*Application of Mass Spectrometric Methods

Terry D. Cyr, Health Canada

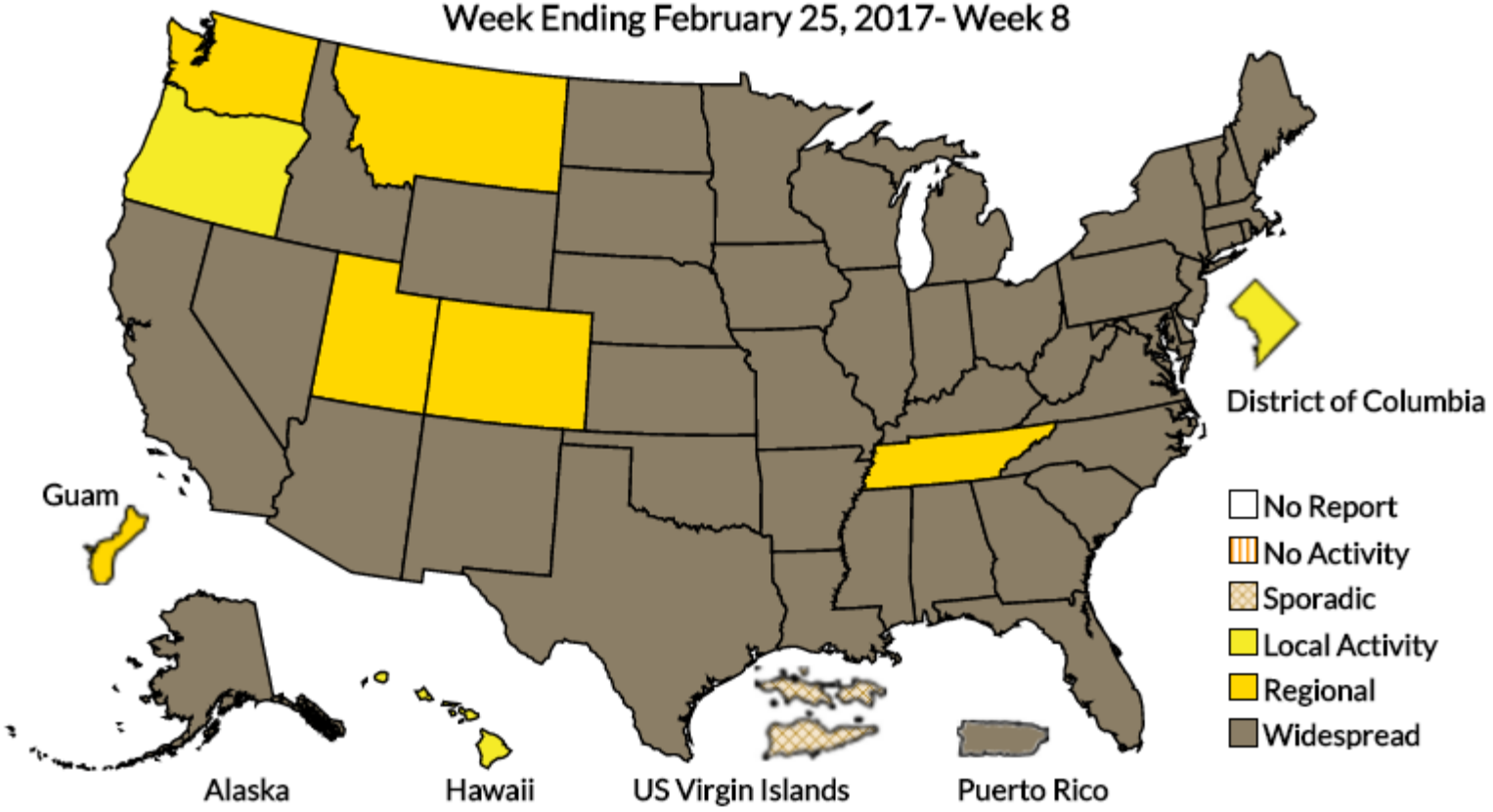
Influenza – annual and pandemic

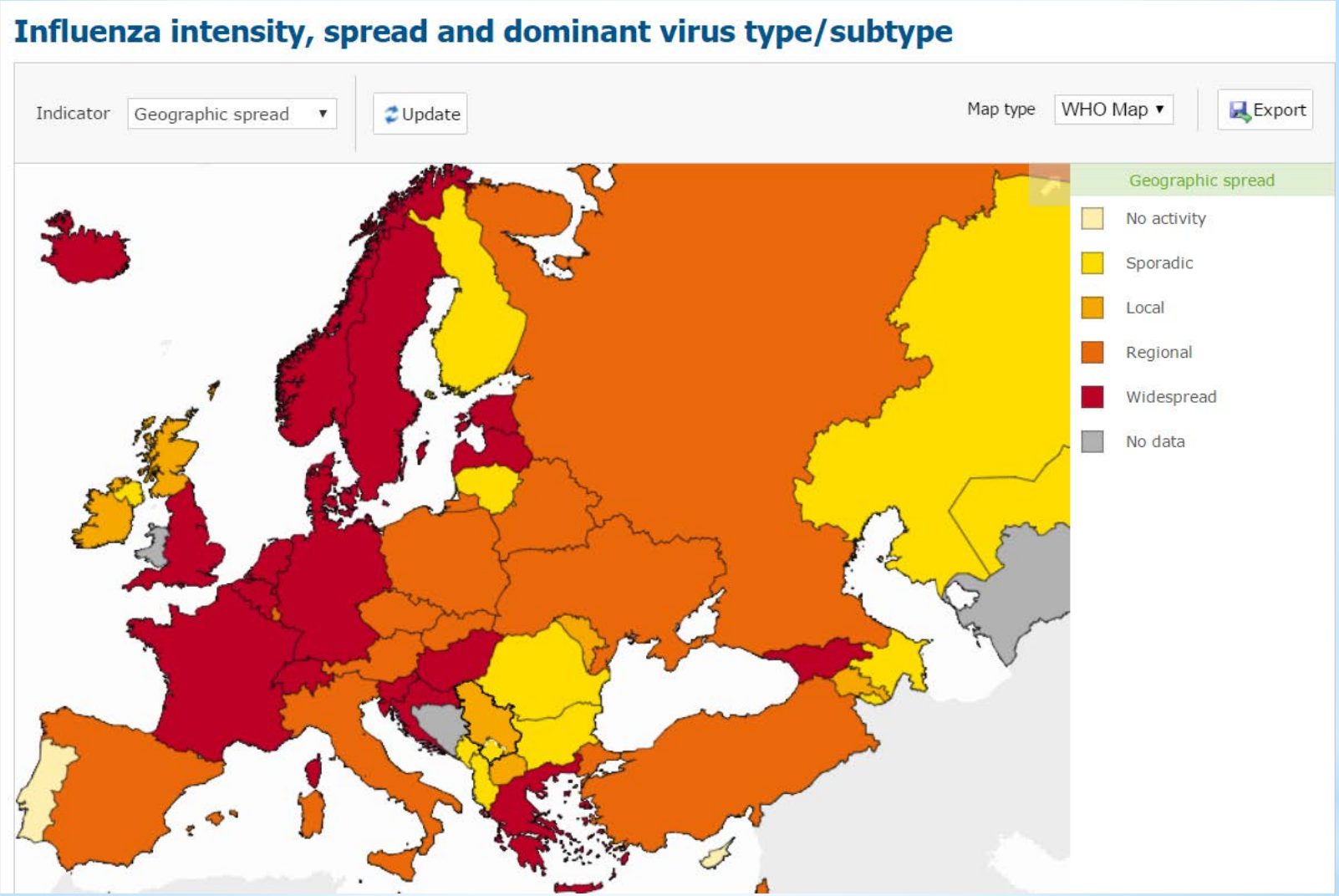
- Protein sequences
- Protein concentrations
- Host Cell Proteins

Weekly US Map: Influenza Summary Update

*Are you vaccinated?

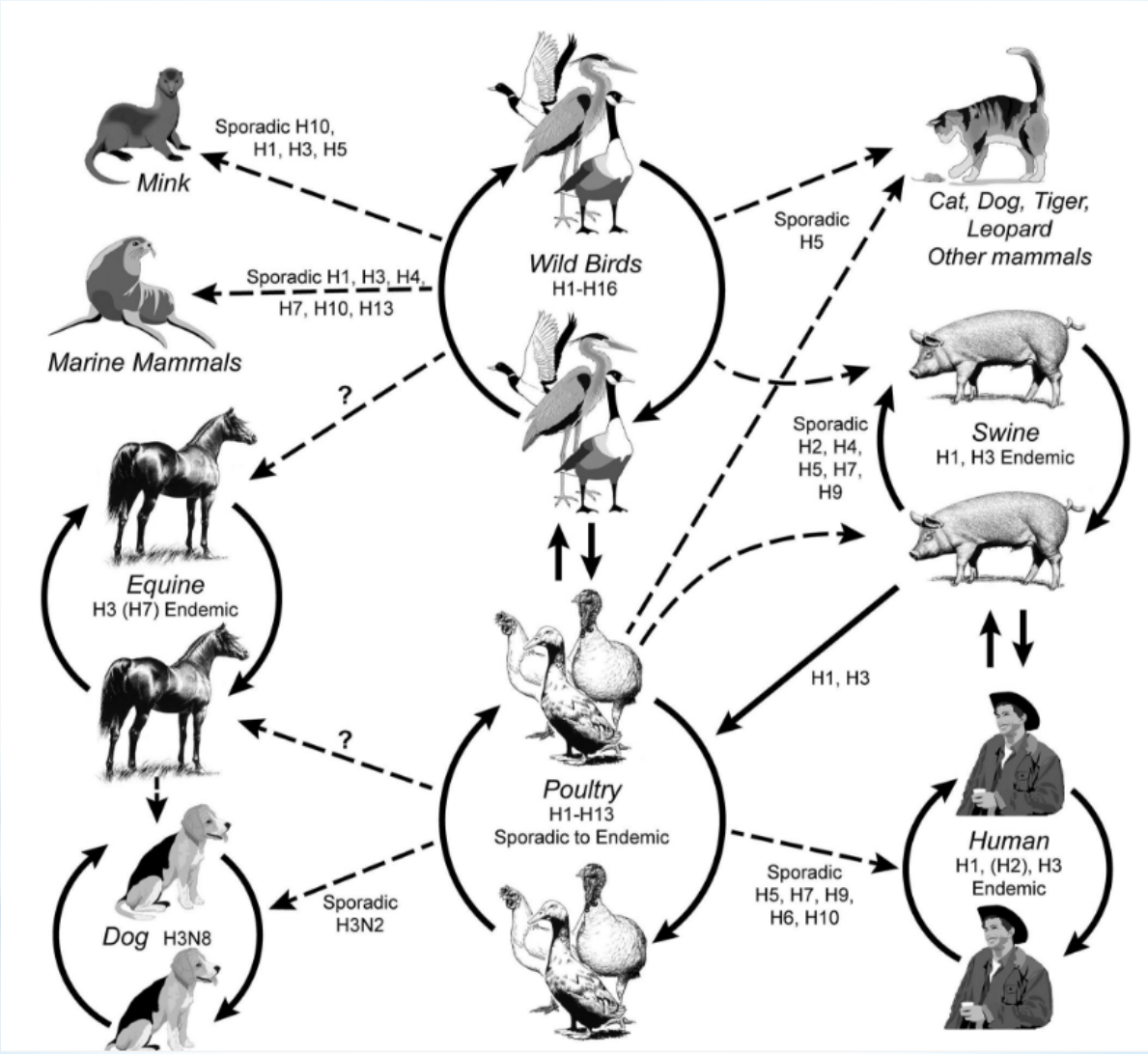
A Weekly Influenza Surveillance Report Prepared by the Influenza Division
Weekly Influenza Activity Estimates Reported by State and Territorial Epidemiologists*





*No place to hide

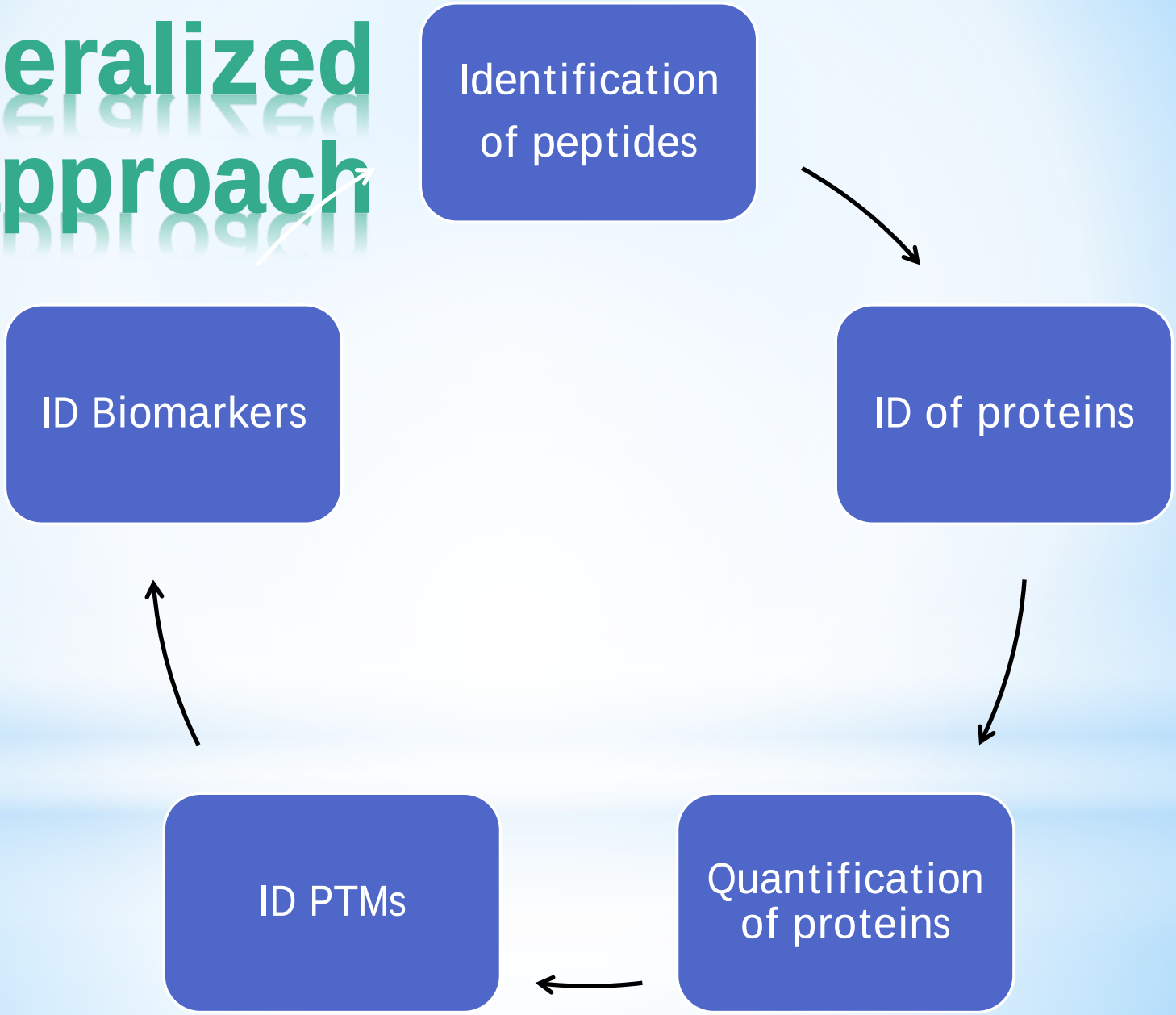
* Influenza A – Interspecies transmission



*GISAID – EpiFlu:Full length strains

*H1N1	23,068
*H3N2	23,609
*H7N9	694
*H5N1	273
*B Victoria	4500
*B Yamagata	5394
	57,538

*Generalized approach

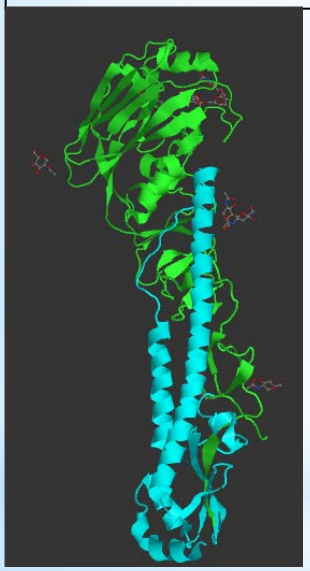


*Annual Influenza Vaccine

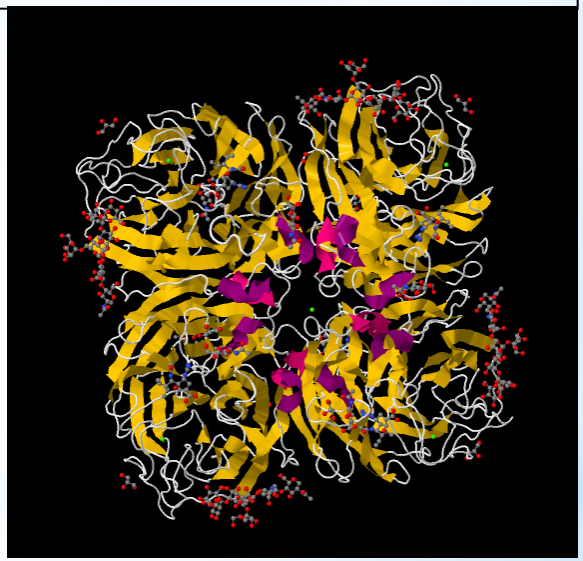
Influenza proteins -

three or four strains 15 µg hemagglutinin/0.5mL ea

A(H1N1) A(H3N2) B



http://www.itqb.unl.pt/labs/protein-modelling/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 LCKLRGVAPL
 51 HLGKCNIAGW ILGNPECESL STASSWSYIV ETPSSDNGTC YPGDFIDYEE
101 LREQLSSVSS FERFEIFPKT SSWPNHDSDK GVTAACPHAG AKSFYKNLIW
151 LVKKGNSYPK LSKSYINDKG KEVLVLWGIH HPSTSADQQS LYQNADAYVF
201 VGSSRYSKTF KPEIAIRPKV RDREGRMNYY WTLVEPGDKI TFEATGNLVV
251 PRYAFAMERN AGSGIIISDT PVHDCNTTCQ TPKGAINTSL PFQNIHPITI
301 GKCPKYVKST KLRLATGLRN IPSIQSRGLF GAIAGFIEGG WTGMVDGWYG
351 YHHQNEQGSG YAADLKSTQN AIDEITNKVN SVIEKMNTQF TAVGKEFNHL
401 EKRIENLNKK VDDGFLDIWT YNAELLVLLE NERTLDYHDS NVKNLYEKVR
451 SQLKNNAKEI GNGCFEFYHK CDNTCMESVK NGTYDYPKYS EEAKLNREEI
501 DGVKLESTRI YQILAIYSTV ASSLVLVVSL GAISFWMCSN GSLQCRICI
```

Hundreds of ambiguous IDs

1 dose vaccine (15 µg HA/500 µl)

DTT
iodoacetamide

DTT
iodoacetamide

Reduction, alkylation, quench reaction

Transfer to filter (10K MWCO)

PNGaseF in H_2O^{18}

PNGaseF in H_2O^{18}

Centrifugation wash steps
Deglycoslyation (+3)

New collection tube

trypsin

chymotrypsin

Protein digestion - centrifuge enzyme solution through filter

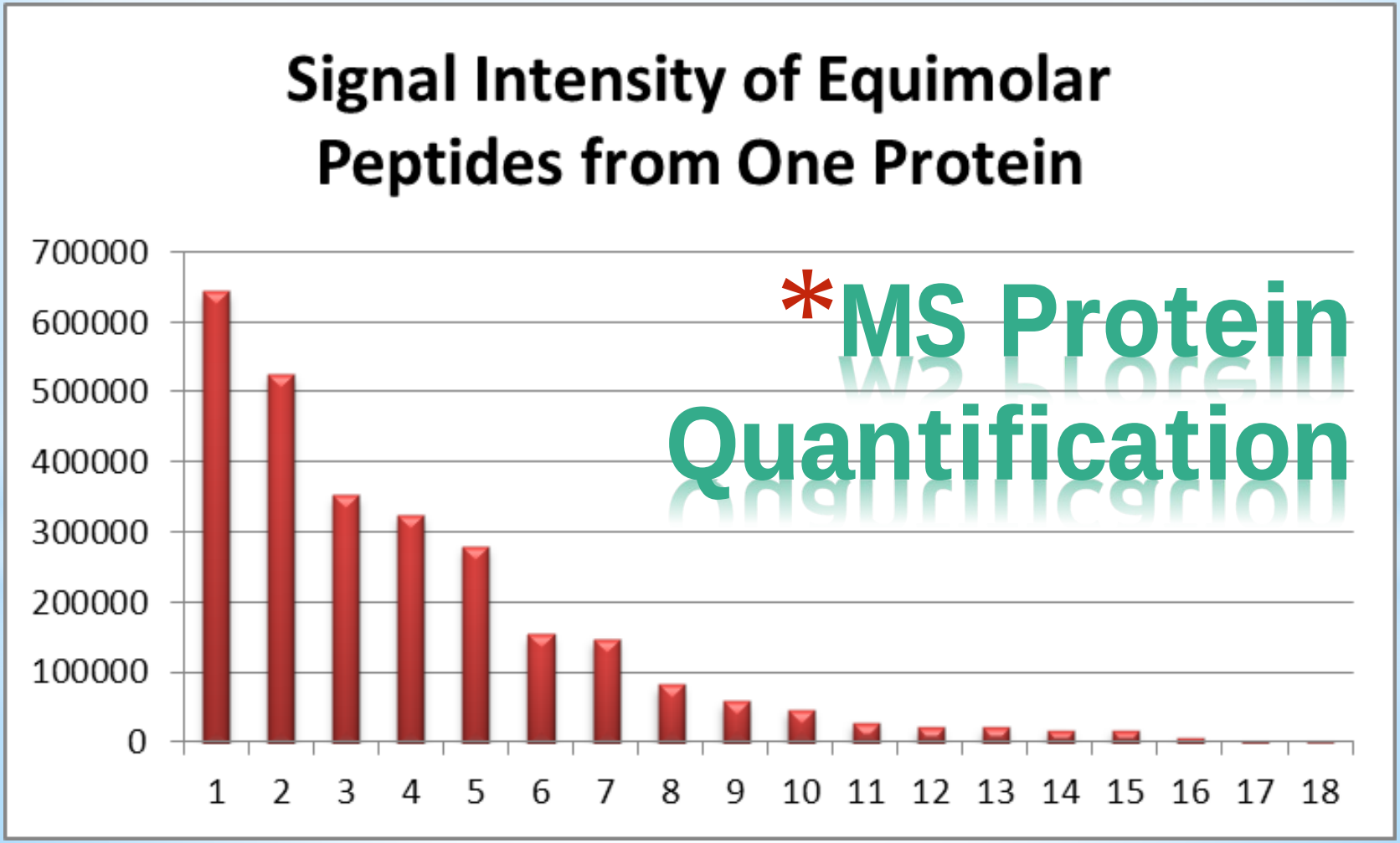
Dry down flowthrough (=peptides)
Resuspend in injection buffer

Synapt : LC-MSMS
Triplicate injections
Autocat exclusion lists

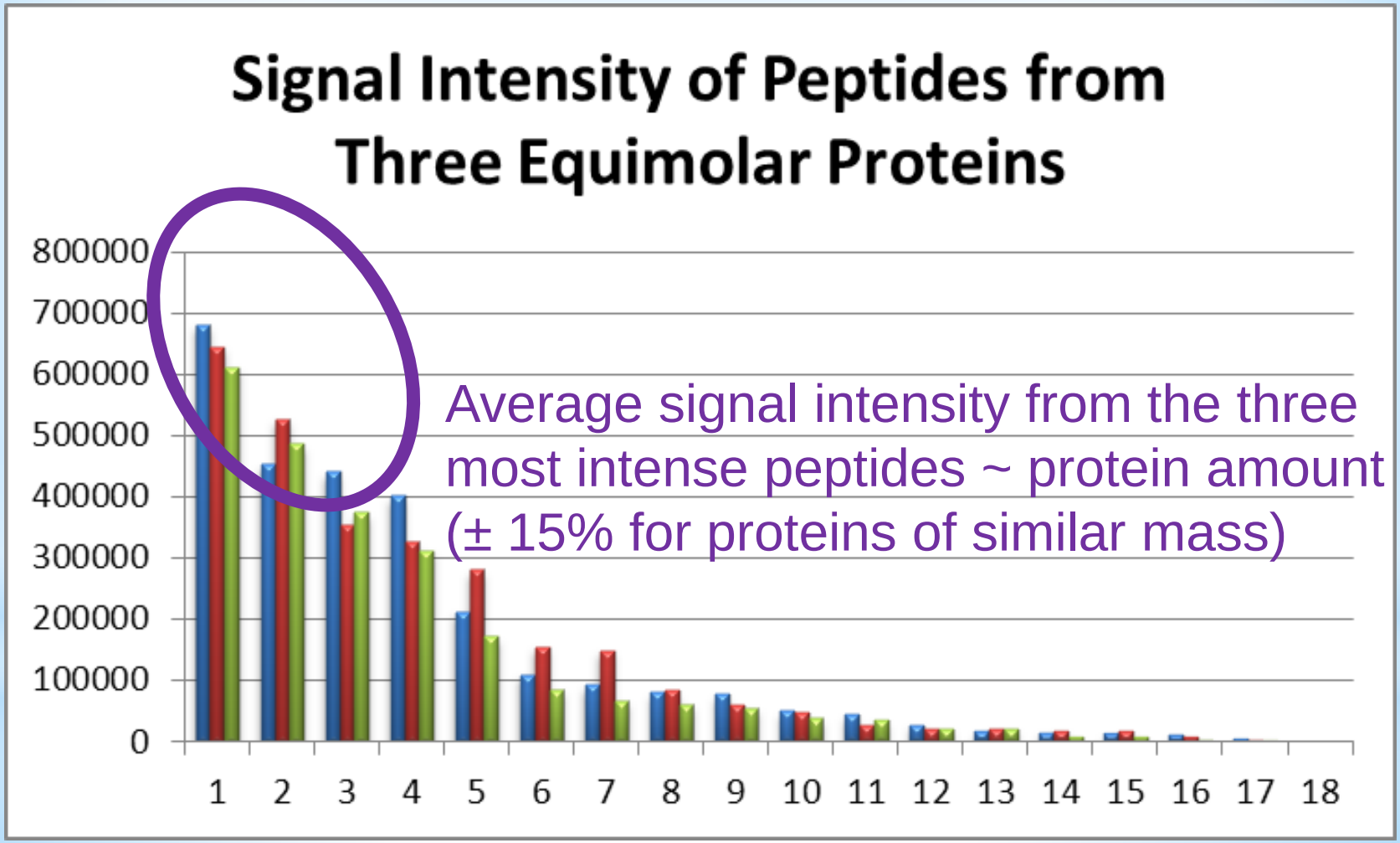
Peak list processing
Merge 6 LC-MSMS runs search
In-house influenza database

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 18O 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.

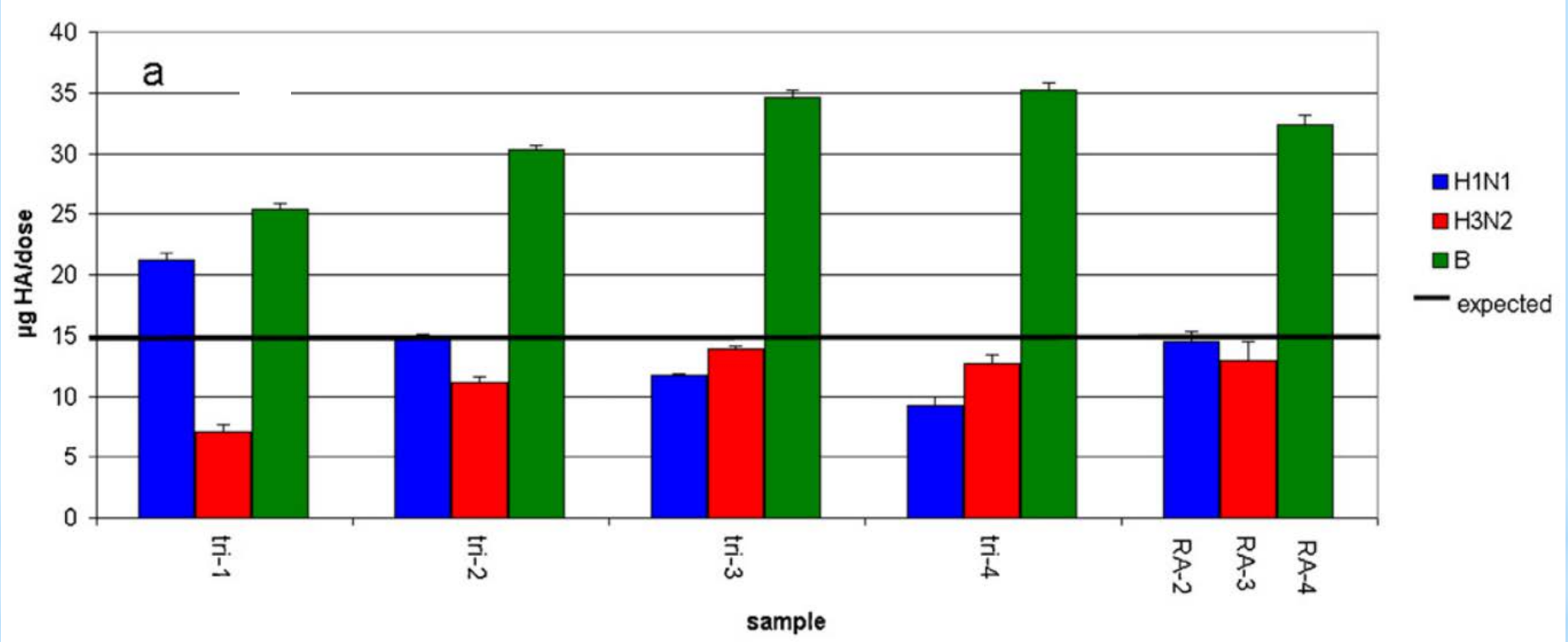


*MS Protein Quantification

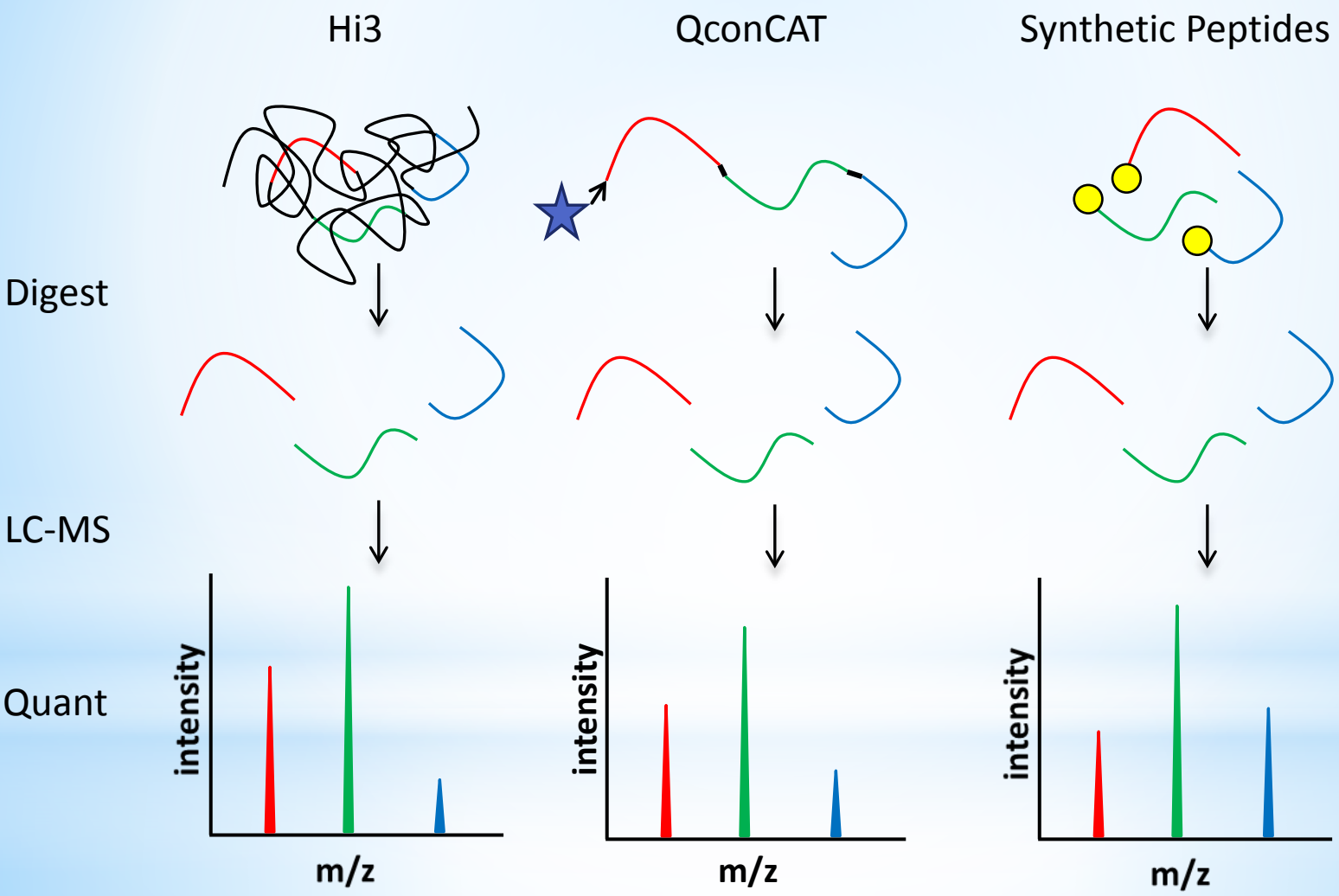


Silva JC, Gorenstein MV, Li GZ, Vissers JP, Geromanos SJ. Absolute quantification of proteins by LCMS^{E}: a virtue of parallel MS acquisition. MCP 2006 5:144–56.

* HA Quantification in Trivalent Vaccine



*Hi3/QconCAT/SpikeTides™



QconCAT Sequence

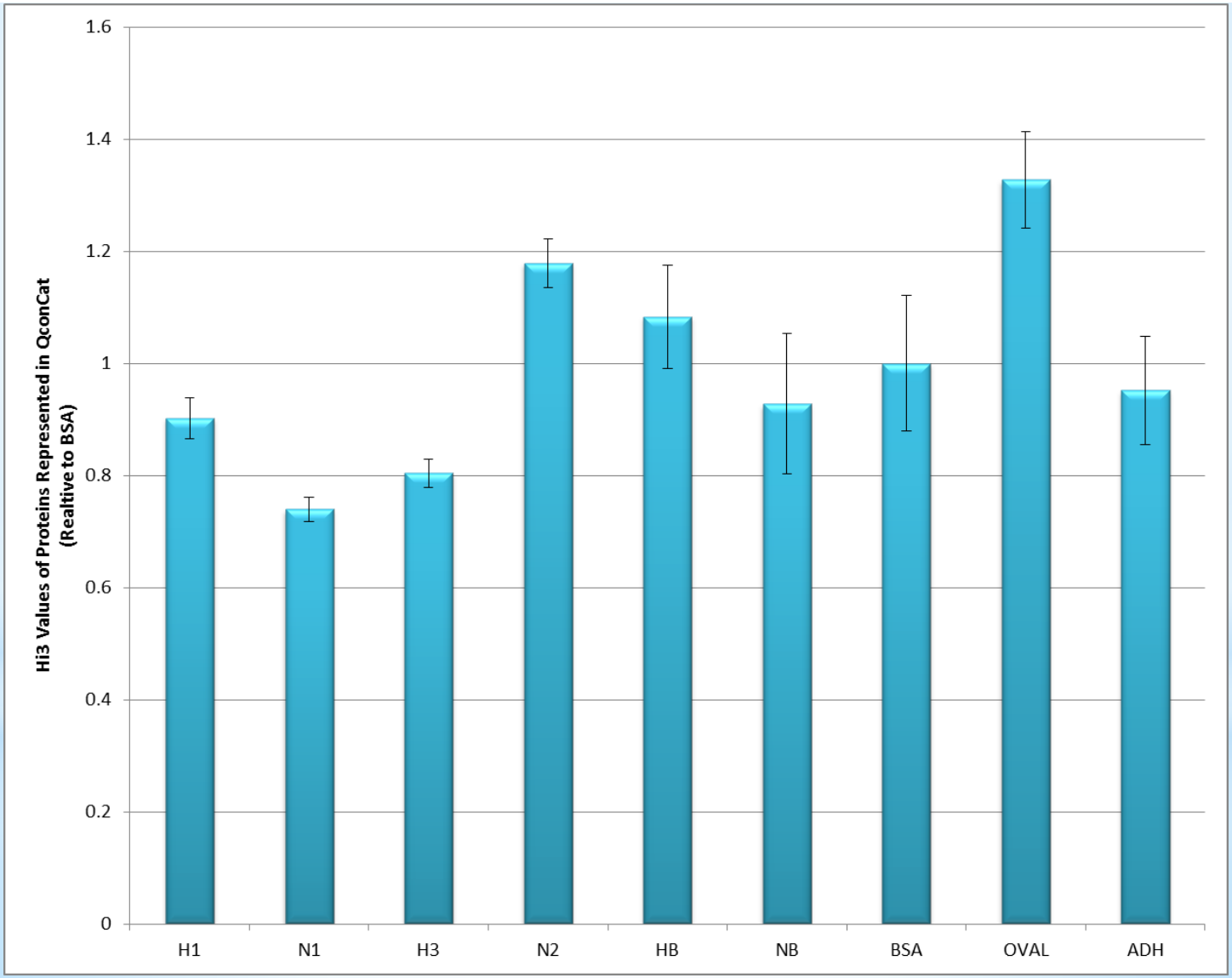
Protein	Peptides
BSA – Bovine Serum Albumin (Bos Taurus)	1- LGEYGFQNALIVR, 2- LVNELTEFAK, 3- DAFLGSFLYEYSR, 4- HLVDEPQNLIK
ADH – Alcohol Dehydrogenase (Saccharomyces cerevisiae)	1- VVGLSTLPEIYEK, 2- LPLVGGHEGAGVVVGMGENVK, 3- SISIVGSYVGNR, 4- ANELLINVK
H1 – Hemagglutinin A/California (H1N1)	1- [illegible] 3- [illegible]
N1 – Neuraminidase A/California (H1N1)	1- [illegible] 3- [illegible]
H3 – Hemagglutinin A/Victoria (H3N2)	1- [illegible] 3- [illegible]
N2 – Neuraminidase A/Victoria (H3N2)	1- [illegible] 3- SGYSGIFSVEGK, 4- GWAFDDGNDVWMGR
HB – Hemagglutinin B/Brisbane	1- LSGAMDELHNEILELDEK, 2- FTSSANGVTTHYVSQIGGFPDQTEDGGLPQSGR, 3- NLNSLSELEVK, 4- ADTISSQIELAVLLSNEGIINSEDEHLLALER
NB – Neuraminidase B/Brisbane	1- GVTLLLPEPEWTYPR, 2- LNVETDTAEIR, 3- YGEAYTDTYHSYANK, 4- GNSAPLIIR
OV – Ovalbumin (Gallus gallus)	1- GGLEPINFQTAADQAR, 2- ISQAVHAAHAEINEAGR, 3- LTEWTSSNVMEER, 4- NVLQPSSVDSQTAMVLVNAIVFK

- 4 peptides
- link peptides with a spacer (ASGK)
- Peptide set are dispersed
- C-terminal polyhistidine tag

QconCAT 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

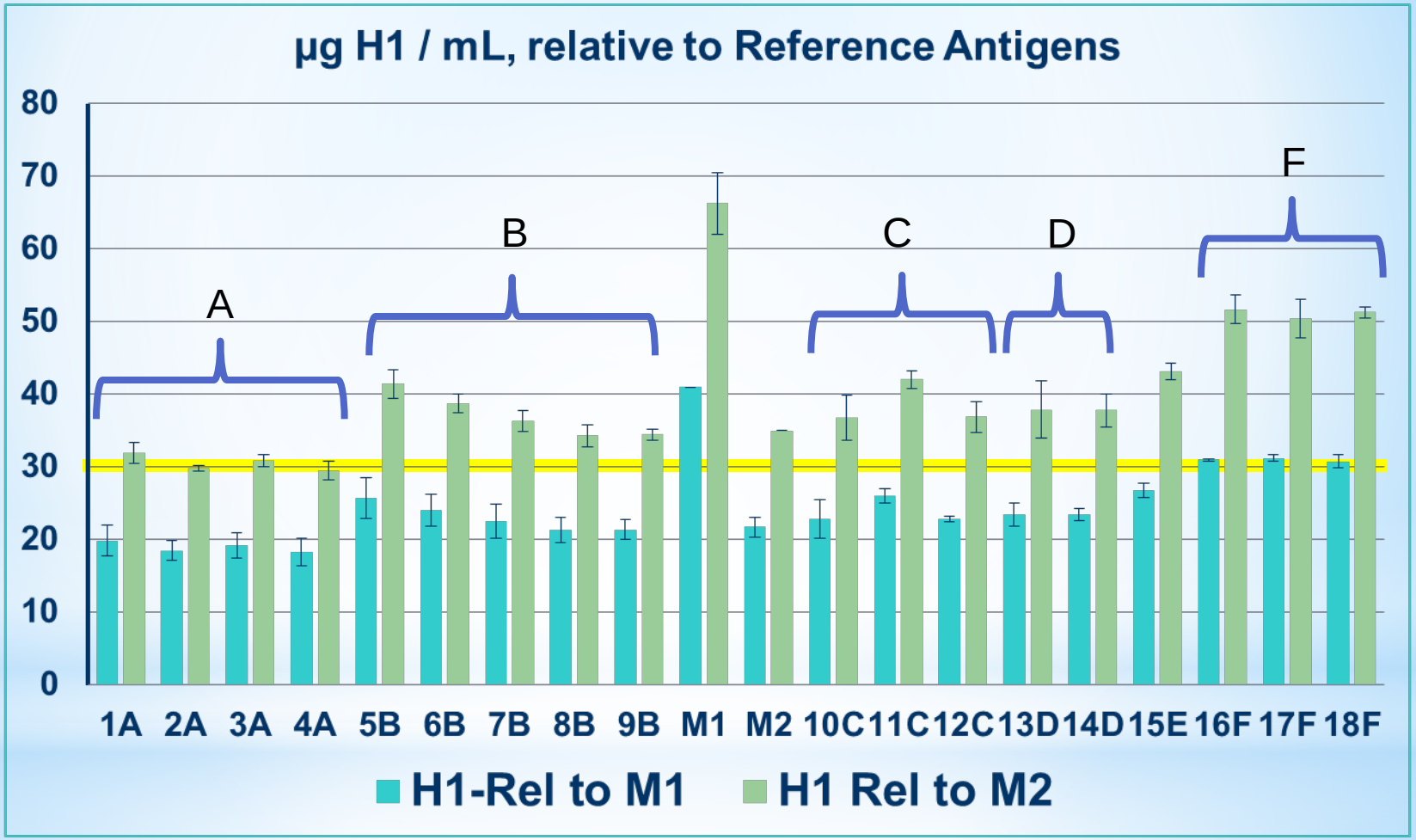
* Hi3 Values relative to BSA in QconCAT



*SpikeTides™

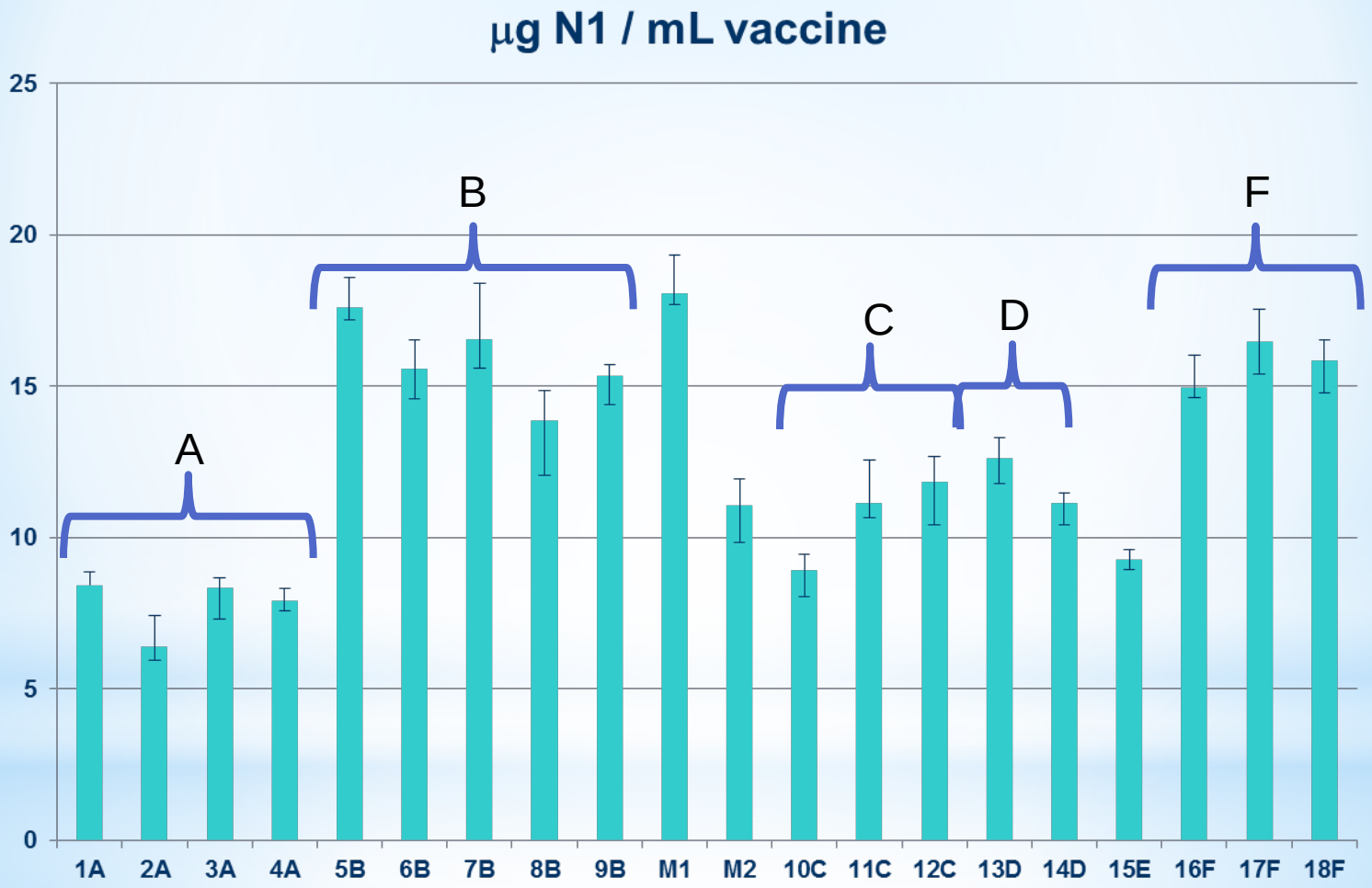
- Low cost commercial peptides *from JPT Peptide Technologies*
- Custom synthesized peptides
- Peptide quantified via a coupled chromophore
- Chromophore tag removed by trypsin

Hemagglutinin – H1

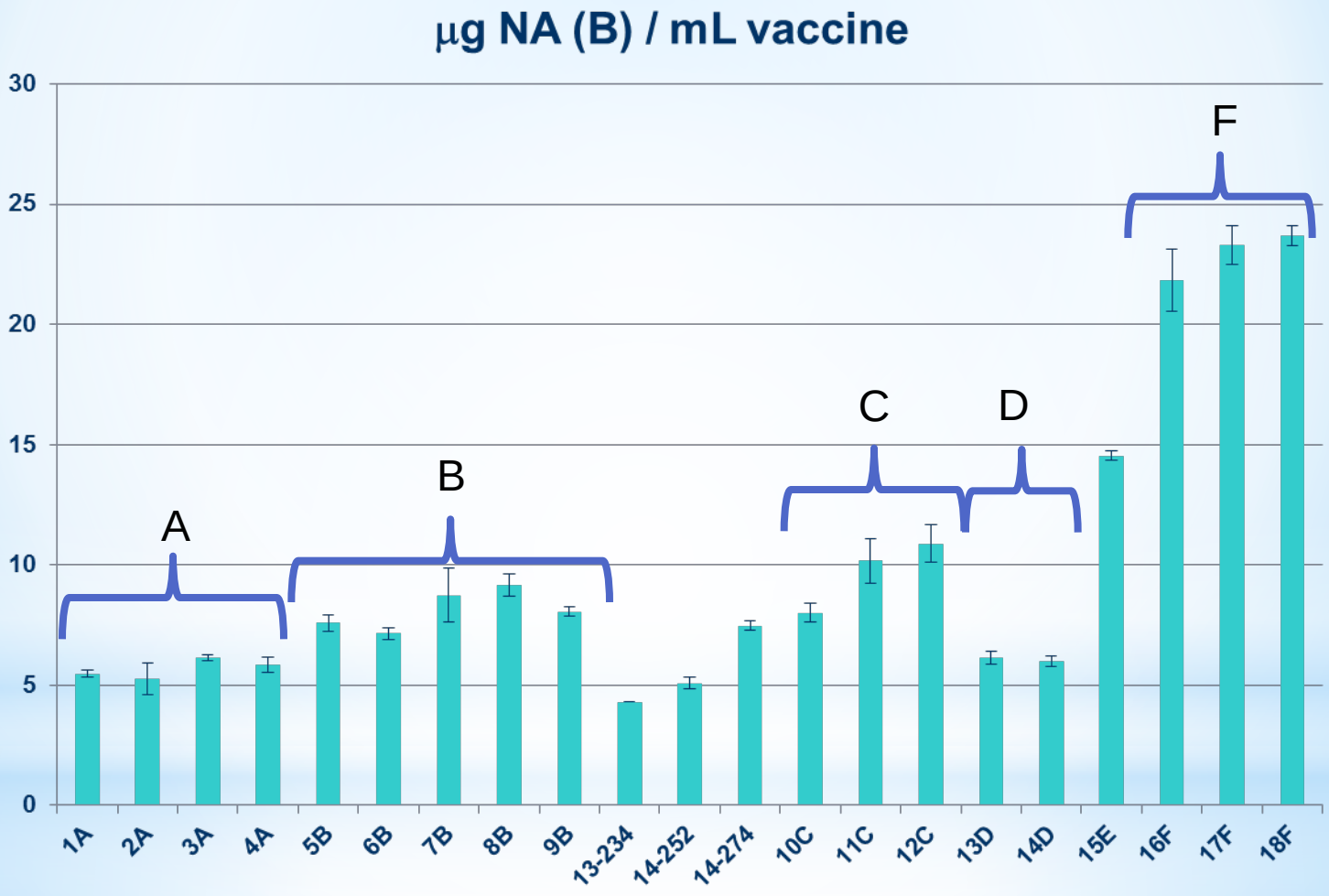


Reference antigens M1 and M2 contain 46 and 35 µg H1/mL, respectively

Neuraminidase - N1

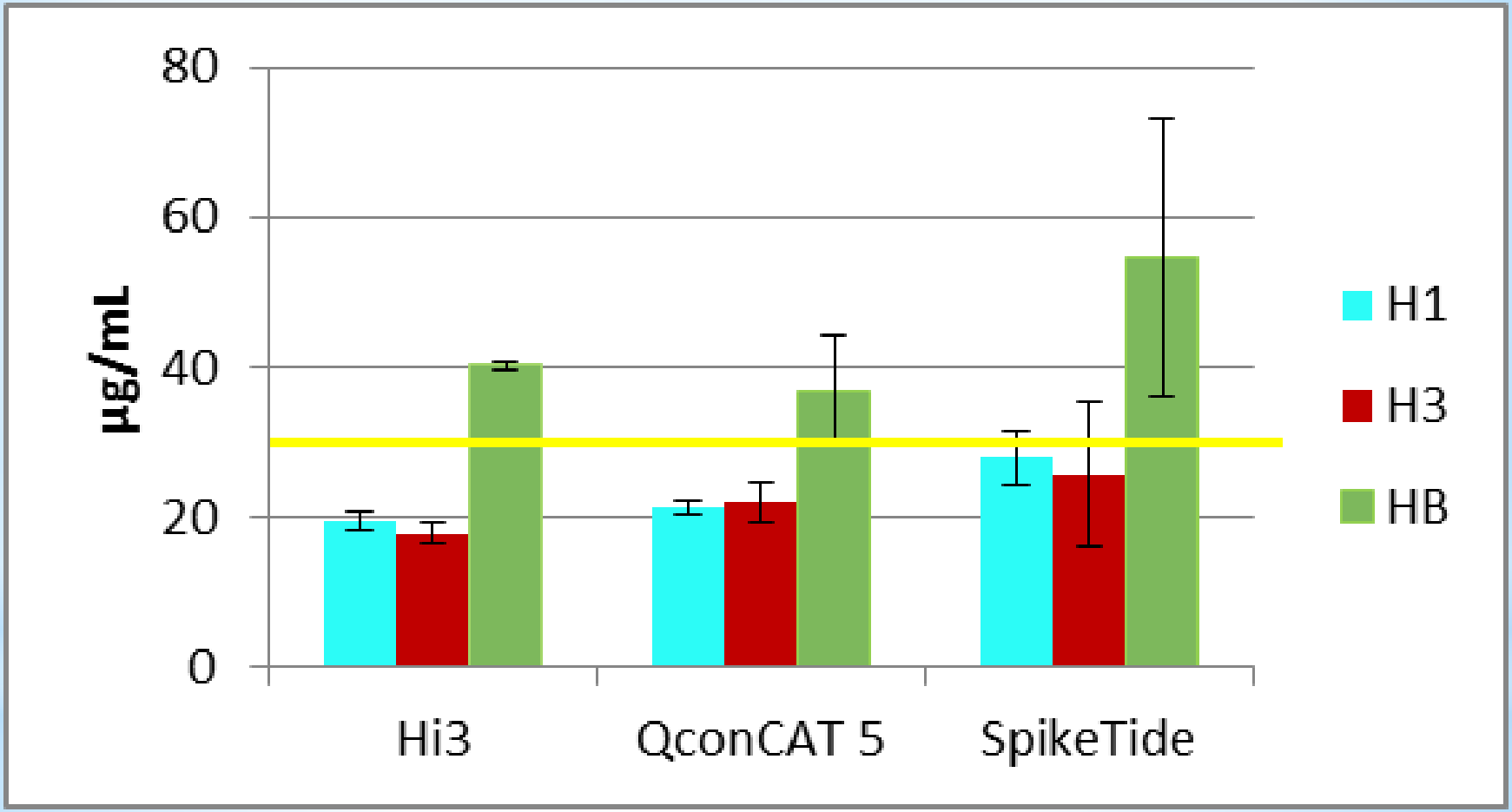


Neuraminidase – NB



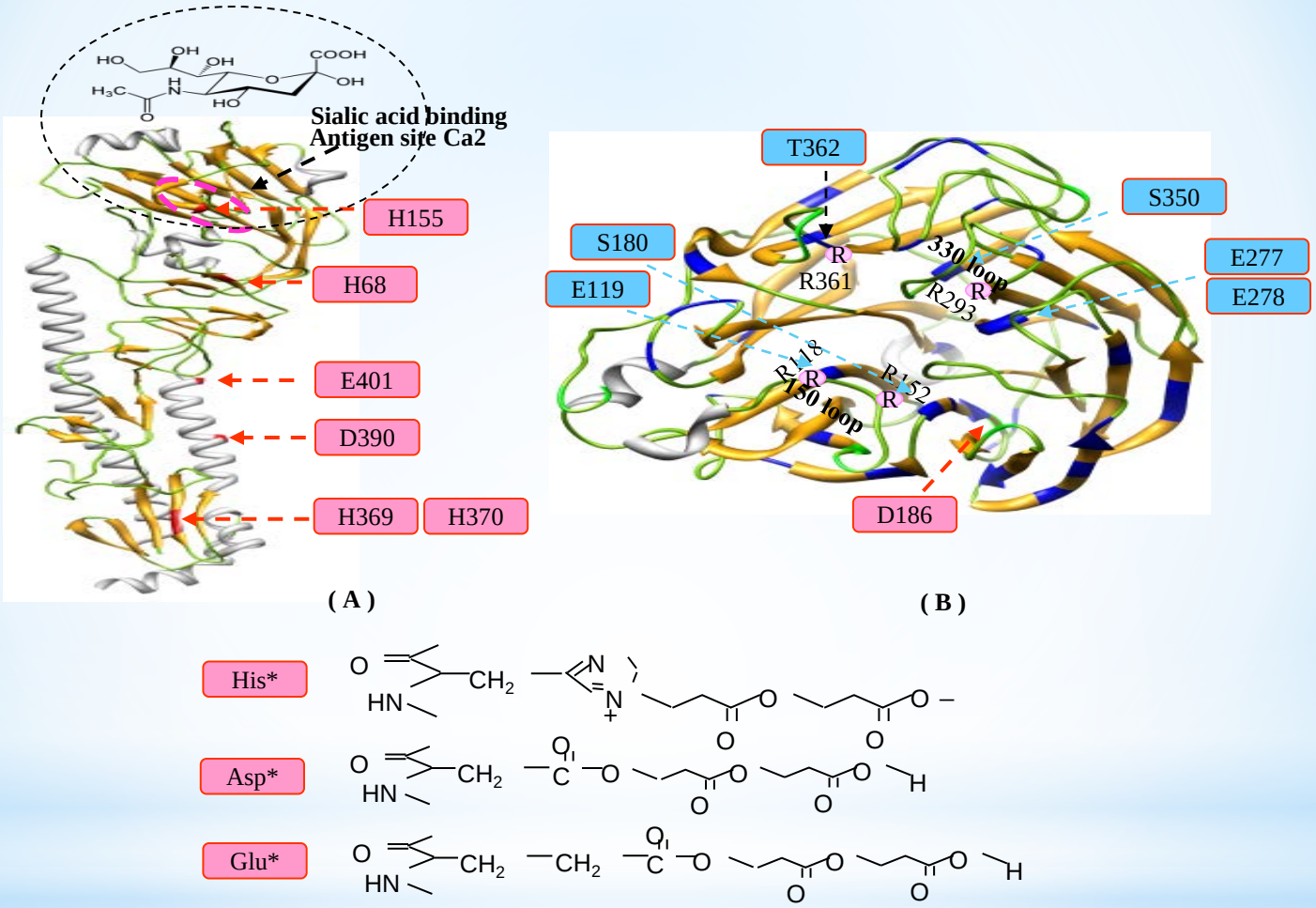
* Quadrivalent vaccines have NB from both B strains.

*Comparison



*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