

**Detection, differentiation, and identification
of botulinum neurotoxin serotypes C, CD, D, and DC by highly
specific immunoassays and mass spectrometry**

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Supplementary Fig: S1

A) Amino acid sequence coverage of BoNT/C after tryptic digest and tandem mass spectrometry analysis

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1  MPITINNFNY SDPVDNKNIL YLDTHLNTLA NEPEKAFRIT GNIWVIPDRF
51  SRNSNPNLNK PPRVTSPPKSG YYDPNYLSTD SDKDTFLKEI IKLFKRINSR
101 EIGEELIYRL STDIPFPGNN NTPINTFDFD VDFNSVDVKT RQGNNWVKTG
151 SINPSVIITG PRENIIDPET STFKLNTNTE AAQEGFGALS IISISPRFML
201 TYSNATNDVG EGRFSKSEFC MDPILILMHE LNHAMHNLYG IAIPNDQTIS
251 SVTSNIFYSQ YNVKLEYAEI YAFGGPTIDL IPKSARKYFE EKALDYYSI
301 AKRLNSITTA NPSSFNKYIG EYKQKLIRKY RFVVESSGEV TVNRNKFVEL
351 YNELTQIFTE FNYAKIYNVQ NRKIYLSNVY TPVTANILDD NVYDIQNGFN
401 IPKSNLNVLF MGQNLSRNP LRVNPNENML YLFTKFCHKA IDGRSLYNKT
451 LDCRELLVKN TDLPPFIGDIS DVKTDIFLRK DINEETEVYI YPDNVSVQV
501 ILSKNTSEHG QLDLLYPSID SESEILPGEN QVFYDNRTQN VDYLNSYYYL
551 ESQKLSDNVE DFTFTRSIEE ALDNSAKVYT YFPTLANKVN AGVQGGLFLM
601 WANDVVEDET TNILRKDTLD KISDVSAIIP YIGPALNISN SVRRGNFTEA
651 FAVTGVTILL EAFPEFTIPA LGAFVIYSKV QERNEIKTI DNCLEQRIKR
701 WKDSYEWMMG TWLSRIITQF NNISYQMYDS LNYQAGAIKA KIDLEYKKYS
751 GSDKENIKSQ VENLKNSLDV KISEAMNNIN KFIRECSVTY LFKNMLPKVI
801 DELNEFDRNT KAKLINLIDS HNIILVGEVD KKKAKVNNSF QNTIPFNIFS
851 YTNNSLLKDI INEYFNNIND SKILSLQNRK NTLVDTSGYN AEVSEEGDVQ
901 LNPIFPFDFK LGSSGEDRGK VIVTQENIV YNSMYESFSI SFWIRINKWV
951 SNLPGYTIID SVKNNSGWSI GIISNFLVFT LKQNEDEQS INFSYDISNN
1001 APGYNKWFV TVTNNMMGMN KIYINGKLID TIKVKELTGI NFSKTITFEI
1051 NKIPDTGLIT SDSNINMWI RDFYIFAKEL DGKDINILFN SLQYTNVVKD
1101 YWGNDLRYNK EYVMVNIDYL NRYMYANSRQ IVFNTRNNN DFNEGYKIII
1151 KRIRGNTNDT RVRGGDILYF DMTINNKAYN LFMKNETMYA DNHSTEDIYA
1201 IGLREQTKDI NDNIIFQIQP MNNTYYYASQ IFKSNFNGEN ISGICSIGTY
1251 RFRLLGGDWYR HNYLVPTVKQ GNYASLLEST STHWGFVPVS E
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B) Amino acid sequence coverage of BoNT/CD after tryptic digest and tandem mass spectrometry analysis

1 MPITINNFNY SDPVDNKNIL YLDTHLNTLA NEPEKAFRII GNIWVIPDRF
51 SRDSNPNLNK PPRVTS^{SPKSG} YYDPNYLSTD SEKDTFLKEI IKLFKRINSR
101 EIGEELIYRL ATDIPFFGNN NTPINTFDFD VDFNSVDVKT RQGNWVKTG
151 SINPSVIITG PRENIIDPET STFKLTNNTF AAQEGFGALS IISISPRFML
201 TYSNATNNVG EGRFSKSEFC MDPILILMHE LNHAMHNLYG IAIPNDQRIS
251 SVTSNIFYSQ YNVKLEYAEI YAFGGPTIDL IPKSARKYFE EKALDYYSI
301 AKRLNSITTA NPSSFNKYIG EYKQKLIRKY RFVVESSGEV AVDRNKFAEL
351 YKELTQIFTE FNYAKIYNVQ NRKIYLSNVY TPVTANILDD NVYDIQNGFN
401 IPKSNLNVLF MGQNLSRNP LRVNPNENML YLFTKFCHKA IDGRSLYNKT
451 LDCRELLVKN TDLPPFIGDIS DIKTDIFLSK DINEETEVID YPDNVSVQV
501 ILSKNTSEHG QLDLLYPIIE GESQVLPGEN QVFYDNRTQN VDYLNSYYYL
551 ESQKLSDNVE DFTFTTSIEE ALDNSGKYYT YFPKLADKVN TGVQGGFLFM
601 WANDVVEFT TNILRKDTLD KISDVSAIIP YIGPALNISN SVRRGNFTEA
651 FAVTGVITILL EAFQEFTIPA LGAFVIYSKV QERNEI^{IKTI} DNCLEQRIKR
701 WKDSYEWMI^G TWLSRITTQF NNISYQMYDS LNYQADAIKD KIDLEYKKYS
751 GSDKENIKSQ VENLKNSLDI KISEAMNNIN K^{FIRECSVTY} LFKNMLPKVI
801 DELNKF^{DLKT} KTELINLIDS HNIILVGEVD RLKAK^{INESF} ENTIPFNIFS
851 YTNNSLLKDI INEYFNSIND SKILSLQNK^K NALVDTSGYN AEVRLEGDVQ
901 VNTIYTND^{FK} LSSSGDKIIV NLNNN^{ILYSA} IYENSSVSFW IKISKDLTNS
951 HNEYTIINSI KQNSGWKLCI R^{NGNIEWILQ} DINRKYKSLI FDYSESLSHT
1001 GYTNKWFFVT ITNNIMGYMK LYINGELKQS ER^{IEDLDEVK} LDKTIVFGID
1051 ENIDENQMLW IRDFNIFSKE LSNEDINIVY EGQILRNVIK DYWGNPLKFD
1101 TEYYMINYNY IDRYIAPK^{NN} ILVLVQYSDI SKLYTKNPIT IK^{SAANKNPY}
1151 SRILNGDDIM FHMLYDSREY MIIRDTDTIY ATQGGQCSKN CVYALKLQSN
1201 LGNYGIGIFS IKNIVSQNKY CSQIFSSFMK NTMLLADIYK PWRFSFENAY
1251 TPVAVTNYET KLLSTSSFWK FISRDPGWVE

C) Amino acid sequence coverage of BoNT/D after tryptic digest and tandem mass spectrometry analysis

1 MTWPVKDFNY SDPVNDNDIL YLRIPQNKLI TTPVKAFMIT QNIWVIPERF
51 SSDTNPSLSK PPRPTSKYQS YYDPSYLPST EQKDTFLKGI IKLFKRINER
101 DIGK**KLINYL VVGSPFMGDS STPEDTFDFT RHTTNIAVEK FENGSWKVTN**
151 IITPSVLIFG PLPNILDYTA SLTLQGQSN PSFEGFGTSL ILK**VAPEFLL**
201 **TFSDVTSNQS SAVLGK**SIFC MDPVIALMHE LTHSLHQLYG INIPSDKRIR
251 PQVSEGFFSQ DGPNVQFEEL YTFGGLDVEI IPQIERSQLR **EKALGHYKDI**
301 **AKRLNNINKT IPSSWISNID KYKKIFSEKY NFDKDNLTGNF VVNIDKFNSL**
351 YSDLTNVMSE VVYSSQYNVK **NRTHYFSRHY LPVFANILDD NIYTIRDGFN**
401 **LTNKGFNIE**N SGQNIERNPA LQKLSSSESVV DLFTK**VCLRL TKNSRDDSTC**
451 **IKVKNNRLPY VADKDSISQE IFENK**IITDE TNVQNYSDKF SLDESILDGQ
501 VPINPEIVDP LLPNVNMEPL NLPGEIIVFY DDITK**YVDYL NSYYYLESQK**
551 **LSNNVENITL TTSVEEALGY SNKIYTFPLS LAEK**VNKG**VQ** AGLFLNWANE
601 VVEDFTTNIM **KD**TLDKISD **VSVIIPYIGP ALNIGNSALR** GNFNQAFATA
651 GVAFLLEGFP EFTIPALGVF TFYSSIQERE **KIIKTIENCL EQRVKRWKDS**
701 **YQWMVSNWLS RITTQFNHIN YQMYDSL**SYQ ADAIKAKIDL EYKKYSGSDK
751 **ENIKSQVENL KNSLDVKISE AMNNINK**FIR **ECSVTYLFKN MLPKVIDELN**
801 **KFDLRTKTEL INLIDSHNII LVGEVDRLKA KVNESFENTM PFNIFSYTNN**
851 **SLLKDIINEY FNSINDSKIL SLQNKKNALV DTSGYNAEVR VGDNVQLNTI**
901 **YTNDFKLSSS** GDKIIVNLNN NILYSAIYEN SSVSFWIKIS **KDLTNSHNEY**
951 **TIINSIEQNS GWKLCIRNGN IEWILQDVNR KYKSLIFDYS ESLSHTGYTN**
1001 **KWFFVTITNN IMGYMKLYIN GELKQSQKIE DLDEVKLDKT** IVFGIDENID
1051 ENQMLWIRDF **NIFSKELSNE DINIVYEGQI LRNVIKDYWG NPLKFDTEYY**
1101 **IINDNYIDRY IAPESNVLVL VQYPDRSKLY TGNPITIKSV SDKNPYSRIL**
1151 **NGDNIILHML YNSRKYMIIR** DTDTIYATQG GECSQNCVYA LK**LQSNLGN**Y
1201 **GIGIFSIKNI** VSKNKYCSQI FSSFRENTML LADIIKPWRF SFK**NAYTPVA**
1251 **VTNYETKLLS TSSFWK**FISR DPGWVE

D) Amino acid sequence coverage of BoNT/DC after tryptic digest and tandem mass spectrometry analysis

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1  MTWPVKDFNY SDPVNDNDIL YLRIPQNKLI TTPVKAFMIT QNIWVIPERF
51  SSDTNPSLSK PPRPTSQYS YYDPSYSLTD EQKDTFLKGI IKLFKRINER
101 DIGKKLINYL VVGSPFMGDS STPEDTFDFT RHTTNIAVEK FENGSWKVTN
151 IITPSVLIFG PLPNILDYTA SLTLQGQSN PSFEGFGTLS ILKVAPEFLL
201 TFSDVTSNQS SAVLGKSIFC MDPVIALMHE LTHSLHQLYG INIPSDKRIR
251 PQVSEGFFSQ DGPNVQFEEL YTFGGSDVEI IPQIERLQLR EKALGHYKDI
301 AKRLNNINKT IPSSWSSNID KYKKIFSEKY NFDKDNLTGNF VVNIDKFNSL
351 YSDLTNVMSE VVYSSQYNVK NRTHYFSKHY LPVFANILDD NIYTIINGFN
401 LTTKGFNIEN SGQNIERNPA LQKLSSESVD DLFTKVCLRL TRNSRDDSTC
451 IQVKNNTLPY VADKDSISQE IFESQIITDE TNVENYSDNF SLDESILDAK
501 VPTNPEAVDP LLPNVNMEPL NVPGESEVFFY DDITKDVDYL NSYYYLEAQK
551 LSNNVENITL TTSVEEALGY SNKIYTFPLS LAEKVNKGVQ AGLFLNWANE
601 VVEDFTTNIM KKDITLTKISD VSAIIPYIGP ALNIGNSALR GNFKQAFATA
651 GVAFLLEGFP EFTIPALGVF TFYSSIQERE KIIKTIENCL EQRVKRWKDS
701 YQWMVSNWLS RITTQFNHIS YQMYDSLQYQ ADAIKAKIDL EYKKYSGSDK
751 ENIKSQVENL KNSLDVKISE AMNNINKFIR ECSVTYLFKN MLPKVIDELN
801 KFDLKTTEL INLIDSHNII LVGEVDRLKA KVNESFENTI PFNIFSYTNN
851 SLLKDMINEY FNSINDSKIL SLQNKKNTLM DTSGYNAEVR VEGNVQLNPI
901 FPFDFKLGS GDDRKGVIIVT QNENIVYNAM YESFSISFWI RINKWVSNLP
951 GYTIIDSVKN NSGWSIGIIS NFLVFTLKQN ENSEQDINFY YDISKNAAGY
1001 NKWFFVTITT NMMGNMMIYI NGKLIDTIKV KELTGINFYSK TITFQMNKIP
1051 NTGLITSDSD NINMWIRDFY IFAKELDDKD INILENSLQY TNVVKDYWGN
1101 DLRDYKEYYM INVNYMNRYM SKKGNIGIVFN TRKNNNDFNE GYKIIIKRII
1151 GNTNDTRVRG ENVLYFNTTI DNKQYSLGMY KPSRNLGTDL VPLGALDQPM
1201 DEIRKYGSFI IQPCNTFDYY ASQLFLSSNA TTNRIGILSI GSYSFKLGDD
1251 YWFNHEYLIP VIKIEHYASL LESTSTHWVF VPASE

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Amino acid sequence coverage of immunoextracted BoNT/C, CD, D, and DC after tryptic digest and liquid chromatography-tandem mass spectrometry analysis. Culture supernatants of *Clostridium botulinum* strains producing BoNT/C (NCTC8548) and BoNT/CD (C6814) were enriched by mAb C394 and BoNT/D (D4947) and BoNT/DC (Eppendorf) were enriched by mAb D63 (both mAb are directed against the light chain of the toxins, see Figure 2). Immunocaptured toxins were digested by trypsin and resulting peptides were analysed by LC-MS/MS. MS/MS evidence was obtained for amino acids marked in red comprising 81% sequence coverage for BoNT/C (Q93HT3_CLOBO) (A), 61% for BoNT/CD (Q5DW55_CLOBO) (B), 65% for BoNT/D (BXD_CLOBO) (C), and 71% for BoNT/DC (C6KZT4_CLOBO) (D).