Development of a qualitative ELISA for the detection of anti-BICD2 autoantibodies in systemic sclerosis

Daniel Wirtz[1], Johannes Schulte-Pelkum[1], Petra Budde[1], Peter Schulz-Knappe[1], Hans-Dieter Zucht[1], Suzana Jordan[2], Oliver Distler[2], Britta Maurer[2] , Nicolas Hunzelmann[3], Karsten Conrad[4], Matthias Schneider[5]

[1]Protagen AG, Dortmund, Germany; [2]Division of Rheumatology, University Hospital Zurich, Switzerland; [3]Universitätsklinikum Köln, Klinik für Dermatologie und Venerologie, Köln, Germany; [4]Carl Gustav Carus Universität, Dresden, Germany; [5]Heinrich Heine Universität, Poliklinik für Rheumatologie, Düsseldorf, Germany

Introduction

Systemic sclerosis (SSc) is a systemic autoimmune disease that manifests as progressive fibrosis of the skin and internal organs[1,2]. We recently conducted autoantibody profiling studies in SSc using the Protagen SeroTag® process and found novel SSc-associated autoantibodies with the potential to improve the diagnosis of SSc and other systemic autoimmune diseases (SARD). Beyond the classical markers such as anti-centromere and anti-Scl70 antibodies, which are generally exclusive of the other, novel autoantibodies were identified. Here, we report the development of the development of a qualitative ELISA for the detection of human BICD2 autoantibodies. BICD2 (Protein bicaudal D homolog 2) is an evolutionarily conserved motor adaptor protein that is involved in dynein-mediated transport by linking the dynein motor complex to various cargoes.

Methods

We developed an ELISA using a highly purified recombinant fragment of human BICD2, the Protagen® MULTILISA® BICD2-ELISA.
The assay principle is described in Fig. 1.
Two lots of kit-material were independently manufactured using two different lots of purified BICD2 protein. Quality control was performed with a quality control panel of 8 samples.

The MULTILISA® BICD2-ELISA was calibrated using receiver operating characteristics (ROC) analysis to 95% specificity.

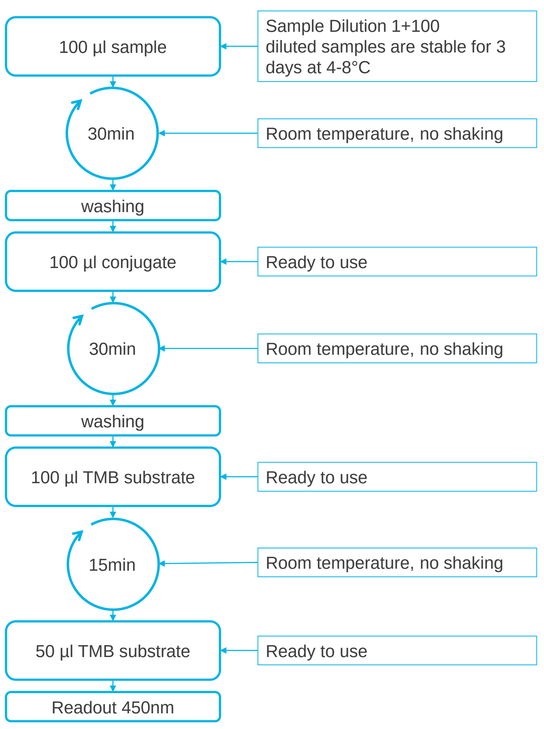
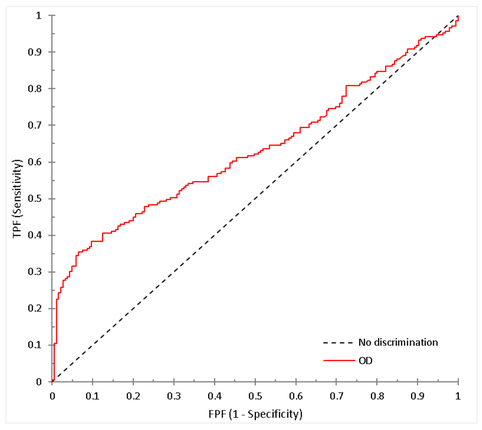


Figure 1: Test procedure of the Protagen® MULTILISA® BICD2-ELISA

Diagnostic performance

A mixed cohort consisting of SSc, SARD and healthy donor samples (n=394) were tested with the Protagen® MULTILISA® BICD2-ELISA. Data were analyzed using ROC analysis (Fig. 2).

Group	N
Arthritis	19
Healthy donor	98
Myositis	19
Sjögren	11
SLE	38
Ssc	209
Pooled	394



TP proportion (Sensitivity)	TN proportion (Specificity)	Predictive value (+)	Predictive value (-)
0.316	0.951	0.91	0.48
0.225	0.989	0.97	0.46

Figure 2: ROC analysis of 394 samples of a mixed cohort tested with Protagen® MULTILISA® BICD2-ELISA. The ROC analysis show 31% sensitivity at 95% specificity and 22% sensitivity at 99% specificity (Table 2). The cutoff was determined at 0.650 OD and fixed by a ready-to-use calibrator.

Linearity analysis

Four positive samples were stepwise diluted with a negative sample and measured using the Protagen® MULTILISA® BICD2-ELISA. The calculated Index (ratio from OD_{sample} and $OD_{calibrator}$) was plotted over sample amount (Fig. 3).
The samples show linear dilution behavior in a range between I=0.2 and I=4. This covers an OD Range between 0.150 OD and 2.600 OD.

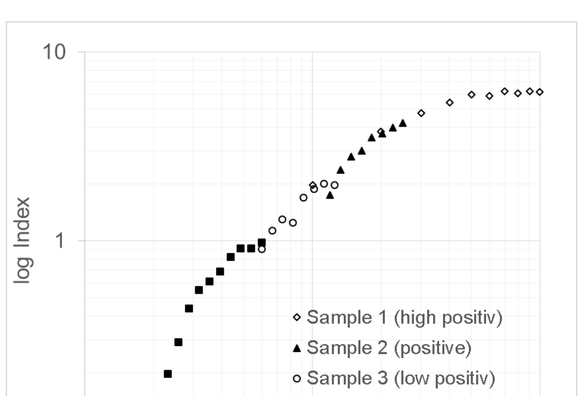


Figure 3: Dilution series of 4 positive sample with a negative sample. Plotted are the calculated Index of the sample against the amount of sample in double logarithmic scale

Added value

173 SSc-samples were analyzed with one lot of the Protagen® MULTILISA® BICD2 ELISA. SSc patients tested positive for anti-BICD2, anti-Scl-70 and anti-CENPB autoantibodies are shown in a Venn-Diagram (Fig. 3).

The prevalence of a-Scl70, a-CENPB and a-BICD2 autoantibodies were comparable in this cohort. The anti-BICD2 assay identifies 7 additional patients, who were tested negative for anti-Scl70 and anti-CENPB.

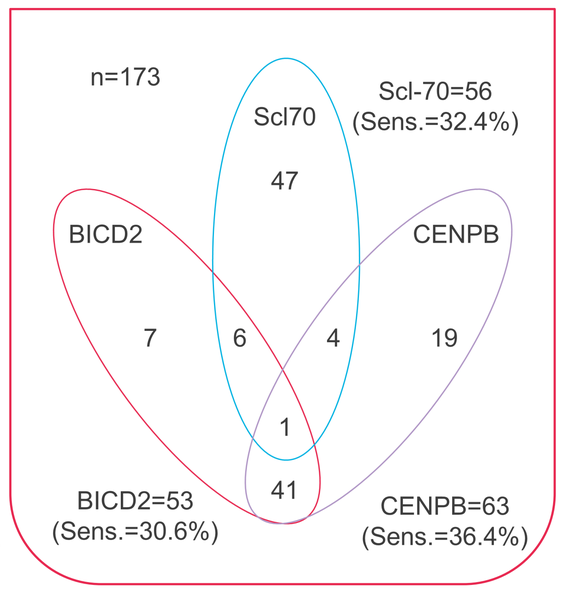


Figure 3: VENN Diagram showing the number of SSc sera with positive results for BICD2, Scl70 and CENPB

Verification data

Two lots of material were used to verify the assay and to determine the lot-to-lot consistency, intra-and inter-assay-variability, robustness against interference substances and the accelerated stability.

Lot-to-lot consistency

Repeatability n=72	Within Lot	Lot-to-Lot	Cal-to-Cal
Slope	1.09	1.003	0.9584
	95% CI 1.04 to 1.11	95% CI 0.95 to 1.03	95% CI 0.958 to 0.958
Intercept	0.006	0.007	0
	95% CI -0.006 to 0.019	95% CI -0.0008 to 0.017	95% CI -1.59*10^-17 to 2.1*10^-17

Intra-/inter-assay variance

Intra-assay n=30 Unit = Index (Sample/Calibrator)	Mean	SD	95% CI		CV
Sample 1 (positive)	1.6	0.1	0.0	0.2	5%
Sample 2 (negative)	0.4	0.0	0.0	0.0	2%
Sample 3 (negative)	0.5	0.0	0.0	0.0	4%
Sample 4 (Intermediate)	1.1	0.1	0.1	0.2	8%
Sample 5 (high positive)	3.1	0.1	0.0	0.2	2%

Inter-assay n=150 Unit = Index (Sample/calibrator)	Mean	SD	95% CI		CV
Sample 1 (positive)	1.6	0.1	0.1	0.1	6%
Sample 2 (negative)	0.4	0.0	0.0	0.0	7%
Sample 3 (negative)	0.5	0.1	0.0	0.1	11%
Sample 4 (Intermediate)	1.2	0.2	0.2	0.3	16%
Sample 5 (high positive)	3.2	0.1	0.1	0.2	4%

Robustness against interference substances

Interference substance (n=108)	Result
Hemoglobin	Up to 300mg/dl
Bilirubin	Up to 18 mg/dl
Intralipid	Up to 1000mg/ml
Rheumatoid factor	Up to 500 IU/ml

Accelerated stability at 37°C

Residual activity [%]	Plate at 37°C [days]	Calibrator at 37°C [days]	Conjugate at 37°C [days]
90%	19	52	20
85%	27	68	30
80%	35	83	41

Assay portability studies

Test portability was verified using bead based assay, and Western blot / Line immunoassay studies. The preliminary results show high correlation of all results to ELISA (data not shown).

Conclusion

We developed a qualitative ELISA for detecting anti-BICD2 autoantibodies, which occur in 30% of SSc patients. The assay was validated by analyzing SSc samples and SARD control samples. The availability of a validated, qualitative BICD2-ELISA may improve the specific detection of SSc and reduce the diagnostic gap.
Verified RuO-Kits of the Protagen® MULTILISA® BICD2-ELISA will be available in November 2015.

References
1. Tan EM: **Autoantibodies to nuclear antigens (ANA): their immunobiology and medicine.** *Adv Immunol* 1982, **33**:167–240.
2. Hudson M, Fritzler MJ: **Diagnostic criteria of systemic sclerosis**. *J Autoimmun* 2014, **48-49**:38–41.
3. Meier FMP, Frommer KW, Dinser R, Walker U a., Czirjak L, Denton CP, Allanore Y, Distler O, Riemekasten G, Valentini G, Muller-Ladner U: **Update on the profile of the EUSTAR cohort: an analysis of the EULAR Scleroderma Trials and Research group database**. *Ann Rheum Dis* 2012, **71**:1355–1360.
4. Müller-Ladner U, Tyndall A, Czirjak L, Denton C, Matucci-Cerinic M: **Ten years EULAR Scleroderma Research and Trials (EUSTAR): what has been achieved?** *Ann Rheum Dis* 2014, **73**:324–7.

Protagen AG Otto-Hahn-Str. 15 44227 Dortmund Germany **T** +49 231 9742 6300 **E** info@protagen.com **W** protagen.com