIF, direct immunofluorescence; ELISA, enzyme-linked immunosorbent assay; F, female; IB, immunoblot; IIF, indirect immunofluorescence; IP, immunoprecipitation; KFA, keratinocyte footprint assay; Ln-332, laminin-332; M, male; na, not available; Neg, negative; np, not performed; SSS, salt-split skin. IP was performed with radiolabelled human keratinocyte extract.³⁴ IB was performed for all sera on extract of cultured keratinocytes, and for sera of patients 7, 9 and 16 also on human keratinocyte matrix proteins. ELISA for anti-Col7 was performed with a kit from MBL (Nagoya, Japan) and ELISA for anti-Ln-332 was performed on affinity-purified Ln-332 as described previously.³⁴ ^{a-c}Described previously by ^aTerra et al.,³⁴ ^bvan den Bos et al.³⁸ and ^cJonkman et al.³⁷

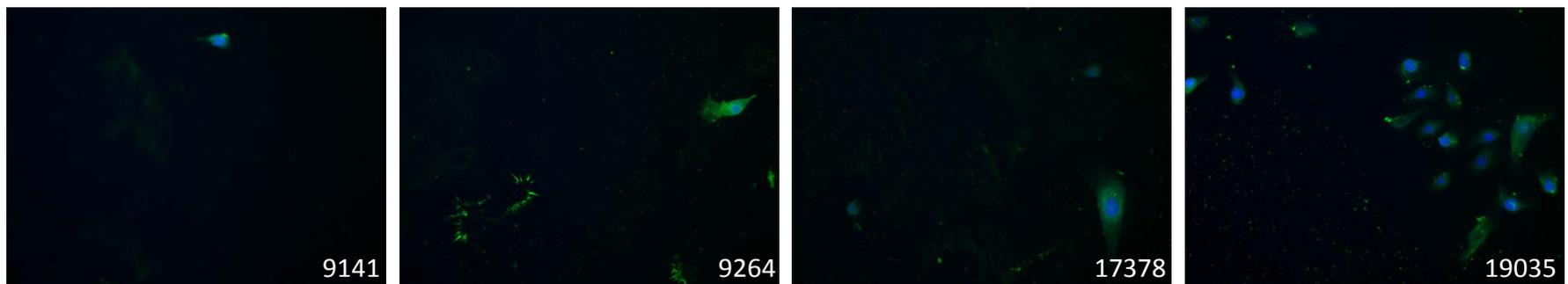




Serum on air-dried frozen cells



EBA



P200

Our patient

- Internal medicine: no evidence for malignancy
- ENT: supraglottic scarring
- Ophthalmologist: abnormalities consistent with cicatricial pemphigoid



Laminin-332 transfected cells

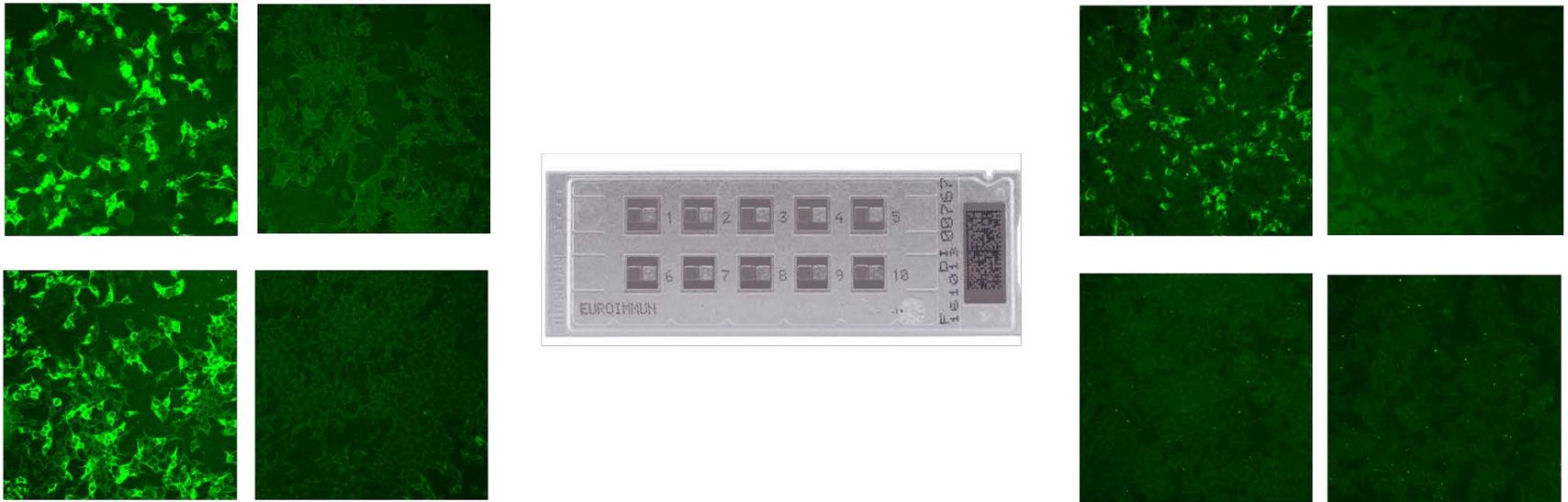


Figure 3. Representative pictures of immunofluorescence microscopy using membrane-bound recombinant laminin 332 on the surface of HEK293 cells (left field) and empty plasmid transfected cells as control (right field). Middle, example of a slide with 10 incubation fields. Red, blue, yellow; laminin 332 positive patients sera. Green, healthy blood donor.

Comparison laminin-332 transfected cells (Lubeck)

	Biochip assay	Footprint assay
Anti-laminin 332 mucous membrane pemphigoid	(51/53) 96%	(53/53) 100%
Mucous membrane pemphigoid	(5/35) 14%	(9/35) 26%
Pemphigus vulgaris	(0/30) 0%	(0/30) 0%
Healthy blood donor	(0/20) 0%	(0/20) 0%

Conclusion

- KFA new and reliable technique to diagnose anti-laminin 332 pemphigoid
- Game changer: comments BJD

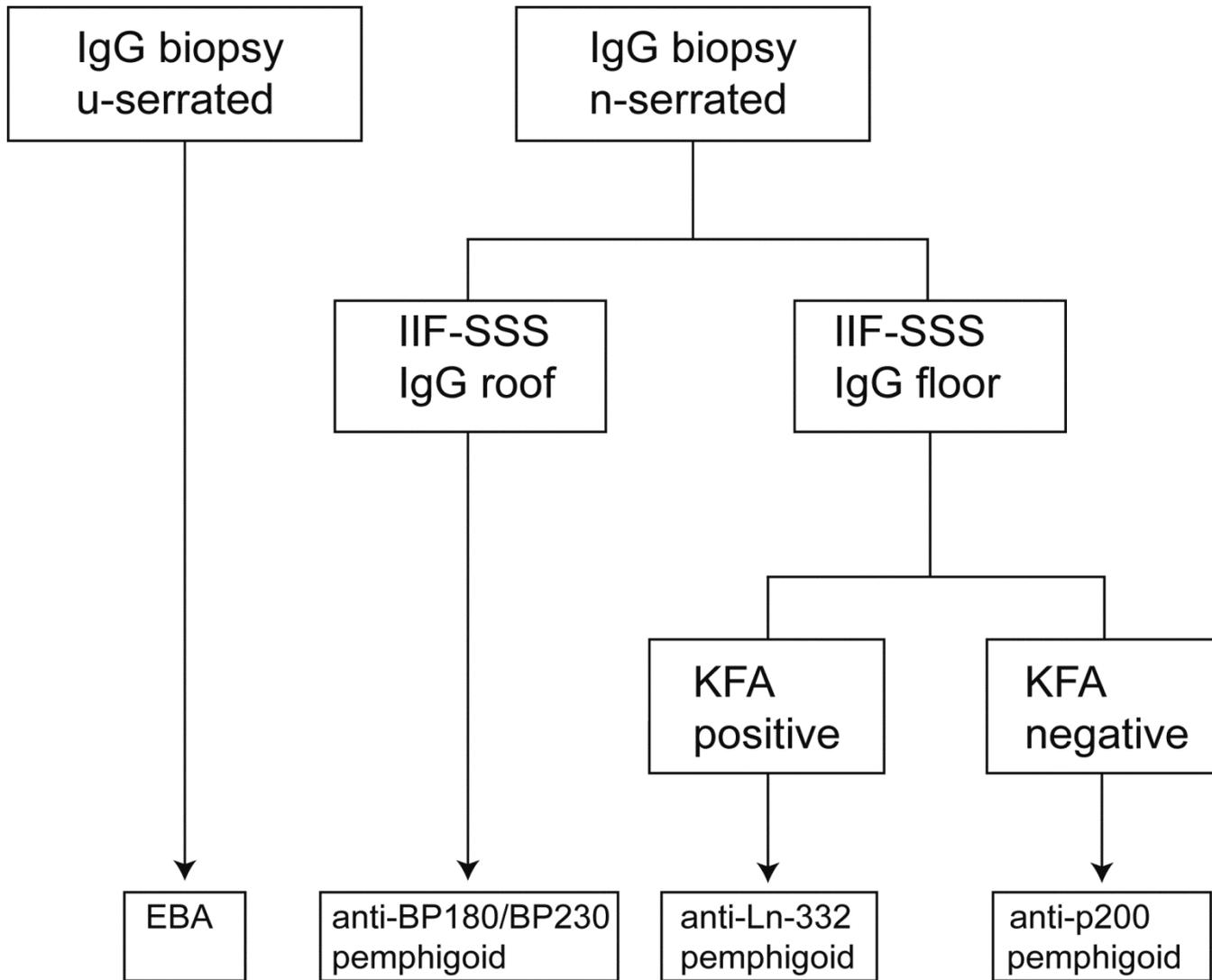
COMMENTARY

BJD
British Journal of Dermatology

Novel diagnostic method to differentiate antilaminin-332 pemphigoid from other forms of pemphigoid

pemphigoid diseases.

The keratinocyte footprint assay as the new diagnostic method provided here may change clinical practice, and we hope aid in the earlier diagnosis of antilaminin-332 pemphigoid. This may lead to faster remission of active disease and less scarring on the mucosa. Recently, a commercial biochip assay that detects antibodies to laminin-332 has been tested, which also detects these autoantibodies.⁴ It would be interesting to compare the sensitivity and specificity of both of these assays. These are useful for diagnostics, but neither is useful for disease activity in terms of quantifying intensity mathematically, as cur-



Keratinocyte Binding Assay



Keratinocyte Binding Assay Identifies Anti-Desmosomal Pemphigus Antibodies Where Other Tests Are Negative

*Federica Giurdanella, Albertine M. Nijenhuis, Gilles F. H. Diercks, Marcel F. Jonkman
and Hendri H. Pas**

*Department of Dermatology, University of Groningen, University Medical Center Groningen, Center for Blistering Diseases,
Groningen, Netherlands*

Research letter

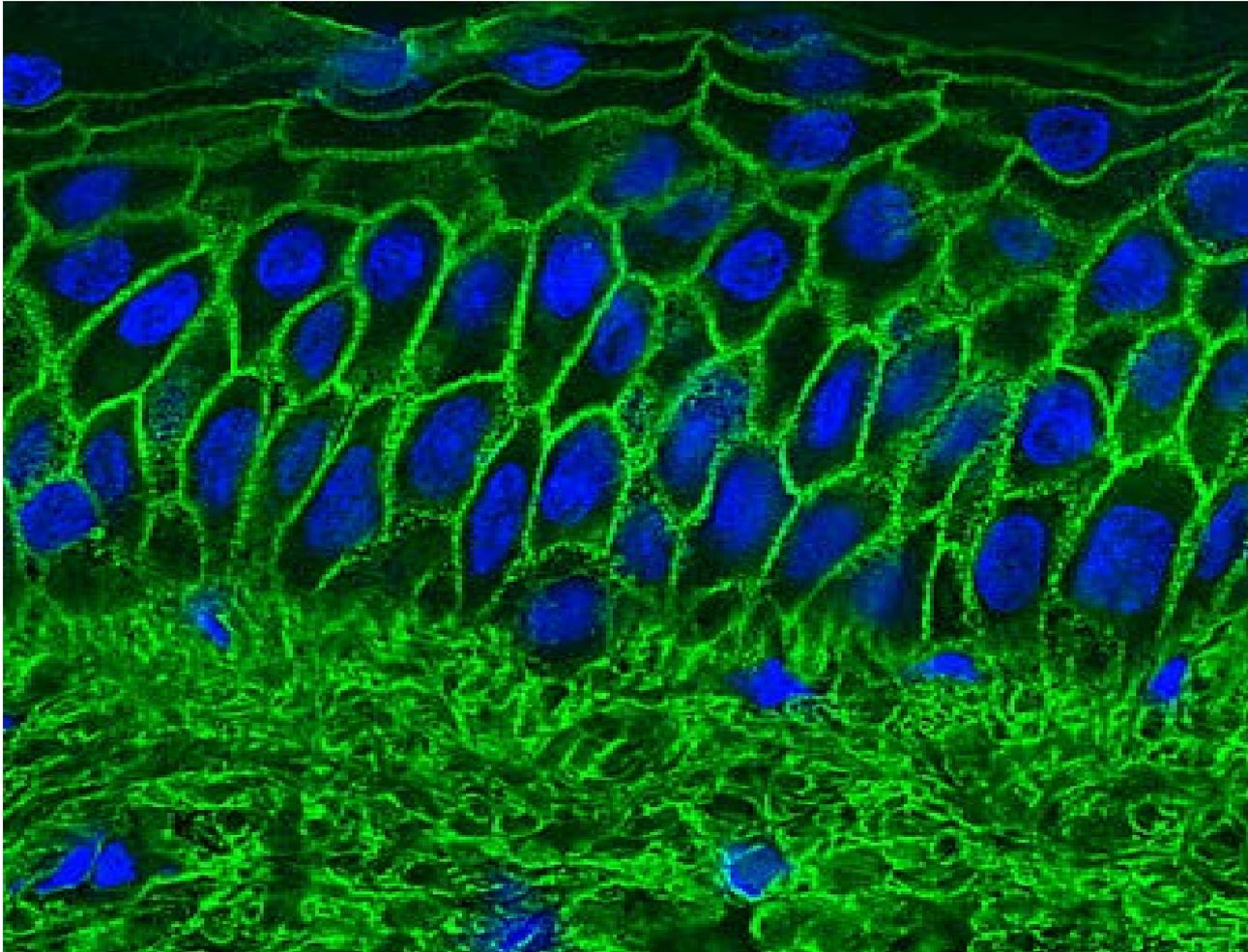
Laboratory diagnosis of pemphigus: direct immunofluorescence remains the gold standard

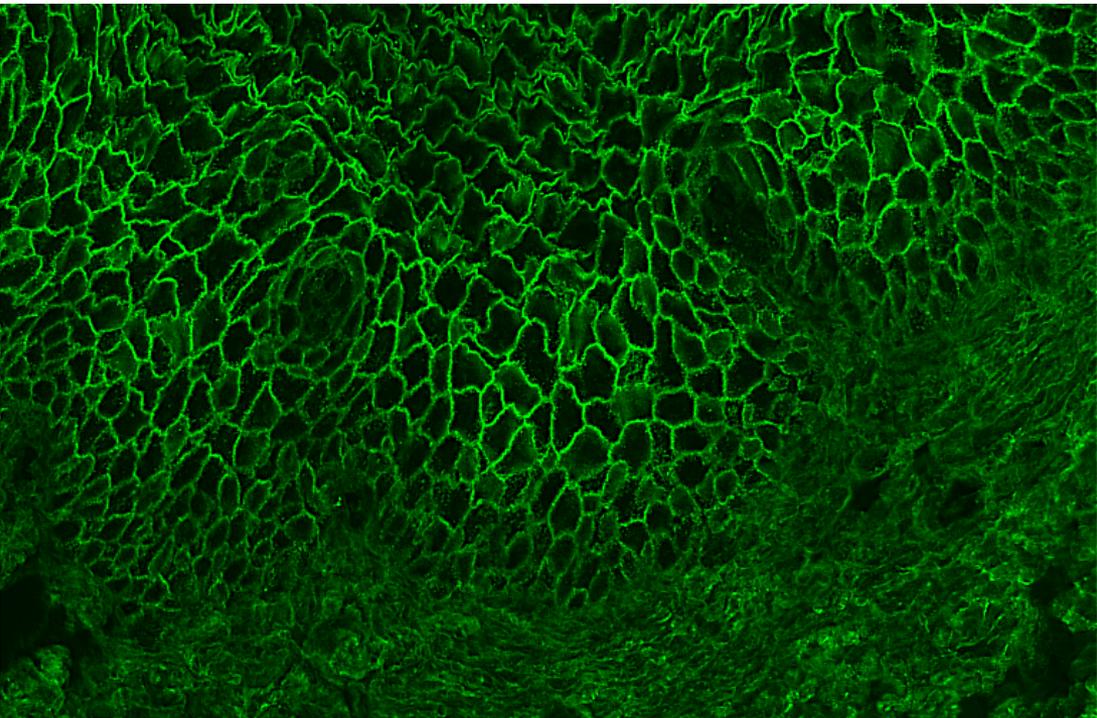
DOI: 10.1111/bjd.14408

stration of autoantibodies plays a crucial role to ascertain the diagnosis. Although several studies have compared the sensitivity of IIF vs. ELISA, data on the relative sensitivity of serum assays compared with the gold-standard DIF are lacking.⁴

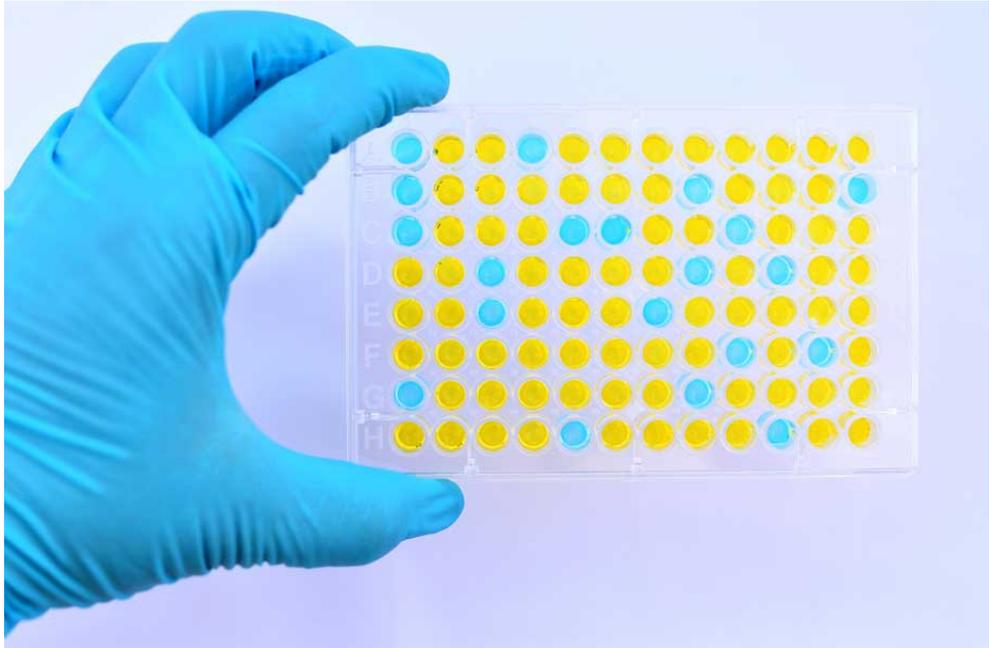
We therefore performed a retrospective analysis of sera from patients with pemphigus with a positive DIF from whom

Direct immunofluorescence



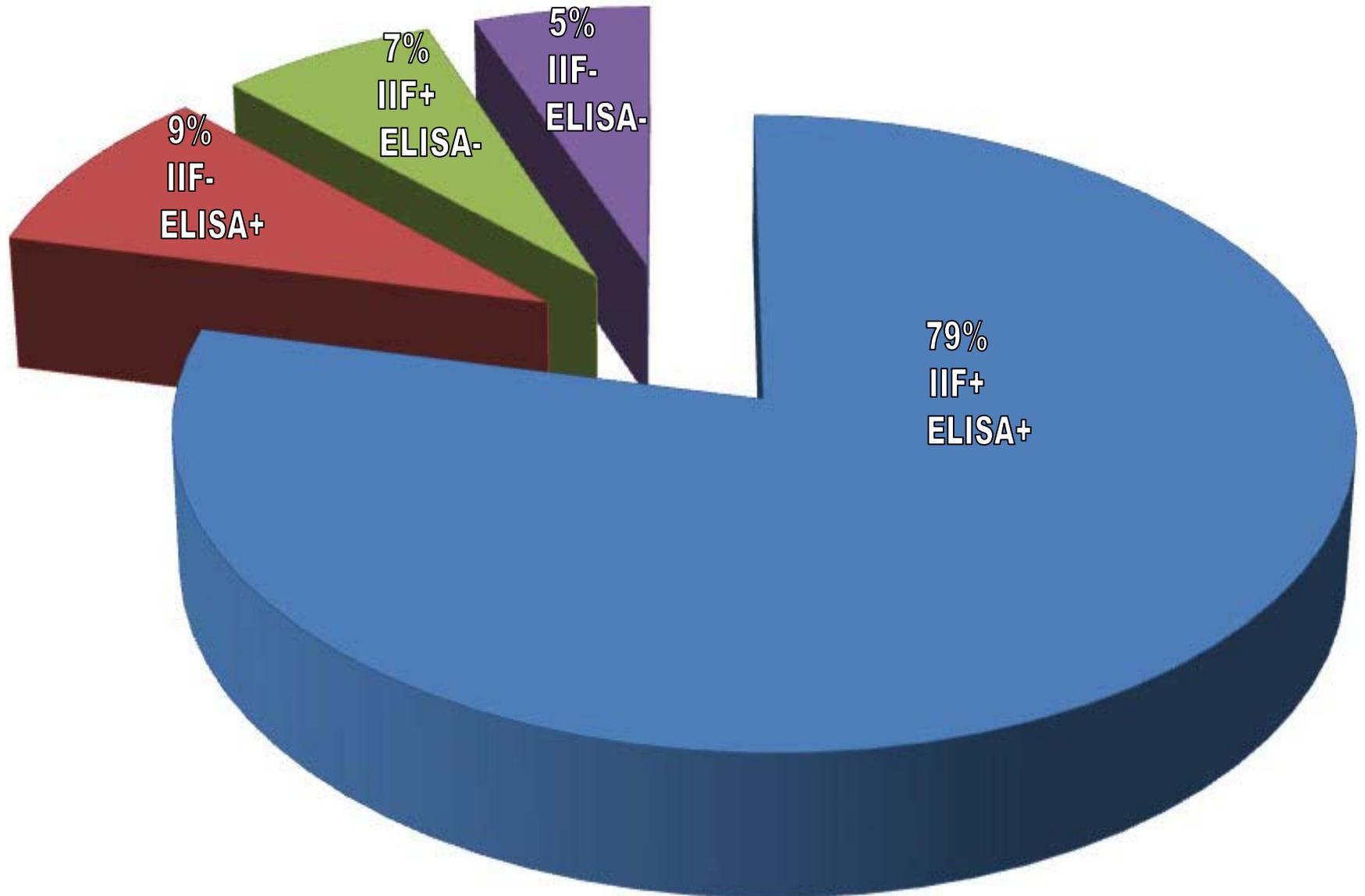


Monkey esophagus



Desmoglein ELISA

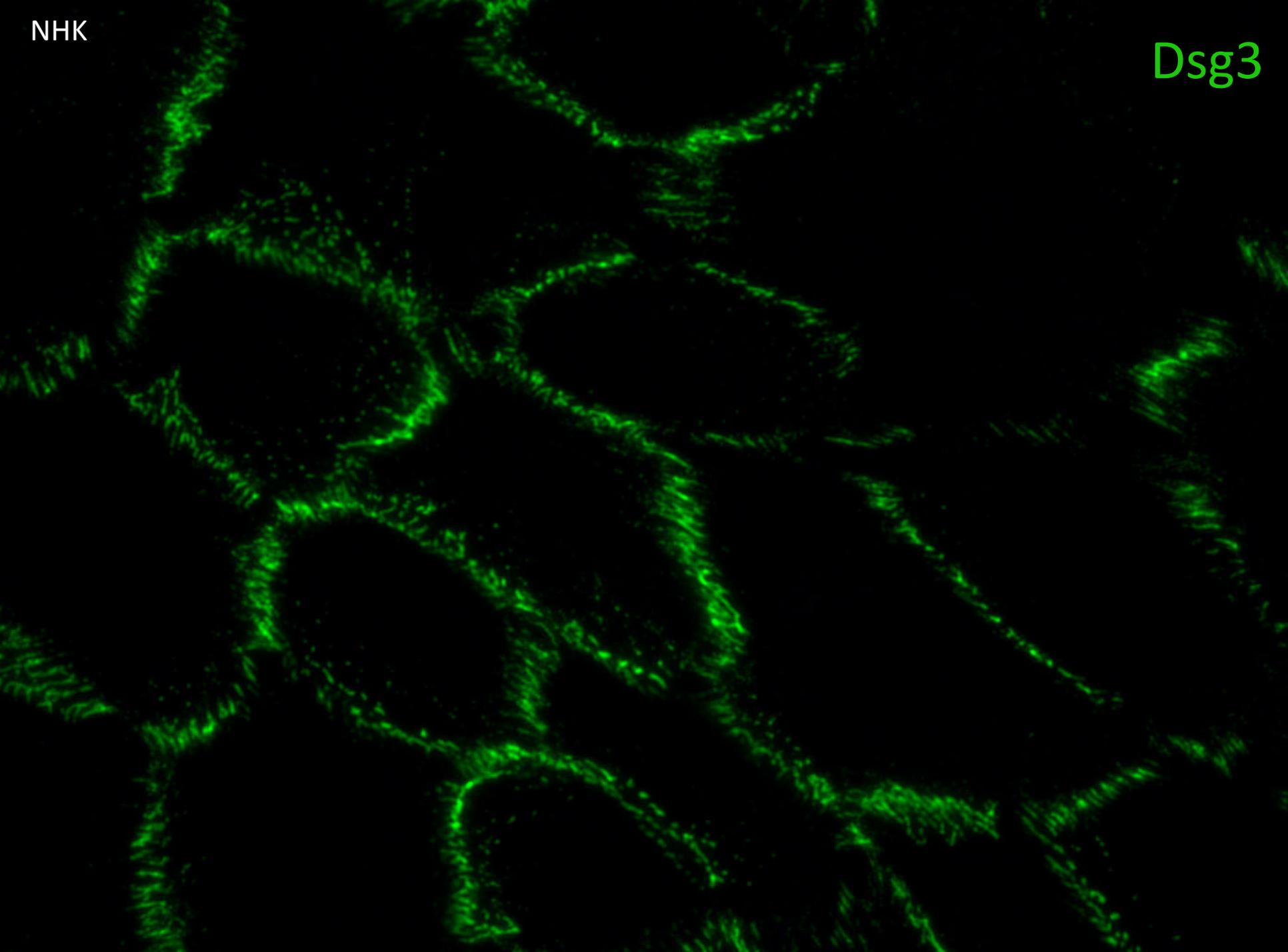
Serology of 234 patients with positive DIF



Inconsistent findings during the diagnostic process bring decision making challenges

NHK

Dsg3



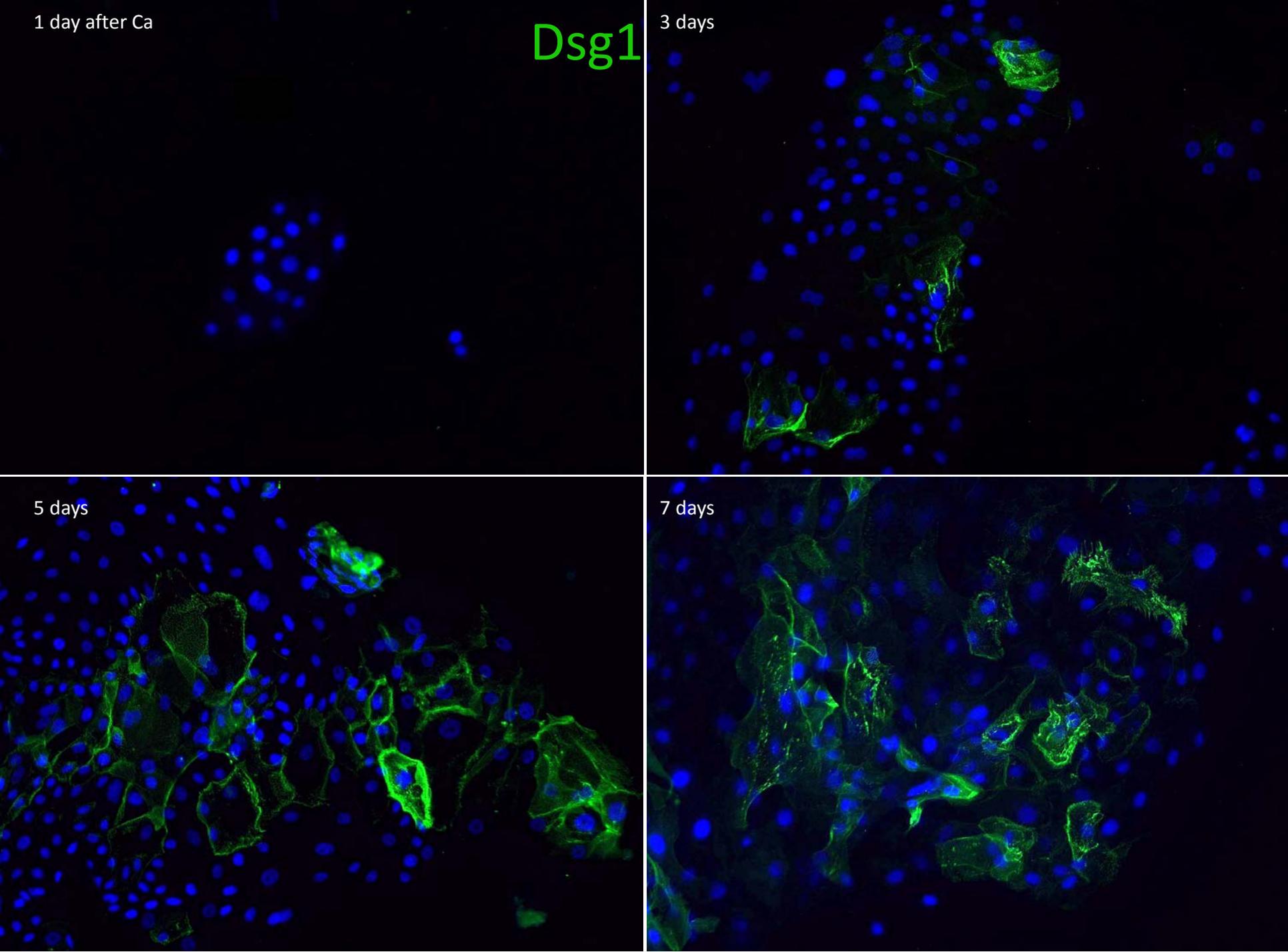
1 day after Ca

Dsg1

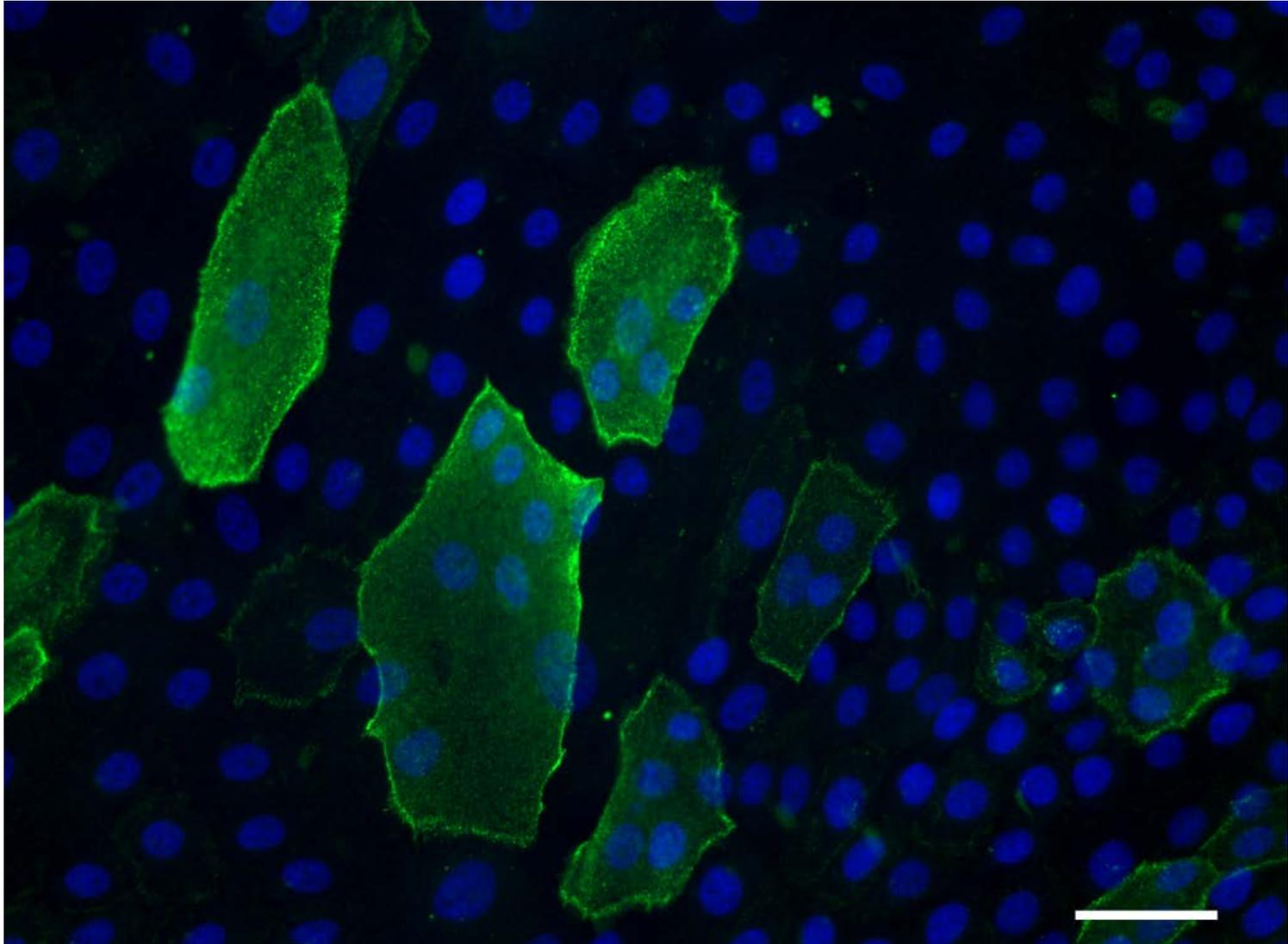
3 days

5 days

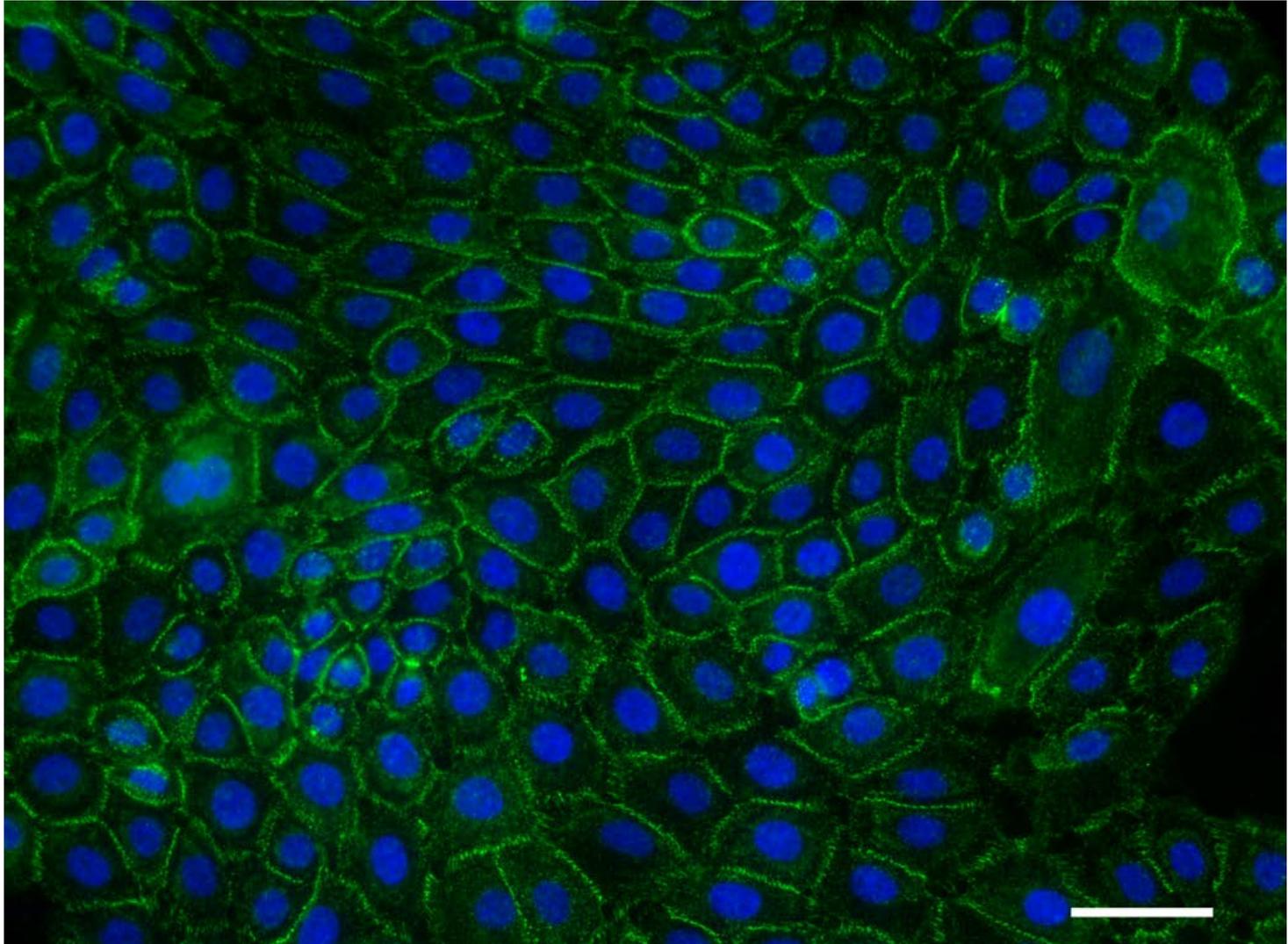
7 days



Pemphigus foliaceus (only DSG1)



Pemphigus vulgaris (DSG 1 and 3)





Keratinocyte Binding Assay Identifies Anti-Desmosomal Pemphigus Antibodies Where Other Tests Are Negative

Federica Giurdanella, Albertine M. Nijenhuis, Gilles F. H. Diercks, Marcel F. Jonkman and Hendri H. Pas*

Department of Dermatology, University of Groningen, University Medical Center Groningen, Center for Blistering Diseases, Groningen, Netherlands

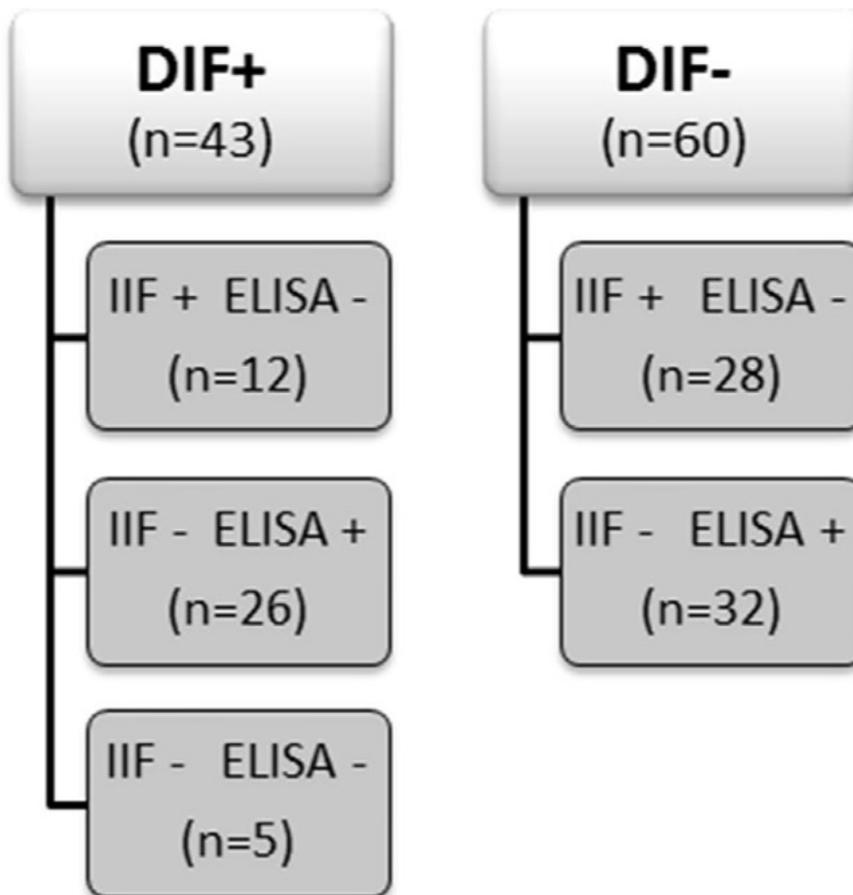


TABLE 3 | Values of sensitivity and specificity calculated for positive and negative controls, biopsy proven pemphigus patients and negative DIF patients groups.

Group	<i>n</i>	KBA+	Sensitivity (%)	Specificity (%)
Positive controls	50	50	100	–
D+ I+ E–	12	10	83.3	–
D+ I– E+	26	26	100	–
D+ I– E–	5	3	60	–
NHS	10	0	–	100
D– I+ E–	28	0	–	100
D– I– E+	32	0	–	100

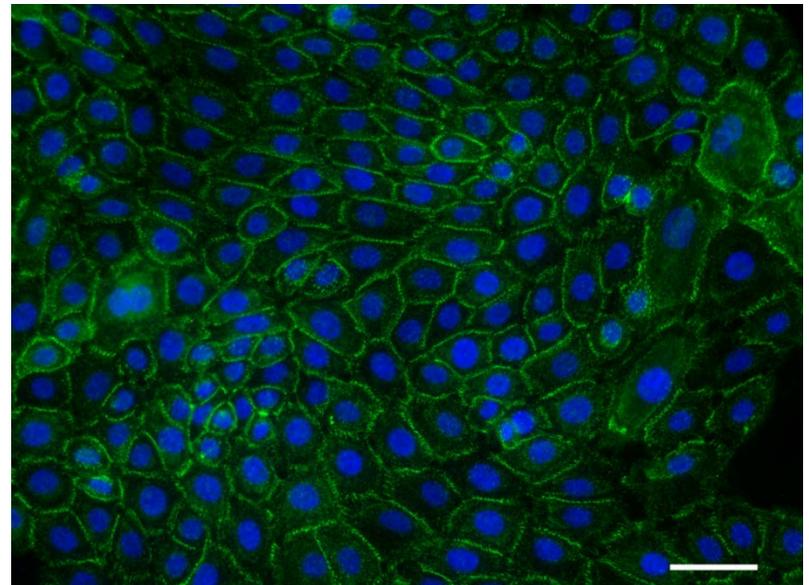
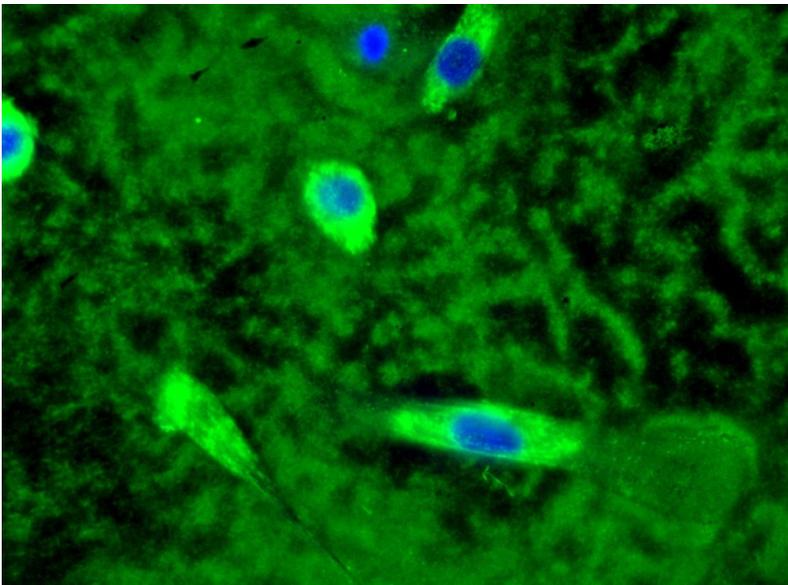
D, direct immunofluorescence; I, indirect immunofluorescence; E, Dsg ELISA; NHS, normal human subjects; KBA, keratinocyte binding assay.

TABLE 1 | Final diagnoses of patients in the direct immunofluorescence negative group.

Definite diagnosis	No. of cases
Lichen planus	10
Bullous pemphigoid	7
Pruritus sine materia	4
Aphthosis	3
Eczema	3
Impetigo bullosa	2
Pityriasis rubra pilaris	2
Prurigo nodularis	2
Pseudoporphyria	2
Vasculitis	2
Brachioradial pruritus	1
Dermatitis factitia	1
Erosive pustular scalp dermatosis	1
Genital ulcer	1
Gingivitis	1
Graft versus host disease	1
Lichen planus pemphigoid	1
Lupus erythematosus	1
Mucous membrane pemphigoid	1
Nummular eczema	1
Urticaria	1
Vulvar carcinoma	1

Conclusion

- Two new serological techniques for pemphigoid and pemphigus based on cultured keratinocytes
- Both are highly sensitive and specific
- Both tests can be used in difficult and challenging cases



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Hoe en wat voor materiaal kan ik opsturen?



Waar kan ik de aanvraagformulieren vinden?



Hoe lang duurt het voordat ik een uitslag kan verwachten?



Wat zijn de kosten?





Medewerkers Laboratorium Immunodermatologie



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Marcel F. Jonkman
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Autoimmune Bullous Diseases

Text and Review

 Springer

