



Data Article

Data about performances of whole and monovalent half-fragments antibodies in immunosorbent assays



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ARTICLE INFO

Article history:

Received 28 December 2020

Revised 15 January 2021

Accepted 18 January 2021

Available online 20 January 2021

Keywords:

Half-fragment antibodies

Reduced antibodies

Immunoassays

Orientation

ABSTRACT

The data here presented are related to the research article entitled “Sensitivity and reproducibility enhancement in enzyme immunosorbent assays based on half fragment antibodies” [1] aimed to compare the performance in ELISA of whole antibodies and their corresponding monovalent half-fragments obtained by reduction. Half-fragment antibodies represent an interesting method to orient antibodies in high-sensitive immunoassays taking advantage of the free sulfhydryl groups of the hinge region [2–4] that allow their oriented binding on maleimide functionalized microplates. Data here presented describe the contribution of both chemical reduction and orientation on the antigen binding capacity of whole and half-fragments antibodies. For this purpose, monoclonal anti-horseradish peroxidase (anti-HRP) or monoclonal anti-fPSA antibodies, and their respective half-fragments, were coated on polystyrene or maleimide functionalized microplates. The antigen binding capability was analyzed by in-house enzyme linked immunosorbent assays. These data would be used for further studies on the

DOI of original article: [10.1016/j.ab.2020.114090](https://doi.org/10.1016/j.ab.2020.114090)

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<https://doi.org/10.1016/j.dib.2021.106778>

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development of oriented immunoassays based on half fragment antibodies.
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Specifications Table

Subject	Biology
Specific subject area	Biosensors, Immunoassays
Type of data	Tables
How data were acquired	ELISA assay: absorbance values were collected using Multiskan GO microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) while fluorescence measurements were obtained using Fluoroskan Ascent microplate reader (Thermo Fisher Scientific, Waltham, MA, USA)
Data format	Raw data
Parameters for data collection	In these experiments the effects of orientation and chemical reduction on the antigen binding capacity of antibodies were assessed separately. For this purpose, we used an antibody against HRP and one against fPSA as study models. Then we compared their antigen binding capacity as whole or reduced antibodies, in oriented or not-oriented condition.
Description of data collection	The antigen binding capacity of the tested antibodies was assessed by enzyme-linked immunosorbent assay. Anti-HRP was used to study the effect of reduction: whole and reduced antibodies were coated on polystyrene microplate and the binding of the HRP was revealed using the substrate o-Phenylenediamine and measuring the absorbance at 450 nm. Anti-fPSA was used to asses both the effects of reduction and orientation: whole and reduced antibodies were coated on polystyrene or maleimide activated microplates. The presence of the bound antigen was revealed using a secondary antibody conjugated with ALP. In this setting a fluorescent substrate was used.
Data source location	University of Pisa, Pisa, Italy
Data accessibility	Repository name: Mendeley Data Data identification number: https://doi.org/10.17632/cgyjydn8xb.2 Direct URL to data: https://data.mendeley.com/datasets/cgyjydn8xb/2
Related research article	V. Susini, L. Caponi, V.L. Rossi, A. Sanesi, N. Romiti, A. Paolicchi, M. Franzini. Sensitivity and reproducibility enhancement in enzyme immunosorbent assays based on half fragment antibodies. Anal. Biochem. Available online 28 December 2020, 114,090, In Press, Corrected Proof. https://doi.org/10.1016/j.ab.2020.114090

Value of the Data

- The data highlight the improved antigen binding capacity of half fragment antibodies due to the chemical reduction of the hinge region.
- These data provide a starting point for researchers interested in the design of high-sensitive immunoassays.
- These data prepare the way for further studies on folding rearrangements of half-fragment antibodies that could happen as a consequence of reduction and that could affect antigen binding capacity.

1. Data Description

The effect of reduction on the antigen binding capacity was studied using mouse antibodies against the enzyme horse-radish peroxidase. Whole anti-HRP antibodies or their half-fragments

obtained by reduction were coated on polystyrene microplate and the enzyme HRP was used as antigen.

Table 1 represents the raw data generated by the enzymatic activity of HRP using OPD substrate: the absorbance value is proportional to the amount of enzyme bound to whole or half-fragment antibodies coated on polystyrene microplates. Data were collected in six-fold and the lower value of absorbance for each quantity of HRP was excluded for the data analysis presented in the associated paper [1]. The dataset “Sensitivity enhancement and the role of orientation in enzyme immunosorbent assay based on half fragment antibodies” [5] displayed the statistical data analysis based on the results showed in Table 1. Indeed, in order to assess if differences between the straight line related to whole anti-HRP and the one related to reduced anti-HRP were significant, the slopes were compared by the F test using GraphPad Prism 8.

To study the effect of antibody orientation, maleimide-activated surface was used to orient an anti-fPSA half-fragment antibody taking advantage of the sulfhydryl groups made free in the hinge region by reduction. Half-fragment antibodies thus generated were coated on polystyrene to evaluate the effects of the reduction on the antigen binding capability. Maleimide-activated microplates allowed us to assess the role of the orientation on the sensitivity of the immunoassays. As a control, whole anti-fPSA were coated both on polystyrene and maleimide-activated microplates. Tables 2 to 5 show the collected raw data.

Table 1

HRP enzyme activity bound by reduced and whole antibodies coated on polystyrene microplate.

HRP (ng)	Absorbance 450 nm Whole anti-HRP						Absorbance 450 nm Reduced anti-HRP					
0.25	0.033*	0.071	0.105	0.149	0.124	0.098	0.065*	0.114	0.143	0.092	0.117	0.150
1.00	0.373	0.404	0.407	0.314	0.323	0.298*	0.481	0.327*	0.474	0.443	0.496	0.462
2.00	0.711	0.723	0.809	0.778	0.682*	0.750	0.459*	1.115	1.236	1.126	0.708	0.997

*Data excluded for the statistical computation.

Table 2

Enzyme linked fluorescent assay for the detection of fPSA using whole antibodies on polystyrene microplates.

fPSA 0 ng/ml	fPSA 0.072 ng/ml	fPSA 0.720 ng/ml	fPSA 1.450 ng/ml	fPSA 3.620 ng/ml	fPSA 7.250 ng/ml
23.15	24.67	28.21	35.82	54.89	73.48
23.76	23.69	27.96	35.55	50.97	50.98
23.92	23.66	32.20	29.24	40.77	49.59
24.27	23.94	28.60	32.14	42.00	56.56

Table 3

Enzyme linked fluorescent assay for the detection of fPSA using whole antibodies on maleimide microplates.

fPSA 0 ng/ml	fPSA 0.072 ng/ml	fPSA 0.720 ng/ml	fPSA 1.450 ng/ml	fPSA 3.620 ng/ml	fPSA 7.250 ng/ml
22.14	22.66	23.27	24.28	26.78	30.43
22.20	22.63	23.33	24.14	26.67	29.03
22.41	22.33	23.01	24.62	26.38	30.54
22.29	22.28	23.16	24.46	27.93	29.11

Table 4

Enzyme linked fluorescent assay for the detection of fPSA using reduced antibodies on maleimide microplates.

fPSA 0 ng/ml	fPSA 0.072 ng/ml	fPSA 0.720 ng/ml	fPSA 1.450 ng/ml	fPSA 3.620 ng/ml	fPSA 7.205 ng/ml
22.04	24.10	38.03	53.28	90.64	141.07
22.08	23.94	36.51	51.88	89.90	136.47
21.89	23.86	36.43	50.15	82.83	124.55
21.95	24.30	36.97	49.99	87.53	127.12

Table 5
Enzyme linked fluorescent assay for the detection of fPSA using reduced antibodies on polystyrene microplates.

fPSA 0 ng/ml	fPSA 0.0720 ng/ml	fPSA 0.720 ng/ml	fPSA 1.450 ng/ml	fPSA 3.620 ng/ml	fPSA 7.250 ng/ml
24.02	24.96	42.08	55.13	110.77	135.76
24.30	27.05	47.54	52.68	100.92	118.98
23.79	25.22	43.20	37.70	75.39	91.91
24.49	25.43	61.64	40.46	91.95	108.16

These results were used for blank subtraction, mean and standard deviation calculations in the dataset “Sensitivity enhancement and the role of orientation in enzyme immunosorbent assay based on half fragment antibodies” [5].

2. Experimental Design, Materials and Methods

2.1. Antibody reduction using 2-mercaptoethylamine

Antibodies were diluted in Dulbecco’s Phosphate Buffered Saline (PBS) containing 10 mM EDTA (PBS - EDTA) to the final concentration of 1 mg/ml and 2-Mercaptoethylamine (2-MEA) freshly prepared were added to the solution to the final concentration of 53 mM. The reduction mixtures were incubated for 90 min at 37 °C under mild agitation. Then 2-MEA and EDTA were removed using a Sephadex G-25 in PD-10 Desalting Column (GE Healthcare, Little Chalfont, UK) according to the manufacturer’s instructions. Reduced antibodies were collected in PBS and used immediately before each experiment.

2.2. Assessment of the antigen binding capacity of whole and reduced anti-HRP antibodies coated on polystyrene microplate

The antigen binding capacity of whole or reduced anti-HRP antibodies was assessed by enzyme-linked immunosorbent assay (ELISA). Microplates were coated with 100µl of intact or reduced antibodies at the concentration of 50µg/ml in PBS - EDTA. Plates were incubated for 2 h and then washed threefold with 200µl of PBS containing TWEEN® 20 0.01% w/v (PBS-T). Nonspecific binding was blocked with 5% (w/v, 200µl) non-fat milk/PBS-T for 1 h and then washed as described above. Antigen, the enzyme HRP (0,25; 1 and 2 ng; 100µl/well), was incubated for 1 h followed by three washing steps. The presence of HRP was revealed using 1 mg/ml o-Phenylenediamine (OPD) in 50 mM phosphate-citric acid pH 5 containing 1 µl/ml of 33% hydrogen peroxide [6]. Absorbance was detected at 450 nm using the plate reader Multiskan GO (Thermo Fisher Scientific, Waltham, MA, USA). All incubation steps were carried out at room temperature.

2.3. Assessment of the antigen binding capacity of physisorbed or oriented anti-fPSA antibodies coated on polystyrene or maleimide microplates

The antigen binding capacity of whole or reduced antibodies was tested both on polystyrene and maleimide activated microplates by an enzyme-linked fluorescent assay (ELFA). Bio-Plex Pro™ Flat Bottom Plates 96-well microplates (BioRad, Hercules, CA, USA) or Pierce™ Maleimide Activated Plates (Thermo Fisher Scientific, Waltham, MA, USA) were rinsed with 200µl of PBS-T and then were coated with 100µl of 50µg/ml whole or reduced anti-fPSA in PBS - EDTA. Plates were incubated for 2 h, and excess coating solution was removed by three washing steps with 200µl of PBS-T. Nonspecific binding of polystyrene microplates was blocked with 200µl of 5%

(w/v) non-fat milk solubilized in PBS-T for 1 h, and then washed as described. To inactivate excess maleimide groups, plates were incubated for 1 h at room temperature with 10 µg/ml freshly prepared cysteine solution. The calibrator S1 of the VIDAS® FPSA assay, diluted at different concentrations in PBS (0,072 ng/ml; 0,725 ng/ml; 1,45 ng/ml; 3,62 ng/ml and 7,25 ng/ml), was used as sample; these dilutions were analysed in four-fold, and PBS was used as negative control. After 1 h incubation at room temperature and three washing steps, 100 µl/well of a secondary antibody conjugated with ALP were added. After a further 1 h incubation, 200 µl/well of VIDAS® optical substrate were added. The fluorescence generated was measured after 20 min on Fluoroskan Ascent (Thermo Fisher Scientific, Waltham, MA, USA) with 20 ms integration time, excitation wavelength at 390 nm and emission at 460 nm. All incubation steps were carried out at room temperature.

Ethics Statement

The work here presented did not involve the use of samples from humans or animals.

CRediT Author Statement

Vanessa Susini: Conceptualization, Writing - review & editing; **Laura Caponi:** Methodology; **Veronica Lucia Rossi:** Resources, Validation; **Antonio Sanesi:** Supervision; **Nadia Romiti:** Investigation; **Aldo Paolicchi:** Funding acquisition; **Maria Franzini:** Project administration, Formal analysis, Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

Acknowledgments

This work was supported by Institutional University funds and by bioMérieux Italia SPA, Via di Campigliano, 58, 50012, Bagno a Ripoli, Florence, Italy.

References

- [1] V. Susini, L. Caponi, V.L. Rossi, A. Sanesi, N. Romiti, A. Paolicchi, M. Franzini, Mechanism of Sensitivity Enhancement in Enzyme Immunosorbent Assays Based on Half Fragment Antibodies, *Anal. Biochem.* In Press, 2020, doi:[10.1016/j.ab.2020.114090](https://doi.org/10.1016/j.ab.2020.114090).
- [2] Z. Balevicius, A. Ramanaviciene, I. Baleviciute, A. Makaraviciute, L. Mikoliunaite, A. Ramanavicius, Evaluation of intact- and fragmented-antibody based immunosensors by total internal reflection ellipsometry, *Sensor Actuat. B-Chem.* 160 (2011) 555–562.
- [3] H. Sharma, R. Mutharasan, Half antibody fragments improve biosensor sensitivity without loss of selectivity, *Anal. Chem.* 85 (2013) 2472–2477.
- [4] A. Kausaite-Minkstiniene, A. Ramanaviciene, J. Kirlyte, A. Ramanavicius, Comparative study of random and oriented antibody immobilization techniques on the binding capacity of immunosensor, *Anal. Chem.* 82 (2010) 6401–6408.
- [5] V. Susini, L. Caponi, V.L. Rossi, A. Sanesi, N. Romiti, A. Paolicchi, M. Franzini, Sensitivity enhancement and the role of orientation in enzyme immunosorbent assay based on half fragment antibodies, *Mendeley Data* (2021) v2, doi:[10.17632/cgyjydn8xb.2](https://doi.org/10.17632/cgyjydn8xb.2).
- [6] OPD Product Information Sheet, ThermoFisher Scientific, Waltham, MA, USA.