* Application of Mass Spectrometric Methods

Terry D. Cyr, Health Canada

Influenza - annual and pandemic Protein sequences Protein concentrations Host Cell Proteins

Weekly US Map: Influenza Summary Update



*Are you vaccinated?

A Weekly Influenza Surveillance Report Prepared by the Influenza Division Weekly Influenza Activity Estimates Reported by State and Territorial Epidemiologists*



Influenza intensity, spread and dominant virus type/subtype



*No place to hide

* Influenza A - Interspecies transmission



Swayne, D.E. (Ed.), Avian Influenza, p. 62. Copyright © John Wiley & Sons (2009)

*H1N1	23,068
*H3N2	23,609
*H7N9	694
*H5N1	273
*B Victoria	4500
*B Yamagata	5394

57,538

*GISAIR - EpiFlu:Full length strains

*Generalized approach

Identification of peptides

ID Biomarkers

ID of proteins

ID PTMs

Quantification of proteins

*Annual Influenza Influenza proteins three or four strains 15 µg hemagglutinin/0.5mL ea A(H1N1) A(H3N2) B

http://www.itqb.unl.pt/labs/proteinmodelling/activities/haemagglutinin http://www.rcsb.org/pdb/explore

~ 100, 000 A and B entries database

Curated influenza plus host cell proteins plus contaminants

*Strain ID - Method Development

- Increased instrument resolution and sensitivity.

Increased peptide IDs, ~50% sequence coverage

1	DTLCIGYHAN	NSTDTVDTVL	EKNVTVTHSV	NLLEDKHNGK	LCKLRGV APL
51	HLGKCNIAGW	ILGNPECESL	STASSWSYIV	ETPSSDNGTC	YPGDFIDYEE
101	LR EQLSSVSS	FER FEIFPK T	SSWPNHDSDK	GVTAACPHAG	AK sfyknliw
151	LVKK GNSYPK	LSKSYINDK <mark>G</mark>	KEVLVLWGIH	HPSTSADQQS	LYQNADAYVF
201	VGSSR YSK TF	KPEIAIRPKV	RDREGRMNYY	WTLVEPGDKI	TFEATGNLVV
251	PRYAFAMER N	AGSGIIISDT	PVHDCNTTCQ	TPKGAINTSL	PFQNIHPITI
301	GKCPKYVKST	K LRLATGLRN	IPSIQSR GLF	GAIAGFIEGG	WTGMVDGWYG
351	YHHQNEQGSG	YAADLK <mark>STQN</mark>	AIDEITNKVN	SVIEKMNTQF	TAVGK EFNHL
401	EKRIENL <mark>NKK</mark>	VDDGFLDIWT	YNAEL LVLLE	NERTLDY HDS	NVKNLY EKVR
451	SQLKNNAK EI	GNGCFEFYHK	CDNTCMESVK	NGTYDYPK ys	EEAKLNR EEI
501	DGVKLESTRI	YQILAIYSTV	ASSLVL VVSL	GAISFWMCSN	GSLQCRICI

Hundreds of ambiguous IDs



Hemagglutinin H1 A/Solomon Islands/3/2006 95% coverage

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1 MKVKLLVLLC TFTATYADTI CIGYHANNST DTVDTVLEKN VTVTHSVNLL
51 EDSHNGKLCL LKGIAPLQLG MCSVAGWILG NPECELLISR ESWSYIVEKP
101 NPENGTCYPG HFADYEELRE QLSSVSSFER FEIFPKESSW PNHTTTGVSA
151 SCSHNGESSF YKNLLWLTGK NGLYPNLSKS YANNKEKEVL VLWGVHHPPN
201 IGDORALYHT ENAYVSVVSS HYSRKFTPEI AKRPKVRDRE GRINYYWTLL
251 EPGDTIIFEA NGNLIAPRYA FALSRGFGSG IINSNAPMDE CDAKCQTPQG
301 AINSSLPFON VHPVTIGECP KYVRSAKLRM VTGLRNIPSI QSRGLFGAIA
351 GFIEGGWTGM VDGWYGYHHQ NEQGSGYAAD QKSTQNAING ITNKVNSVIE
401 KMNTQFTAVG KEFNKLERRM ENLNKKVDDG FIDIWTYNAE LLVLLENERT
451 LDFHDSNVKN LYEKVKSQLK NNAKEIGNGC FEFYHKCNDE CMESVKNGTY
501 DYPKYSEESK LNREKIDGVK LESMGVYQIL AIYSTVASSR VLLVSLGAIS
551 FWMCSNGSLQ CRICI
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Neuraminidase N1 A/Fukushima/141/2006 80% coverage

1	QKIITIGSIS	IAIGIISLIL	QIGNIISIWA	SHSIQTGSQN	HTGICNORII
51	TYENSTWVNN	TYVNINNTNV	VAEKDKTSVT	LAGNSSLCSI	SGWAIYTKDN
101	SIRIGSKGDV	FVIREPFISC	SHLECRTFFL	TQGALLNDKH	SNGTVKDRSP
151	YRALMSCPLG	EAPSPYNSRF	ESVAWSASAC	HDGMGWLTIG	ISGPDNGAVA
201	VLKYNGIITE	TIKSWKKRIL	RTQESECVCM	NGSCFTIMTD	GPSNGAASYK
251	IFKIEKGKVT	KTIELNAPNF	HYEECSCYPD	TGTVMCVCRD	NWHGSNRPWV
301	SFNQNLDYQI	GYICSGVFGD	NPRPKDGEGS	CNPVTVDGAD	GVKGFSYKYG
351	NGVWIGRTKS	NRLRKGFEMI	WDPNGWTNTD	SDFSVKQDVV	AITDWSGYSG
401	SFVQHPELTG	LDCIRPCFWV	ELVRGLPREN	TTIWTSGSSI	SFCGVNSGTA
451	NWSWPDGAEL	PFTIDK	_		

Hemagglutinin H3 A/Wisconsin/67/2005 86% coverage

1 MKTIIALSYI LCLVFAQKLP GNDNSTATLC LGHHAVPNGT IVKTITNDQI 51 EVTNATELVQ SSSTGGICDS PHQILDGENC TLIDALLGDP QCDGFQNKKW 101 DLFVERSKAY SNCYPYDVPD YASLRSLVAS SGTLEFNDES FNWTGVTONG 151 TSSSCKRRSN NSFFSRLNWL TQLKFKYPAL NVTMPNNEKF DKLYIWGVHH 201 PVTDNDQIFL YAQASGRITV STKRSQQTVI PNIGSRPRIR NIPSRISIYW 251 TIVKPGDILL INSTGNLIAP RGYFKIRSGK SSIMRSDAPI GKCNSECITP 301 NGSIPNDKPF ONVNRITYGA CPRYVKONTL KLATGMRNVP EKOTRGIFGA 351 IAGFIENGWE GMVDGWYGFR HQNSEGIGQA ADLKSTQAAI NQINGKLNRL 401 IGKTNEKFHQ IEKEFSEVEG RIQDLEKYVE DTKIDLWSYN AELLVALENQ 451 HTIDLTDSEM NKLFERTKKQ LRENAEDMGN GCFKIYHKCD NACIGSIRNG 501 TYDHDVYRDE ALNNRFQIKG VELKSGYKDW ILWISFAISC FLLCVALLGF 551 IMWACQKGNI RCNICI

Hemagglutinin B/Malaysia/2506/2005 (corrected sequence) 95% coverage Neuraminidase B/Malaysia/2506/2004 83% coverage

1 MKAIIVLLMV VTSNADRICT GITSSNSPHV VKTATQGEVN VTGVIPLTTT 51 PTKSHFANLK GTETRGKLCP KCLNCTDLDV ALGRPKCTGN IPSARVSILH 101 EVRPVTSGCF PIMHDRTKIR QLPNLLRGYE HIRLSTHNVI NAENAPGGPY 151 KIGTSGSCPN VTNGNGFFAT MAWAVPKNDN NKTATNSLTI EVPYICTEGE 201 DQITVWGFHS DIETQMAKLY GDSKPQKFTS SANGVTTHYV SQIGGFPNQT 251 EDGGLPQSGR IVVDYMVQKS GKTGTITYQR GILLPQKVWC ASGRSKVIKG 301 SLPLIGEADC LHEKYGGLAK SKPYYTGEHA KAIGNCPIWV KTPLKLANGT 351 KYRPPAKLLK ERGFFGAIAG FLEGGWEGMI AGWHGYTSHG AHGVAVAADL 401 KSTQEAINKI TKNLNSLSEL EVKNLQRLSG AMDELHNEIL ELDEKVDDLR 451 ADTISSQIEL AVLLSNEGII NSEDEHLLAL ERKLKKMLGP SAVEIGNGCF 501 ETKHKCNQTC LDRIAAGTFD AGEFSLPTFD SLNITAASLN DDGLDNHTIL 551 LYYSTAASSL AVTLMIAIFV VYMVSRDNVS CSICL

Neuraminidase N2 A/Wisconsin/67/2005 81% coverage

51 MLCEPTIIER MITEIVYLTM TTIEKEICPK LAEYRWWSKP QCMITGFAPF 101 SKDNSIRLSA GGDIWVTREP YVSCDPDKCY QFALGQGTTL NNVHSMDTVH 151 DRTPYRTLLM NELGVPFHLG TKQVCIAWSS SSCHDGKAWL HVCVTGDDKN 201 ATASFIYNGR LVDSIVSWSK EILRTQESEC VCINGTCTVV MTDGSASGKA 251 DTKILFIEEG KIVHTSTLSG SAQHVEECSC YPRYLGVRCV CRDNWKGSNR 301 PIVDINIKDY SIVSSYVCSG LVGDTPRKMD SSSSSHCLDP NNEEGGHGVK 351 GWAFDDGNDV WMGRTISEKL RSGYETFKVI EGWSNPNSKL QINRQVIVDR 401 GNRSGYSGIF SVEGKSCINR CFYVELIRGR KEETEVLWTS NSIVVFCGTS 451 GTYGTGSWPD GADINLMPI	1	MNPNQKIITI	GSVSLTISTI	CFFMQIAILI	TTVTLHFKQY	EFNSPPNNQV
101 SKDNSIRLSA GGDIWVTREP YVSCDPDKCY QFALGQGTTL NNVHSMDTVH 151 DRTPYRTLLM NELGVPFHLG TKQVCIAWSS SSCHDGKAWL HVCVTGDDKN 201 ATASFIYNGR LVDSIVSWSK EILRTQESEC VCINGTCTVV MTDGSASGKA 251 DTKILFIEEG KIVHTSTLSG SAQHVEECSC YPRYLGVRCV CRDNWKGSNR 301 PIVDINIKDY SIVSSYVCSG LVGDTPRKMD SSSSSHCLDP NNEEGGHGVK 351 GWAFDDGNDV WMGRTISEKL RSGYETFKVI EGWSNPNSKL QINRQVIVDR 401 GNRSGYSGIF SVEGKSCINR CFYVELIRGR KEETEVLWTS NSIVVFCGTS 451 GTYGTGSWPD GADINLMPI	51	MLCEPTIIER	NITEIVYLTN	TTIEKEICPK	LAEYRNWSKP	QCNITGFAPF
151 DRTPYRTLIM NELGVPFHLG TKQVCIAWSS SSCHDGKAWL HVCVTGDDKN 201 ATASFIYNGR LVDSIVSWSK EILRTQESEC VCINGTCTVV MTDGSASGKA 251 DTKILFIEEG KIVHTSTLSG SAQHVEECSC YPRYLGVRCV CRDNWKGSNR 301 PIVDINIKDY SIVSSYVCSG LVGDTPRKND SSSSSHCLDP NNEEGGHGVK 351 GWAFDDGNDV WMGRTISEKL RSGYETFKVI EGWSNPNSKL QINRQVIVDR 401 GNRSGYSGIF SVEGKSCINR CFYVELIRGR KEETEVLWTS NSIVVFCGTS 451 GTYGTGSWPD GADINLMPI	101	SKDNSIRLSA	GGDIWVTREP	YVSCDPDKCY	QFALGQGTTL	NNVHSNDTVH
201 ATASFIYNGR LVDSIVSWSK EILRTQESEC VCINGTCTVV MTDGSASGKA 251 DTKILFIEEG KIVHTSTLSG SAQHVEECSC YPRYLGVRCV CRDNWKGSNR 301 PIVDINIKDY SIVSSYVCSG LVGDTPRKND SSSSSHCLDP NNEEGGHGVK 351 GWAFDDGNDV WMGRTISEKL RSGYETFKVI EGWSNPNSKL QINRQVIVDR 401 GNRSGYSGIF SVEGKSCINR CFYVELIRGR KEETEVLWTS NSIVVFCGTS 451 GTYGTGSWPD GADINLMPI	151	DRTPYRTLLM	NELGVPFHLG	TKQVCIAWSS	SSCHDGKAWL	HVCVTGDDKN
251 DTKILFIEEG KIVHTSTLSG SAQHVEECSC YPRYLGVRCV CRDNWKGSNR 301 PIVDINIKDY SIVSSYVCSG LVGDTPRKND SSSSSHCLDP NNEEGGHGVK 351 GWAFDDGNDV WMGRTISEKL RSGYETFKVI EGWSNPNSKL QINRQVIVDR 401 GNRSGYSGIF SVEGKSCINR CFYVELIRGR KEETEVLWTS NSIVVFCGTS 451 GTYGTGSWPD GADINLMPI	201	ATASFIYNGR	LVDSIVSWSK	EILRTQESEC	VCINGTCTVV	MTDGSASGKA
301 PIVDINIKDY SIVSSYVCSG LVGDTPRKND SSSSSHCLDP NNEEGGHGVK 351 GWAFDDGNDV WMGRTISEKL RSGYETFKVI EGWSNPNSKL QINRQVIVDR 401 GNRSGYSGIF SVEGKSCINR CFYVELIRGR KEETEVLWTS NSIVVFCGTS 451 GTYGTGSWPD GADINLMPI	251	DTKILFIEEG	KIVHTSTLSG	SAQHVEECSC	YPRYLGVRCV	CRDNWKGSNR
351 GWAFDDGNDV WMGRTISEKL RSGYETFKVI EGWSNPNSKL QINRQVIVDR 401 GNRSGYSGIF SVEGKSCINR CFYVELIRGR KEETEVLWTS NSIVVFCGTS 451 GTYGTGSWPD GADINLMPI	301	PIVDINIKDY	SIVSSYVCSG	LVGDTPRKND	SSSSSHCLDP	NNEEGGHGVK
401 GNRSGYSGIF SVEGKSCINR CFYVELIRGR KEETEVLWTS NSIVVFCGTS 451 GTYGTGSWPD GADINLMPI	351	GWAFDDGNDV	WMGRTISEKL	RSGYETFKVI	EGWSNPNSKL	QINRQVIVDR
451 GTYGTGSWPD GADINLMPI	401	GNRSGYSGIF	SVEGKSCINR	CFYVELIRGR	KEETEVLWTS	NSIVVFCGTS
	451	GTYGTGSWPD	GADINLMPI			

1	MLPSTIQTLT	LFLTSGGVLL	SLYVSASLSY	LLYSDILLKF	PSTEITAPTM
51	PLDCANASNV	QAVNRSATKG	VTLLLPEPEW	TYPRLSCPGS	TFQKALLISP
101	HRFGETKGNS	APLIIREPFI	ACGPKECKHF	ALTHYAAQPG	GYYNGTRGDR
151	NKLRHLISVK	LGKIPTVENS	IFHMAAWSGS	ACHDGKEWTY	IGVDGPDNNA
201	LLKIKYGEAY	TDTYHSYANN	ILRTQESACN	CIGGNCYLMI	TDGSASGVSE
251	CRFL KIREGR	IIKEIFPTGR	IKHTEECTCG	FASNKTIECA	CRDNSYTAKR
301	PFVKLNVETD	TAEIRLMCTE	TYLDTPRPDD	GSITGPCESN	GDKGSGGIKG
351	GFVHQRMASK	IGRWY SRTMS	KTKRMGMGLY	VKYDGD PWAD	SDALAFSGVM
401	VSMEEPGWYS	FGFEIKDKKC	DVPCIGIEMV	HDGGKETWHS	AATAIYCLMG
451	SGQLLWDTVT	GVNMAL			

Hemagglutinin and neuraminidase sequences in the 2007–2008 trivalent vaccine showing identified sequence in bold red. N-Glycosylation sites are highlighted in green, as determined by incorporation of 180 during enzymatic deglycosylation with PNGase F. Blue = partially glycosylated, as evidenced by the identification of both the modified and unmodified peptides. Note that the low observation of partially glycosylated peptides by this approach suggests that the glycosylation of the identified peptides is near 100%.

*Signal intensity is not representative of peptide / protein amount.







Silva JC, Gorenstein MV, Li GZ, Vissers JP, Geromanos SJ. Absolute quantification of proteins by LCMS^E: a virtue of parallel MS acquisition. MCP 2006 5:144–56.

* HA Quantification in Trivalent Vaccine



*Hi3/QconCAT/SpikeTidesTM



QconCAT Sequence

Protein	Peptides
BSA - Bovine Serum Albumin	1- LGEYGFQNALIVR, 2- LVNELTEFAK,
(Bos Taurus)	3- DAFLGSFLYEYSR, 4- HLVDEPQNLIK
ADH - Alcohol Dehydrogenase	1- VVGLSTLPEIYEK, 2- LPLVGGHEGAGVVVGMGENVK,
(Saccharomyces cerevisiae)	3- SISIVGSYVGNR, 4- ANELLINVK
H1 - Hemagglutinin	1- EVILVIEW CHILLINGTEADOOGLYONIADAVA/EVICES 2. STONIAIDEITNIK
A/California (H1N1)	3 4 peptides
N1 - Neuraminidase A/California (H1N1)	$\frac{1}{3}$ - link peptides with a spacer (ASGK)
H3 - Hemagglutinin A/Victoria (H3N2)	 Peptide set are dispersed C terminal polybistiding tag
N2 - Neuraminidase	1-
A/Victoria (H3N2)	3- SGYSGIFSVEGK, 4- GWAFDDGNDVWMGR
HB - Hemagglutinin	1- LSGAMDELHNEILELDEK, 2- FTSSANGVTTHYVSQIGGFPDQTEDGGLPQSGR,
B/Brisbane	3- NLNSLSELEVK, 4- ADTISSQIELAVLLSNEGIINSEDEHLLALER
NB - Neuraminidase	1- GVTLLLPEPEWTYPR, 2- LNVETDTAEIR,
B/Brisbane	3- YGEAYTDTYHSYANK, 4- GNSAPLIIR
OV - Ovalbumin (Gallus gallus)	 1- GGLEPINFQTAADQAR, 2- ISQAVHAAHAEINEAGR, 3- LTEWTSSNVMEER, 4- NVLQPSSVDSQTAMVLVNAIVFK

OconCAT Final Sequence:

MAGR ~ BSA-1 ~ ADH-1 ~ H1-1 ~ H3-1 ~ HB-1 ~ N1-1 ~ N2-1 ~ NB-1 ~ OV-1 ~ OV-2~ NB-2 ~ N2-2 ~ N1-2 ~ HB-2 ~ H3-2 ~ H1-2 ~ ADH-2 ~ BSA-2 ~ HB-3 ~ N1-3 ~ N2-3 ~ NB-3 ~ OV-3 ~ BSA-3 ~ ADH-3 ~ H1-3 ~ H3-3 ~ H3-4 ~ H1-4 ~ ADH-4 ~ BSA-4 ~ OV-4 ~ NB-4 ~ N2-4 ~ N1-4 ~ HB-4 ~ LAAALEHHHHHH

* Hi3 Values relative to BSA in QconCAT



*SpikeTidesTM

- *Low cost commercial peptides *from JPT Peptide Technologies*
- *Custom synthesized peptides
- *Peptide quantified via a coupled chromophore
- *Chromophore tag removed by trypsin

Hemagglutinin - H1



Reference antigens M1 and M2 contain 46 and 35 μ g H1/mL, respectively

Neuraminidase - N1

$\mu \textbf{g}$ N1 / mL vaccine



Neuraminidase - NB

μ g NA (B) / mL vaccine



* Quadrivalent vaccines have NB from both B strains.



 \bigcirc



*MS Applications

*Issues that can be addressed [what?/how much?]

- * What proteins are in a sample?
- *What are the rel.conc. of proteins between samples?
- * Are the proteins degraded?
- * Are there unexpected proteins (HCP) present?

*Other types of questions are more problematic

* Why is? [may be answered by what/how much] * Who ...

* When ...





Virus deactivation with propriolactone

Mass Spectrometry Terry D Cyr Marybeth Creskey Lisa Walrond Yi-Min She

* Thank you Merci Danke Dankeschön Danke sehr