



## A cross-reactive monoclonal antibody as universal detection antibody in autoantibody diagnostic assays



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### ABSTRACT

Diagnostics of Autoimmune Diseases involve screening of patient samples for containing autoantibodies against various antigens. To ensure quality of diagnostic assays a calibrator is needed in each assay system. Different calibrators as recombinant human monoclonal antibodies as well as chimeric antibodies against the autoantigens of interest are described. A less cost-intensive and also more representative possibility covering different targets on the antigens is the utilization of polyclonal sera from other species. Nevertheless, the detection of human autoantibodies as well as the calibration reagent containing antibodies from other species in one assay constitutes a challenge in terms of assay calibration. We therefore developed a cross-reactive monoclonal antibody which binds human as well as rabbit sera with similar affinities in the nanomolar range. We tested our monoclonal antibody S38CD11B12 successfully in the commercial *Serazym*® Anti-Cardiolipin- $\beta$ 2-GPI IgG/IgM assay and could thereby prove the eligibility of S38CD11B12 as detection antibody in autoimmune diagnostic assays using rabbit derived sera as reference material.

### 1. Introduction

In vitro diagnostics of Autoimmune Diseases (AD) comprises the detection of autoantibodies against a broad range of different autoantigens. Many systemic and organ specific autoantibodies are of major importance for clinical characteristics, for primary and differential diagnosis and prognostic of autoimmune diseases respectively [1]. Despite increasing possibilities and importance of AD diagnostics, the harmonization and standardization of available assays still underlie several problems [2]. A lack of assay reproducibility and consequential reliability has been frequently described concerning autoantibody assays such as those for antiphospholipid syndrome (APS) diagnostics [3–5].

In many of those cases the choice of a relevant positive reference serum (positive control) already causes unwanted problems. For the diagnostics of AD an ideal calibrator would be a polyclonal immunoglobulin preparation representing the natural occurring autoantibody subpopulations [6]. However polyclonal reference material derived from human patients is of limited availability and therefore

expensive and difficult to collect in sufficient amounts. Alternatively, monoclonal antibodies (mAbs) against the autoantigen converted into chimeric antibodies can be used as controls in enzyme-immunoassays [7,8]. But they would be limited to special assays and are also difficult to produce. Recombinant human mAbs could be developed by phage display technologies like among others the HuCAL technology and might be a promising solution [9–11]. The problem is that mAbs only react against one epitope and do not cover the entire spectrum of possible binding sites as they are present in a polyclonal serum.

Therefore an easier way would be to use polyclonal antibodies generated in another species (e.g. rabbits) as positive control and detect the human antibodies and the rabbit control antibodies with one reagent binding the antibodies of the different species with comparable binding capacity. We tried this last possibility and generated a cross-reactive monoclonal antibody through immunization with recombinant rabbit Immunoglobulin G (IgG)-Fc fragment derived from expression in the transgenic host *Leishmania tarentolae*. The resulting mAb S38CD11B12 binds human and rabbit IgG with similar affinity. We used the antibody in an immunoassay for the APS diagnostics. This

**Abbreviations:** AD, Autoimmune Diseases; APS, antiphospholipid syndrome; mAb, monoclonal antibody; h, hours; PBS, Phosphate-buffered saline; NiNTA, nickel-nitrilotriacetic acid; ELISA, Enzyme-linked immunosorbent assay; hu, human; ra, rabbit; IgG, Immunoglobulin G; NCS, neonatal calf serum; RT, room temperature; HRP, horseradish peroxidase; TMB, Tetramethylbenzidine;  $k_a$ , Association rate;  $k_d$ , dissociation rate;  $K_D$ , equilibrium constant; GPI, glycoprotein I

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commercial assay allows the detection of human autoantibodies against cardiolipin- $\beta$ 2GPI. Polyclonal rabbit anti-cardiolipin- $\beta$ 2GPI serves as reference material. The newly produced anti-human/rabbit IgG antibody could be applied in this commercial immunoassay in a similar way as the original detection reagents from the assay system. This mAb improves not only standardization in APS diagnostics, as it is shown here for the example of an anti-cardiolipin- $\beta$ 2GPI assay, but might also be a solution in autoimmune diagnostics in general when using rabbit-derived serum as reference material.

## 2. Material and methods

### 2.1. Production of recombinant rabbit IgG-Fc fragments used for immunization to generate monoclonal human/rabbit IgG antibodies

The expression plasmid for constitutive, secretoric expression of recombinant rabbit IgG-Fc in parasite cells was constructed using a commercial vector pLEXSY-sat2 (Jena Bioscience, Jena, Germany). The sequence encoding the CH2-CH3 domain of rabbit IgG-Fc (UniProt ID P01870) was amplified by PCR using a synthetic gene (Geneart, Regensburg, Germany) as template. The oligonucleotide primers used for rabbit IgG-Fc were forward 5- GCGTCTAGACCCG AGC ACC TGT AGC AAA C-3 (sense, *Xba*I site underlined, first codon highlighted), and reverse 5- CGCGGTACC TTT ACC AGG GCT GCG ACT AA-3 (antisense, *Kpn*I site underlined, no stop codon). The PCR product was digested with *Xba*I and *Kpn*I and cloned into the corresponding restriction sites of vector pLEXSY-sat2 resulting in a C-terminally His-tagged expression construct. *Leishmania tarentolae* cells were transfected by electroporation. pLEXSY-sat2 containing the DNA sequence of rabbit IgG-Fc was first digested with *Swa*I restriction enzyme and the linear expression cassette (without *E. coli*-relevant origin and including the antibiotic selection marker neomycin (NTC) was purified by gel extraction. Parasite cells in logarithmic growth phase were centrifuged (2500 xg, 5 min), resuspended in LEXSY BHI liquid medium at a final concentration of  $1 \times 10^8$  cells/mL and kept on ice for 10 min prior to the addition of 2.5  $\mu$ g of the linearized expression cassette. Following electroporation (Eppendorf Multiporator, 2 mm cuvette, 1000 V, 160  $\mu$ s) and incubation on ice for 10 min, cells were resuspended in fresh BHI culture medium and grown in suspension for the next 24 h. Single colonies derived from NTC resistant cells were selected after 9 days of growth on solid medium (BHI-agar containing 100  $\mu$ g/mL NTC) and maintained in suspension culture with constant antibiotic concentration. Genomic integration of the expression cassette in rabbit IgG-Fc transfected strains was verified by diagnostic PCR with oligonucleotide primers flanking the expression cassette (Jena Bioscience, Jena, Germany).

For production of recombinant rabbit IgG-Fc, transformed parasite cells derived from a single colony were grown at 26 °C in 1 L Erlenmeyer flasks on orbital shakers (120 rpm). Cells were separated by low-speed centrifugation 72 h post infection, at 3500 xg (Sorvall Lynx 6000, Thermo Fisher Scientific, Germany; fixed angle rotor 6  $\times$  1000 mL) for 20 min at 4 °C. Clarified cell culture supernatant was concentrated by tangential flow filtration using Vivaflow 200 modules (Sartorius, Göttingen, Germany) and the concentrated supernatant containing secreted recombinant protein was processed as described below.

Recombinant rabbit IgG-Fc from concentrated *L. tarentolae* cell culture supernatant was purified by nickel-nitrilotriacetic acid (NiNTA) affinity chromatography. The supernatant was incubated with 3 mL of pre-equilibrated NiNTA agarose (Qiagen, Hilden, Germany) on a rotating wheel for 16 h at 4 °C. The NiNTA agarose with bound protein was packed in a 10 mL chromatography column (Tricorn 10/100; GE Healthcare, Freiburg, Germany). Weakly bound and contaminating proteins were washed from the agarose gel with washing buffer by using 10 x the column volume (20 mM Tris, 0.3 M NaCl, pH 7.4). The recombinant His-tagged protein was finally eluted from the packed bed

with 3 x the column volume elution buffer (20 mM Tris, 0.3 M NaCl, 250 mM imidazole, pH 8.0). Eluted fractions were analyzed by UV absorbance at 280 nm using a BioDrop Touch Duo spectrophotometer (BioDrop, Serva Electrophoresis, Heidelberg, Germany). Protein containing fractions were pooled and dialyzed against Phosphate-buffered saline (PBS, pH 7.4).

### 2.2. Immunization and production of monoclonal antibodies

For immunization two fourteen weeks old mice (strain Balb/c) were injected intraperitoneally with 100  $\mu$ g of recombinant rabbit IgG Fc-fragment per mouse and additional 65  $\mu$ L of complete Freund's adjuvant in a final volume of 250  $\mu$ L (diluted in PBS) for each mouse. Immunizations were performed according to international ethical guidelines and approved by the Brandenburg Ministry of Environment, Health, and Consumer Protection (reference number V3-2347-A16-4-2012). After 24 days, mice were boosted with 70  $\mu$ g antigen dissolved in PBS. Blood samples from both animals were taken at day 30. Serum was prepared by centrifugation and analyzed positive for an immune response via indirect Enzyme-linked immunosorbent assay (ELISA) as described below. A third and fourth injection with 70  $\mu$ g antigen each was performed after one year with four days in between them. Four days after the final boost the spleen was removed and splenocytes were harvested under sterile conditions. Splenocytes were fused with myeloma cells (cell line SP2/0-Ag14) by electrofusion in the presence of polyethylene glycol. The cell fusion and also the selection processes were performed as described before [12]. Monoclonal cell clones were cultivated and supernatants were repeatedly tested in indirect ELISA experiments. One clone was chosen and cultivated in large scale for collecting supernatant. MAb was purified by protein A affinity chromatography as described elsewhere [12].

### 2.3. ELISA to determine antibody specificity

To determine the reactivity of the mAb S38CD11B12 cell culture supernatant as well as purified mAb S38CD11B12 were tested for binding of human (hu) and rabbit (ra) IgG (Merck KGaA, Darmstadt, Germany) by indirect ELISA. Human and rabbit IgG were coated as antigen on the solid phase of 96 well microtiter plates (5  $\mu$ g/mL dissolved in PBS, 50  $\mu$ L per well, overnight at 4 °C, Greiner Bio-One International GmbH, Frickenhausen, Germany). Free binding sites were blocked by PBS with 5% of neonatal calf serum (NCS) at room temperature (RT) (100  $\mu$ L per well) for 45 min. After washing with tap water either cell culture supernatant or mAb S38CD11B12 in a dilution series (3-fold from 5  $\mu$ g/mL to  $8.5 \times 10^{-5}$   $\mu$ g/mL in PBS/5% NCS) were incubated with the immobilized antigen at RT for 1 h. After washing, plates were incubated at RT with a horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, USA) 1 to 5000 diluted in PBS/5% NCS for 45 min. Unbound conjugated molecules were removed by washing with tap water. Tetramethylbenzidine (TMB, Carl Roth GmbH, Karlsruhe, Germany) served as substrate for HRP enzyme and 50  $\mu$ L of substrate solution were used for each well (0.12 mg/mL TMB in 50 mM NaH<sub>2</sub>PO<sub>4</sub> with 0.04% carbamideperoxide CH<sub>4</sub>N<sub>2</sub>O·H<sub>2</sub>O<sub>2</sub>). The reaction was stopped after 3 min by adding 50  $\mu$ L of 1 M H<sub>2</sub>SO<sub>4</sub> to each well. The optical density was measured at 450 nm and a reference wavelength of 620 nm (Multiskan™ FC Mikrotiterplatten-Photometer, Thermo Fisher Scientific, Germany) and the background was subtracted from each value.

To analyze the specificity of mAb S38CD11B12 reactivity against IgG molecules of several other species (sheep, cattle, rat, goat, chicken, camel, llama) a second ELISA was performed. Therefore, the sera were coated on microtiterplates in a dilution of 1:200 in PBS (overnight, 4 °C). MAb S38CD11B12 was incubated at a concentration of 1  $\mu$ g/mL for 1 h at RT. As detection antibody a goat-anti-mouse IgG - HRP antibody was used again for 45 min at RT (see above). To prove the

successful coating of sera to the plate species-specific HRP-conjugated antibodies in dilutions of 1:5000 (rabbit anti-goat IgG-HRP, goat anti-bovine IgG-HRP, goat anti-rat IgG-HRP and donkey anti-sheep IgG-HRP from Jackson ImmunoResearch Laboratories) and 1:1000 (donkey anti-chicken IgY (IgG)-HRP from Jackson ImmunoResearch Laboratories, HRP-conjugated NEM17.12 (mouse-anti-camelid IgG1/IgG2/IgG3) from new/era/mabs GmbH [13]) were used. HRP-enzyme reaction was performed as described above. Background activity was subtracted from OD values. All OD values were normalized to an internal positive control (coated raIgG at 5 µg/mL, S38CD11B12 at 1 µg/mL and HRP-conjugated goat anti-mouse antibody).

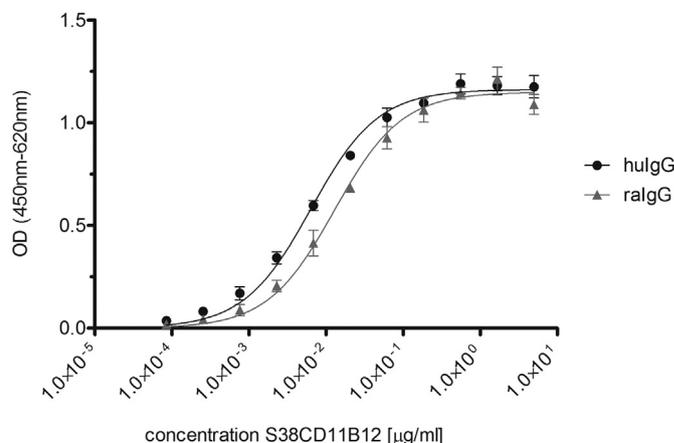
#### 2.4. Kinetic binding evaluation using surface plasmon resonance (SPR) analysis

All kinetic parameters were recorded by SPR Navi 2210A (BioNavis Tampere-region, Finland). MAb S38CD11B12 was immobilized on a 3D SPR Sensorchip CMD 500I (BioNavis). PBS was used as running buffer in all measurements. Immobilization was performed with 100 µg/mL of S38CD11B12 in 5 mM 2-(*N*-morpholino) ethanesulfonic acid (MES) buffer. Before immobilization, both flow channels on the sensorchip were washed with a solution of 2 M NaCl/10 mM NaOH and activation of the sensorchip was performed by injecting 0.05 M NHS/0.2 M EDC solution. For immobilization, mAb S38CD11B12 was injected to flow channel 1. Flow channel 2 served as reference channel and running buffer only was applied. After immobilization, 140 ng/cm<sup>2</sup> of mAb S38CD11B12 could be detected on the surface of the sensorchip in flow channel 1. Following immobilization, the chip was inactivated by injection of 1 M ethanolamine. The analytes were injected in both flow channels, whereas the second channel served as control channel. Human IgG diluted in running buffer was injected at six concentrations (12.5 nM, 25 nM, 75 nM, 250 nM, 500 nM, 1000 nM). RaIgG diluted in running buffer was injected at six concentrations (0.78 nM, 3.13 nM, 6.25 nM, 25 nM, 50 nM, 100 nM). Sensograms are fitted with a one to one binding model in data analysis software TraceDrawer™ for MP-SPR Navi™. The SPR interaction signal was used for calculations after the reference signal (from channel two) was subtracted. Calculations were validated using TraceDrawer™ software. For kinetic binding calculations we used the one to one fitting model. Association rate ( $k_a$ ), dissociation rate ( $k_d$ ) and equilibrium constant ( $K_D$ ) were calculated. The  $K_D$  value results from the following equation [14].

$$K_D = \frac{k_d}{k_a}$$

#### 2.5. Application of S38CD11B12 in Serazym® anti-cardiolipin-β2 glycoprotein I (GPI) IgG/IgM assay

The suitability of mAb S38CD11B12 for the application in auto-antibody detection assays was exemplarily investigated with the help of the commercial Serazym® anti-cardiolipin-β2GPI IgG/IgM assay from Seramun Diagnostica GmbH (Germany). The assay detects auto-antibodies against cardiolipin-β2-GPI from patients sera. Therefore cardiolipin-β2-GPI serves as capture antigen in an enzyme-immunoassay system to capture human autoantibodies from the sera. As internal control/standard this assay uses a polyclonal rabbit anti-cardiolipin-β2-GPI antibody. The conjugate mixture serves as detection reagent. Since there is the need to detect both captured human IgG (serum samples) as well as rabbit IgG (control/standard) the conjugate mixture consists of a combination of anti-human IgG and anti-rabbit IgG antibodies which are conjugated to horseradish peroxidase for visualization of binding reaction. Sera from 16 patients suffering from APS and from 16 healthy individuals (anonymized left-over samples) were tested on the one hand with a detection conjugate mix of anti-human IgG-HRP and anti-rabbit IgG-HRP (Seramun Diagnostica GmbH, Germany) and on the other hand with HRP-labelled mAb S38CD11B12



**Fig. 1.** Reactivity of mAb S38CD11B12 to human IgG (huIgG) and rabbit IgG (raIgG) molecules detected by indirect ELISA. Whole IgG was coated to microtiterplates. mAb S38CD11B12 was serially (3 fold) diluted starting at 5 µg/mL. Background signal was subtracted. The graph shows the results of 3 independent experiments with value ranges (error bars).

(400 ng/mL). The assay procedure was performed as indicated in the instruction of the manufacturer (Serazym® Anti-Cardiolipin/β2-GPI IgG/IgM ELISA, catalogue No. E-054). Samples were tested in duplicates and OD values were measured using Tecan Spectra microplate reader (Tecan Deutschland GmbH, Crailsheim, Germany).

### 3. Results

#### 3.1. Monoclonal antibody S38CD11B12 cross-reacts with human and rabbit IgG

Recombinant raIgG-Fc fragment was successfully used as antigen for immunization. Screening of resulting hybridoma cell clones was performed with human and rabbit IgG in order to identify cross-reactive binders. Potential antibody candidates were produced and purified by protein A mediated affinity chromatography. One murine mAb of subclass IgG2a (S38CD11B12) was chosen as a promising candidate due to its similar dose-dependent reactivity to human as well as rabbit IgG in ELISA experiments (Fig. 1).

Binding kinetics of S38CD11B12 to either immobilized human or rabbit IgG were analyzed by SPR. Dose response sensograms are shown for both antigens at different concentrations (Fig. 2a and b). Since the  $K_D$  value is related to the affinity of an antibody to its antigen, we compared the  $K_D$  values calculated for binding of human and rabbit IgG. As it is shown in Fig. 2,  $K_D$  values are quite similar for both antigens pointing out a slightly higher affinity of S38CD11B12 to rabbit IgG than to human IgG. Still  $K_D$  values for human IgG and rabbit IgG differ only by a factor of three and values are both in the low nanomolar range.

To show the specificity of mAb S38CD11B12 it was tested for cross-reactivity with IgG molecules of other species. Therefore, sera of different species were analyzed for S38CD11B12 binding. As coating control species-specific HRP-conjugated antibodies were used. Immunoglobulin molecules from all tested species could be shown to be immobilized to the plates (Fig. 3). MAb S38CD11B12 turned out to be quite specific for human and rabbit IgG, showing no cross-reactivity to sheep, chicken, goat, cattle and rat IgG molecules. However, S38CD11B12 detected both camelid sera (llama and camel) (Fig. 3). The cross-reactivity to camelid sera can be interpreted in terms of high homology between the amino acid sequence of camelid and human IgG [15,16].

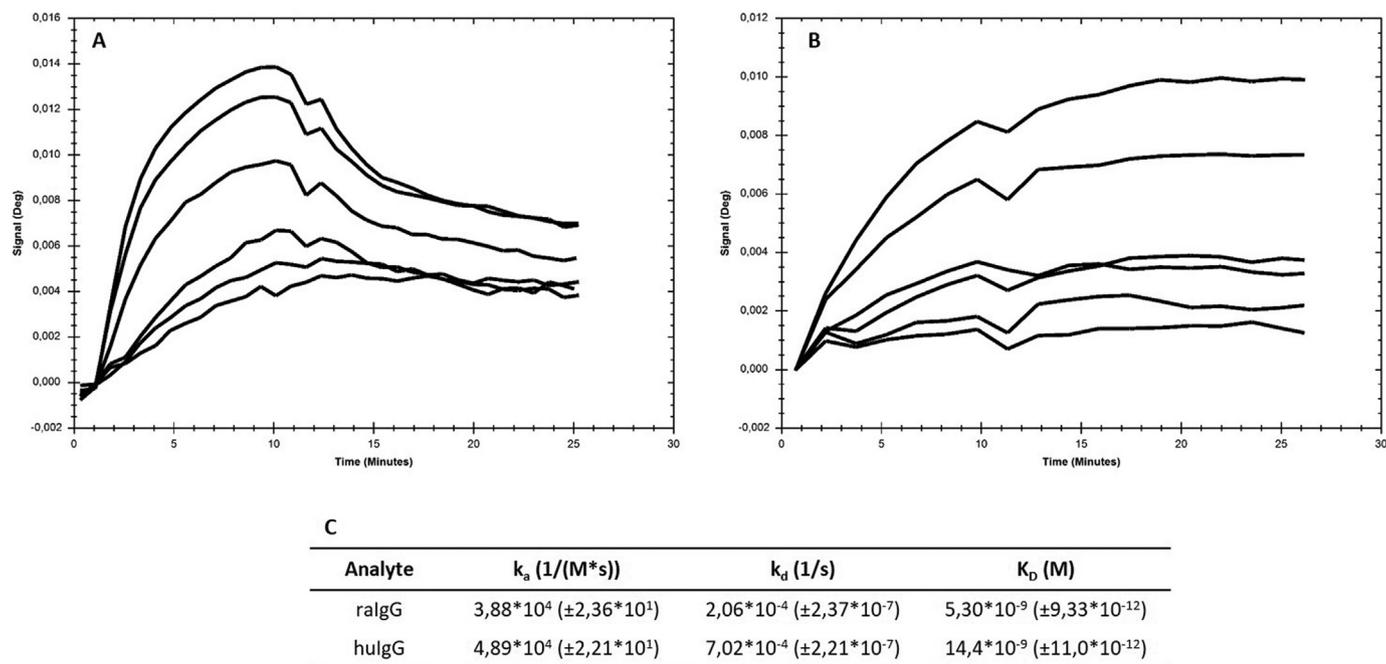


Fig. 2. Binding kinetics of mAb S38CD11B12 to huIgG and ralGg by SPR analysis. A) Sensogram of immobilized S38CD11B12 with huIgG at different concentrations (12.5 nM–1000 nM) B) Sensogram of immobilized S38CD11B12 with rabbit IgG at different concentrations (0.78 nM–100 nM). C) Association rate ( $k_a$ ), dissociation rate ( $k_d$ ) and equilibrium constant ( $K_D$ ) values of interaction between S38CD11B12 and huIgG as well as ralGg.

3.2. MAb S38CD11B12 as detection antibody in Serazym® anti-cardiolipin-β2-GPI IgG/IgM assay

MAb S38CD11B12 was used in a commercial anti-cardiolipin-β2-GPI IgG/IgM assay to demonstrate its applicability as cross-reactive secondary antibody in AD diagnostics. Therefore 16 serum samples from healthy individuals (negative samples) and 16 samples from patients suffering from APS (positive samples) were tested with either a mixture of anti-human IgG-HRP and anti-rabbit IgG-HRP (detection conjugate mix) or HRP-conjugated mAb S38CD11B12 as detection reagents. As shown in Fig. 4 mAb S38CD11B12 reacted similar to the detection conjugate mixture from the assay kit. The entire positive sample panel was recognized as positive by using HRP-conjugated S38CD11B12 proving the ability of this new mAb to detect human IgG in serum samples from different individuals without a loss of sensitivity in comparison to a polyclonal anti-human-IgG-HRP conjugate. All negative serum samples were detected negative indicating no unspecific binding of S38CD11B12 to any kit components. OD values of negative samples were even lower than those detected by the conjugate mix

which improves the distinction between negative and positive sera.

This benefit was demonstrated by calculation of P/N (Positive/Negative) ratios (Table 1). Therefore signals from positive sera in anti-cardiolipin-β2-GPI IgG/IgM assay were divided by the mean signal of all negative serum samples for the HRP-conjugated mAb S38CD11B12 as well as for the conjugate mixture. The higher the P/N ratio, the better the differentiation between negative and positive samples. As shown in Table 1, P/N ratios for all positive samples tested in both detection systems were calculated. It turned out that P/N ratios were higher in 14 of 16 cases when using S38CD11B12 as detection antibody suggesting that conjugated S38CD11B12 is more suitable as detection reagent than the conjugate mixture. Since the conjugate mixture consists of two different polyclonal antibodies unspecific binding as cause for the higher P/N ratios in most of the cases is probably higher compared to a single mAb.

We also tested mAb S38CD11B12 in Serazym® Anti-Cardiolipin-β2-GPI IgG/IgM assay calibration. HRP-conjugated mAb S38CD11B12 was tested with polyclonal rabbit anti-β2-GP-1 as calibrator in the commercial assay system. Although there is still a need to titrate the mAb

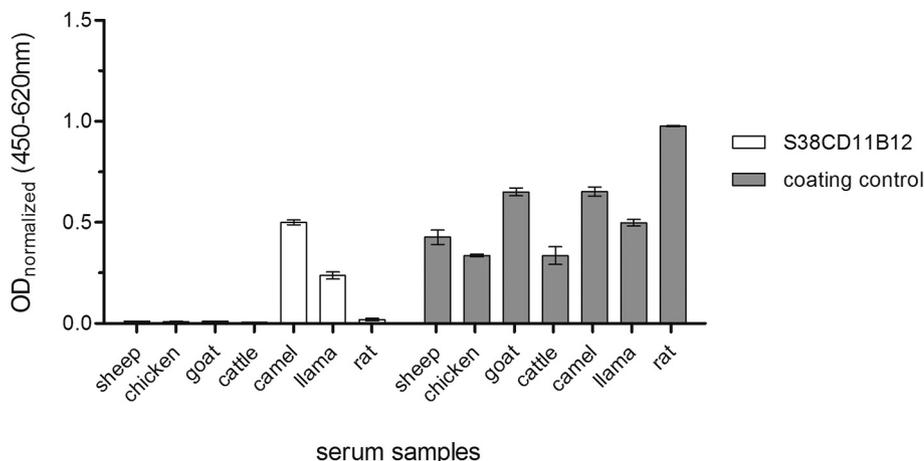
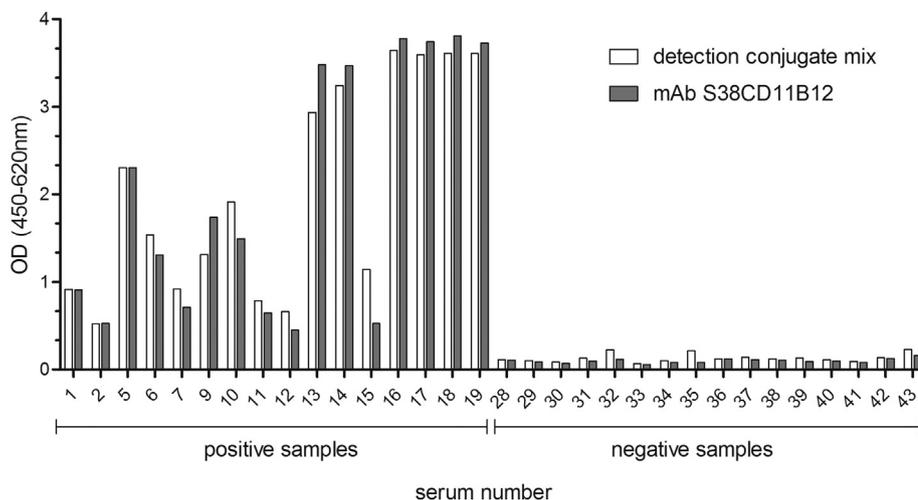


Fig. 3. Cross reactivities of mAb S38CD11B12 to different animal sera analyzed by indirect ELISA (n = 3). Sera were coated to microtiterplates in a dilution of 1:200 (in PBS). MAb S38CD11B12 was incubated with a concentration of 1 µg/mL and detected by goat anti-mouse IgG-HRP antibody. Species-specific HRP-conjugated antibodies served as positive coating controls. Background signals (signals from unspecific binding of goat anti-mouse IgG-HRP to the sera) were already subtracted. Optical densities were normalized to OD values of internal positive controls (S38CD11B12 binding ralGg).

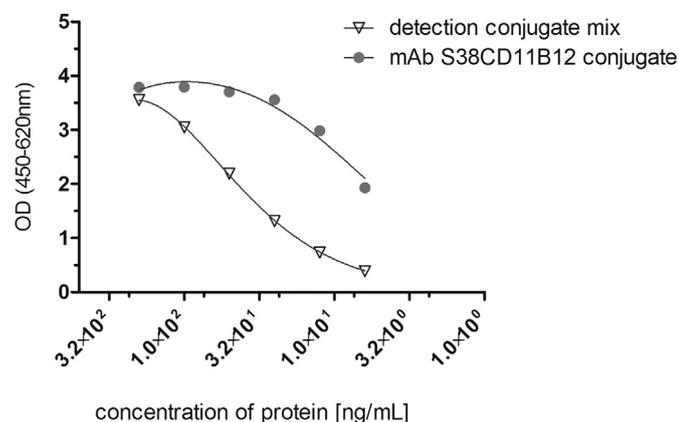


**Fig. 4.** Application of mAb S38CD11B12 in Serazym® Anti-Cardiolipin-β2-GPI IgG/IgM assay. Sixteen sera from patients suffering from APS (positive samples) and 16 sera from healthy individuals (negative samples) were tested using polyclonal rabbit anti- β2-GP-1 IgG/IgM serum as calibrator. The detection conjugate mix (white bars) consisted of a mixture of anti-huIgG and antiraIgG (Seramun GmbH, Germany). It was replaced by mAb S38CD11B12 (black bars) to investigate its applicability in autoantibody assays. Samples were tested as duplicates.

**Table 1**

Positive/Negative (P/N) ratios for conjugate mix and HRP-coupled cross-reactive mAb S38CD11B12. P/N ratios of S38CD11B12 were higher (highlighted in dark grey) in 14 serum samples and lower (highlighted in grey) in two serum samples than the P/N ratios of the conjugate mixture from the kit.

Serum number	P/N ratio (conjugate mix)	P/N ratio (S38CD11B12)
1	6.89	9.02
2	3.93	5.22
5	17.36	22.91
6	11.55	13.00
7	6.93	7.05
9	9.89	17.28
10	14.38	14.82
11	5.92	6.41
12	4.97	4.47
13	22.12	34.54
14	24.41	34.46
15	8.61	5.23
16	27.44	37.54
17	27.06	37.17
18	27.19	37.83
19	27.17	37.03



**Fig. 5.** Application of mAb S38CD11B12 conjugate in Serazym® Anti-Cardiolipin-β2-GPI IgG/IgM assay to detect calibration solution. Polyclonal rabbit anti- β2-GP-1 IgG/IgM was used as calibrator in different concentrations starting a serial dilution at 200 ng/mL and detected by conjugate mixture from Serazym® Anti-Cardiolipin-β2-GPI IgG/IgM assay and also by mAbS38CD11B12 conjugate.

S38CD11B12 before using it in calibration, we can already show the ability of mAb S38CD11B12 to detect the calibrator (see Fig. 5). It can be used to detect human autoantibodies as well as calibrators derived from polyclonal rabbit serum in one assay.

**4. Conclusion**

We have developed a cross-reactive mAb S38CD11B12 recognizing rabbit and human IgG and showed its applicability in an anti-cardiolipin-β2-GPI IgG/IgM assay using rabbit serum as reference material.

The diagnosis of autoimmune diseases e.g. antiphospholipid syndrome is still challenging. Especially the choice of reference material is crucial for test reproducibility. A group of established international standards derived from well characterized human positive and normal sera are the so-called ‘Harris standards’ [17]. Unfortunately human reference material from patients is reliable but unlasting and also expensive. An alternative represent mAbs either as chimeric antibodies or as recombinant antibodies with the advantage of infinite production. The problem with mAbs is their limited epitope coverage which opens the possibility of false negative test results and decreases the sensitivity of the assay [18].

By contrast to those solutions animal derived polyclonal serum is broadly reactive against multiple epitopes, less expensive and of easier access for many diagnostic test suppliers. The utilization of standards derived from animals in assays analyzing human sera however need to include the detection of immunoglobulins from two different species (human and the animal species). To avoid problems that could occur by using a mixture of two different detection antibodies and the resulting decrease in accuracy and increase in expense, we developed a cross-reactive mAb which recognizes human IgG and rabbit IgG with similar affinities. Our mAb S38CD11B12 binds human IgG as well as rabbit IgG with high affinities and specificity which allow the simultaneously detection of human serum samples as well as rabbit polyclonal reference material in one assay. By SPR analysis we could show that affinities of S38CD11B12 to both antigens (human IgG and rabbit IgG) are similar in the low nanomolar range. Analysis of S38CD11B12 in the commercial Serazym® Anti-Cardiolipin-β2-GPI IgG/IgM assay exemplarily showed a successful application of S38CD11B12 in an autoimmune diagnostic assay. We therefore promote mAb S38CD11B12 as a cost effective, low tech possibility to perform standardization of autoantibody diagnostic assays when using rabbit derived antisera as reference material. An interesting fact of our results is the strong cross-reactivity of the anti-rabbit/human IgG antibody S38CD11B12 with camelid immunoglobulins. Camel species and primates and lagomorphs are phylogenetically not closely related. Nonetheless do immunoglobulin genes have significant nucleotide homology so that the

immunological cross reactions are not so surprising [19]. Our antibody may have therefore additional application possibilities and the cross-reactivity has to be kept in mind in other cases. We postulate, that this strategy is also transferable to generate other cross-reactive binders between different species needed in autoimmune diagnostics.

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### Declaration of Competing Interest

Katja Hanack had a management role and is shareholder of new/era/mabs GmbH. The company is generating customized antibodies. Silvia and Tomas Porstmann were shareholders at Seramun Diagnostica GmbH. Tomas Porstmann is now retired and Silvia Porstmann is still employed by Seramun Diagnostica GmbH. All other authors declare no conflict of interests.

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The experiments were performed and analyzed by Steffi Lütkecosmann and Thomas Faupel. Steffi Lütkecosmann, Silvia Porstmann, Thomas Faupel and Burkhard Micheel wrote the manuscript. Katja Hanack, Tomas Porstmann and Silvia Porstmann designed the study, discussed the data and proof read the manuscript.

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