

Supplementary Material

Supplementary Table S1. Methods by ID used by the participants. This table summarizes the principles and combinations of methods with their respective antibodies used to detect BoNT according to the acronym given (Abbreviation). (*) Methods selected for training during EuroBioTox and before this proficiency test. Of note, while EL.R-1A was the method selected for training, both the antibodies used for EL.R-1A and 1B were available in the EuroBioTox repository.

Method	Toxin	Assignment	Abbreviation (Method ID)
ELISA commercial	BoNT/E	ELISA BioSentinel E	EL.BioSen-E
ELISA RKI	BoNT/A	ELISA RKI 1A*	EL.R-1A
		ELISA RKI 2A	EL.R-2A
		ELISA RKI 3A	EL.R-3A
	BoNT/B	ELISA RKI 1B	EL.R-1B
		ELISA RKI 3B	EL.R-3B
	BoNT/E	ELISA RKI 1E	EL.R-1E
		ELISA RKI 2E	EL.R-2E
	BoNT/F	ELISA RKI 1K	EL.R-1F
		ELISA RKI 2F	EL.R-2F
		ELISA RKI 3F	EL.R-3F
	BoNT/A	ELISA in-house 2A	EL.2-A
	BoNT/B	ELISA in-house 2B	EL.2-B
	BoNT/E	ELISA in-house 2E	EL.2-E
	BoNT/A, B, E	ELISA in-house A + B + E	EL.-A/B/E
ELISA CEA	BoNT/A	ELISA CEA A*	EL.C-A
	BoNT/B	ELISA CEA B	EL.C-B
	BoNT/E	ELISA CEA E	EL.C-E
LFA commercial	BoNT/A, B, E	LFA CEA Multiplex	LFA.CEA-Multi
	BoNT/A	LFA Miprolab A	LFA.Mipro-A
	BoNT/B	LFA Miprolab B	LFA.Mipro-B
	BoNT/A, B	LFA Bot Tox, Tetracore	LFA.Tetra
	BoNT	LFA RAID Tox, Alexeter	LFA.Alex
		LFA ProStrips, Advnt	LFA.Advnt
Endopep-ELISA	BoNT/A, B	Endopep-ELISA Bead A + B	EpEL.-A/B
LC-MS/MS	BoNT/A	MS/MS (LC)	MS/MS
	BoNT/B	MS/MS (LC)	MS/MS
	BoNT/E	MS/MS (LC)	MS/MS
	BoNT/F	MS/MS (LC)	MS/MS
LC-MS/MS (HR)	BoNT/A-F	MS/MS (LC)	MS/MS
Endopep-MS	BoNT/A	Endopep-MS	EpMS
	BoNT/B	Endopep-MS	EpMS
	BoNT/E	Endopep-MS	EpMS
	BoNT/F	Endopep-MS	EpMS
Mouse Bio Assay	BoNT, A, B, E, F	Mouse bioassay	MBA
Microarray (ELISA-based)	BoNT/A-F	Microarray (ELISA-based) Bead in-house	MAR.1
	BoNT/A-F	Microarray (ELISA-based) pBDi	MAR.2
	BoNT/A-F	Microarray (ELISA-based) Bead commercial	MAR.3
Hemidiaphragm assay	BoNT, A-F	Hemidiaphragm assay	HDA or MPN

Supplementary Table S2. Number of cases obtained by the method. Note that the amount of data per method (n) shows a high dispersion of values, which has an impact on the mean and standard deviation values.

Measurand	Method	n
BoNT/A	ELISA CEA A	6
	ELISA RKI 1A	26
	ELISA RKI 3A	3
	Endopep-MS	3
	Hemidiaphragm assay	3
BoNT/B	ELISA CEA B	14
	ELISA RKI 1B	21
	ELISA RKI 3B	16
	ELISA RKI 3Bi	1
	Endopep-MS	3
	Hemidiaphragm assay	4

Supplementary Table S3. List of monoclonal and polyclonal antibodies used for sandwich ELISA provided by RKI. Monoclonal (mAb) and polyclonal (pAb) antibodies were used to detect BoNT/A, B, E, and F to characterize the PT samples.

BoNT serotypes	Coating antibody	Detection antibody
BoNT/A	mAb A778 + mAb A2807	mAb A120-Bio
BoNT/B	mAb B279	polyclonal horse antiserum Behring-Bio (Novartis)
BoNT/E	pAb KE97	mAb E1447-Bio
BoNT/F	mAb F189	pAb HF59

Supplementary Table S4. Summary statistics of homogeneity data. Data were processed according to ISO 13528:2015, B.2.2 (a) and ISO 13528:2015, B.2.3 (b). s_s : between-unit standard deviation (sampling standard deviation); σ_p : Standard deviation for proficiency assessment; s_r : repeatability standard deviation; σ_{all} : "Allowed" sampling standard deviation.

Measurand	Material	Assigned value	s_s	σ_p	s_r	σ_{all}	unit	$s/\sigma_p^{(a)}$	s_r/σ_p	Conclusion ^(b)
BoNT/A4	S10	387	176	969	124	291	ng/mL	018	013	homogeneity sufficient
BoNT/A1	S12	120	0921	299	0796	0898	ng/mL	031	027	homogeneity sufficient
BoNT/A1	S13	0646	00414	0162	00591	00485	ng/mL	026	037	homogeneity sufficient
BoNT/A3	S14	174	0998	436	142	131	ng/mL	023	033	homogeneity sufficient
BoNT/B1	S04	662	0263	166	0743	0497	ng/mL	016	045	homogeneity sufficient
BoNT/B1	S08	199	0113	0497	0170	0149	ng/mL	023	034	homogeneity sufficient
BoNT/B5	S10	189	00818	473	125	142	ng/mL	0017	026	homogeneity sufficient
BoNT/B1	S11	0662	00538	0165	00735	00496	ng/mL	033	044	homogeneity sufficient
BoNT/B1	S15	0153	00114	00382	000646	00114	ng/mL	030	017	homogeneity sufficient
BoNT/B1	S16	0390	00201	00975	00250	00292	ng/mL	021	026	homogeneity sufficient
BoNT/B1	S17	0128	000667	00321	00105	000964	ng/mL	021	033	homogeneity sufficient
BoNT/B2	S18	132	00755	0329	00990	00987	ng/mL	023	030	homogeneity sufficient
BoNT/E1	S06	0593	00184	0148	00803	00445	ng/mL	012	054	homogeneity sufficient
BoNT/F1	S02	532	0508	133	0627	0399	ng/mL	038	047	homogeneity sufficient