Variation of Site-Specific Glycosylation Profiles for Influenza Glycoproteins from Different Vaccines and Recombinant Sources

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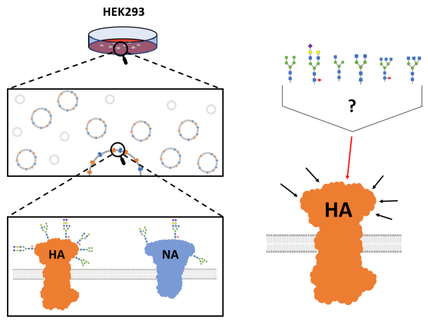
Overview

- Site-specific MS methods can be used to assess reproducibility in manufactured glycoproteins.
- Similarity in glycosylation profile is high among replicates and low between strains.
- ~90% of unique glycosylation sites on vaccines are characterized and the primary distributions include high mannose, and monofucosylated complex type glycans.

Introduction

Site-specific glycosylation analysis of influenza glycoproteins hemagglutinin (HA) and neuraminidase (NA) was conducted using high resolution mass spectrometry. Variation among glycosylation profiles was assessed to determine reproducibility among various conditions.



Methods

Recombinant Proteins

TABLE 1 Recombinant proteins analyzed for site-specific glycosylation

Abbreviation	Protein	Strain	Subtype	Vendors	Number of sequons	Protein mass* (kDa)
HA-CA09	HA	A/California/04/2009	H1N1	Creative Biomart	8	63
HA-NC99	HA	A/New Caledonia/20/1999	H1N1	Sino Biological	10	63
HA-JP57	HA	A/Japan/305/1957	H2N2	Creative Biomart	8	63
HA-HK14	HA	A/Hong Kong/485197/2014	H3N2	Biovision	13	64
HA-HK97	HA	A/Hong Kong/483/1997	H5N1	BioVision, US Biological, Sino Biological	8	64
NA-AZ08	NA	A/Arizona/13/2008	H1N1	Sino Biological	9	52
NA-TH04	NA	A/Thailand/1(KAN-1)/2004	H5N1	BioVision, US Biological, Sino Biological	3	49
NA-NL03	NA	A/Netherlands/219/2003	H7N7	Creative Biomart	11	52

*Unglycosylated

Vaccines

Table 2. Monovalent and quadrivalent vaccines analyzed for site-specific glycosylation

Vendor	Strain(s)	Subtype	Source
NIBSC	A/NewCaledonia/20/1999	H1N1	Egg
NIBSC	A/Philippines/2/1982	H3N2	Egg
NIBSC	A/Switzerland/9715293/2013	H3N2	Egg
Creative Biomart	A/Panama/2007/1999	H3N2	Egg
Creative Biomart	A/NewCaledonia/20/1999	H1N1	Egg
Creative Biomart	A/Shandong/9/1993	H3N2	Egg
Afluria Quadrivalent 2021	A/Victoria/2570/2019 A/Cambodia/e0826360/2020 B/Victoria/705/2018 B/Phuket/3073/2013	H1N1 H3N2 B/Victoria B/Yamagata	Egg
Afluria Quadrivalent 2022	A/Victoria/2570/2019 A/Darwin/6/2021 B/Austria/1359417/2021 B/Phuket/3073/2013	H1N1 H3N2 B/Victoria B/Yamagata	Egg
Flucelvax Quadrivalent 2022	A/Delaware/55/2019 A/Darwin/11/2021 B/Singapore/WUH4618/2021 B/Singapore/INFTT-16-0610/2016	H1N1 H3N2 B/Victoria B/Yamagata	MDCK cell culture
Flublok Quadrivalent 2022	A/Wisconsin/588/2019 A/Darwin/6/2021 B/Austria/1359417/2021 B/Phuket/3073/2013	H1N1 H3N2 B/Victoria B/Yamagata	SF9 recombinant

Sample processing and MS:

Proteins were digested using RapiGest surfactant in 50 mM ABC, 20 mM DTT, and 55 mM IAA. Digests were purified and desalted using a MonoSpin column procedure. Digests were analyzed on a Thermo Scientific Fusion Lumos mass spectrometer using an optimized stepped HCD energy-ion trap fragmentation sequence.

Results and Discussion

Influenza Recombinant Proteins

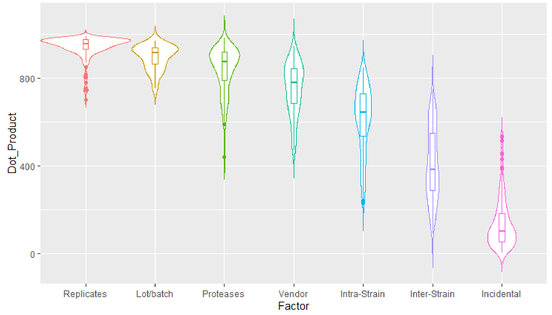


Figure 1. Violin plots depicting similarity of glycosylation distribution (Dot_Product) based on various factors. High dot product = high similarity.

Discussion

Variation in glycosylation distribution was assessed between different factors (Fig 1). Most factors, such as using different proteases (Table 1 & Fig 2), maintained high similarity. However, factors such as inter-strain differences (Fig 3 & 4) and different glycosylation sites from unrelated proteins produced lower similarity.

Table 3. Protease combinations used for digestion

Proteases	Cleavage Sites
Trypsin + Lys-C	KR
Trypsin + Glu-C	KRED
Trypsin + Chymo	KRFWYL
Chymo + Glu-C	FWYLED
Chymotrypsin	FWYL
Alpha-lytic	TASV

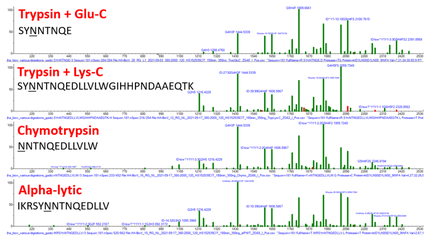


Figure 2. Glycosylation patterns compared between different protease digestions among the same glycosylation site.

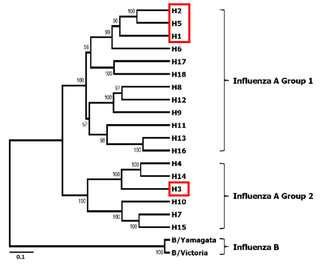


Figure 3. Evolutionary divergence in HA subtype[2]

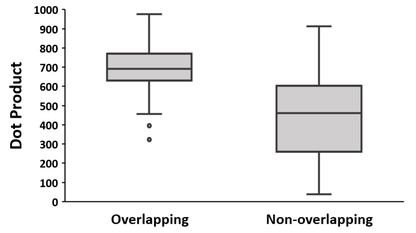


Figure 4. Similarity of glycosylation for homologous HA regions

Influenza Vaccines

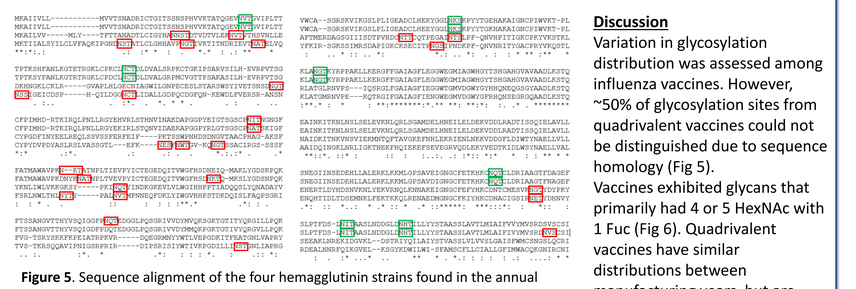
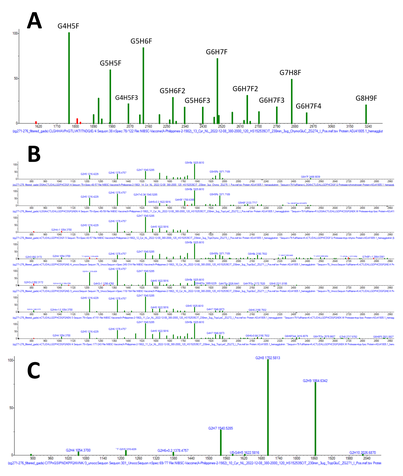


Figure 5. Sequence alignment of the four hemagglutinin strains found in the annual quadrivalent flu shot. Many sequons share a sequence with other strains, with some being distinguishable (red) and others being indistinguishable (green).

Discussion

Variation in glycosylation distribution was assessed among influenza vaccines. However, ~50% of glycosylation sites from quadrivalent vaccines could not be distinguished due to sequence homology (Fig 5).
Vaccines exhibited glycans that primarily had 4 or 5 HexNAc with 1 Fuc (Fig 6). Quadrivalent vaccines have similar distributions between manufacturing years, but are different between vendors (Fig 7).

Monovalent Vaccines



Quadrivalent Vaccines

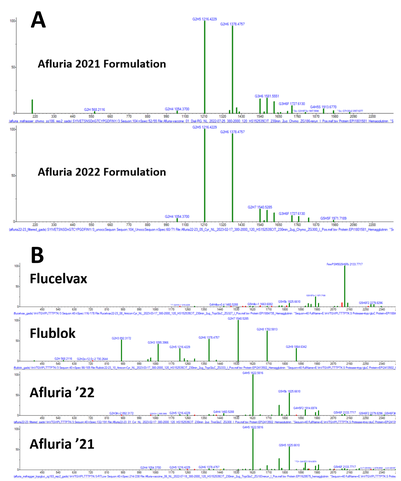


Figure 6. Glycopeptide distribution spectra for monovalent vaccines. Common distributions include: A) Monofucosylated complex glycans B) High mannose with complex and hybrid glycans C) High mannose glycans

Figure 7. Glycopeptide distribution spectra comparing quadrivalent vaccines between A) two formulations of the same vendor and B) three different vendors.

Conclusions

- Glycosylation profiles were most similar between replicates (median similarity score = 957 out of 1000), lots and batches (916), and different protease digestions (875). Profiles were least similar between vendors (781), glycosylation sites within the same protein (643), influenza strains (383), and unrelated proteins (102).
- Homologous sequence regions between different influenza strains have similar glycosylation distribution compared to non-conserved regions.
- Most glycans are high-mannose or monofucosylated complex in egg-based quadrivalent vaccines. No distributions were detected for NA due to low expression.

References and Disclaimers

[1]Remoroza, C. A., Burke, M. C., Liu, Y., Mirokhin, Y. A., Tchekhovskoi, D. V., Yang, X., & Stein, S. E. (2021). Representing and Comparing Site-Specific Glycan Abundance Distributions of Glycoproteins. Journal of Proteome Research, 20(9), 4475-4486.
[2]Jang, Y. H., & Seong, B. L. (2014). Options and obstacles for designing a universal influenza vaccine. Viruses, 6(8), 3159-3180.