Supplementary Data

"Simultaneous quantification of five bacterial and plant toxins from complex matrices using a multiplexed fluorescent magnetic suspension assay"

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Table S1. Comparison of multiplex detection systems for proteotoxins*

Detection system	Antigens	Detection limit [ng/L]	Matrix	Time [min]	Ref
Magnetic xMAP-technology	BoNT/A BoNT/B Ricin SEB Abrin	21 73 2 3 546	food	210	this manus-cript
NRL – biosensor	BoNT/A toxoid BoNT/B toxoid Ricin SEB Cholera toxin several bacteria, viruses and small toxins	20,000 200,000 8,000 100 1,600	food	10 – 45	1
Immunoassay on optical silicon chip	BoNT/A BoNT/B BoNT/E BoNT/F	2,500 5,000 2,500 5,000	food	35	2
Semi-homogeneous fluidic force discrimination assay	Ricin A chain SEB	5,000 0.001	clinical	10	3
Conventional xMAP-technology	Ricin SEB several viruses and bacteria	29,100 200	environmental	~75	4
RAPTOR	Ricin SEB several bacteria	50,000 10,000	environmental	3 – 10	5
ArrayTube – system	Ricin SEB several viruses and bacteria	100 200	buffer	60	6
Immunomagnetic, fluorogenic detection	BoNT/A Ricin A chain SEB Cholera toxin diverse bacteria and viruses	2,000 1,000 100 2,000	buffer	30	7
Microfluidic electrophoretic chip-based immunoassay	Ricin SEB Shiga toxin 1	1,320,000 8,400 35,000	buffer	< 20	8

Table S1. (continued)

Detection system	Antigens	Detection limit [ng/L]	Matrix	Time [min]	Ref
Hydrogel-based protein-microarray	Ricin SEB Diphteria toxin LF (anthrax toxin) Viscumin Tetanus toxin	100 1,000 1,000 4,000 2,000 10,000	buffer	~1000	9
CombiMatrix VLSI array	Ricin bacteria and proteins	300	buffer	12	10
Bidiffractive grating (BDG) biosensor	BoNT/A or /B Ricin SEB	100,000 5,000 < 1,000	buffer	15	11
QTL-biosensor 2200R	BoNT Ricin SEB	< 10,000 < 1,000 < 1,000	buffer	10	12
Multiplexed sandwich ELISA with quantum dots	Ricin SEB Cholera toxin Shiga like toxin 1	30,000 30,000 30,000 30,000	buffer	~1000	13
Conventional xMAP-technology	BoNT/A toxoid Ricin SEB Cholera toxin	1,100 3,300 14 4	buffer	120	14
AMP-biosensor	BoNT/A BoNT/B	1,000 10,000	buffer	75	15
Bead based immunoassay with eTag reporter	BoNT/A toxoid Protein ovalbumin	125,000 125,000	buffer	~66	16

* Table S1 gives an overview over different multiplex detection systems suitable for laboratory-based and on-site detection of high molecular weight proteotoxins. The table includes information on the matrices analysed, the detection limits obtained for individual proteotoxins in multiplexed settings and the experimental time per analysis.

Table S2. Immunisation schema for rabbit RB77 (anti-BoNT/B IgG)*

immunisation	day	antigen	quantity	adjuvants
1	0	BoNT/B-Dynabeads (**5 µg** BoNT/B/ 6.5 × 10^8 Dynabeads)	5 µl	-
2	37		10 µl	CFA
3	92		40 µl	IFA
4	125	BoNT/B	2 ng	IFA
5	144		10 ng	IFA
6	207		10 ng	IFA
7	232	BoNT/B-Dynabeads (**15 µg** BoNT/B/ 6.5 × 10^8 Dynabeads)	30 µl	IFA
8	257		30 µl	IFA
9	293		30 µl	IFA
10	335	BoNT/B	4 ng	IFA
11	424		4 ng	IFA
12	544		10 ng	IFA
13	557		20 ng	IFA
14	649		20 ng	IFA
15	705		20 ng	IFA
16	762		23 ng	IFA
17	814		23 ng	IFA

* Detailed immunisation schema for rabbit RB77 immunised with BoNT/B coupled to Dynabeads and free native BoNT/B. Tab. S2 and Fig. S1 together show that an increase in absolute amount of BoNT/B coupled to Dynabeads leads to a specific titer development. From immunisation 9 onwards, the rabbit was regularly bled in order to collect large amounts of serum.

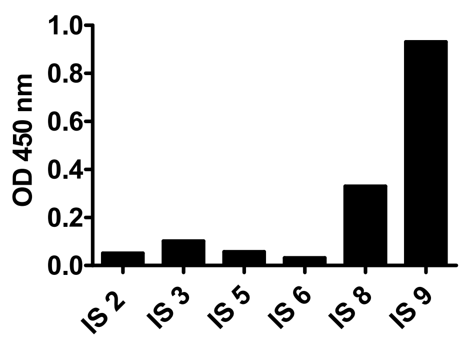

Fig. S1. Titer development of rabbit RB77 (anti-BoNT/B IgG). The rabbit was immunised according to Table S2 and 10–16 days after each immunisation (IS) the rabbit serum was tested in indirect ELISA (dilution 1:1000) against BoNT/B. Shown are the sera obtained after immunisation 2 through 9. From IS 9 on the titer remained on a constantly high level, no further increase in titer was observed after switching from BoNT/B-Beads to soluble BoNT/B.

Table S3. Immunisation schema for mouse M51 (anti-ricin IgG)*

immunisation	day	antigen	quantity	adjuvants
1	0	Ricin-Dynabeads (42 µg Ricin/ 6.5×10^8 Dynabeads)	5 µl	CFA
2	28		5 µl	IFA
3	43		5 µl	IFA
4	57		10 µl	IFA
5	72	Ricin	1 µg	IFA
6	98		2.5 µg	IFA
7	147		2.5 µg	-
8	148		3.75 µg	-
9	196		4 µg	-
10	197		8 µg	-
11	198		16 µg	-

* Detailed immunisation schema for mouse M51 immunised with ricin coupled to Dynabeads followed by soluble ricin. Fusion of spleen cells from mouse M51 delivered mAb R18, R109 and R70 (compare Fig. 1 and Tab. 2, main manuscript). Two other mice were immunised similarly, but used earlier for fusion (after immunisation 7) and delivered the other hybridoma clones described in Tab. 2 of the main manuscript.

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