Supporting Information for: Quantification of Immunoreactive Viral Influenza Proteins by Immunoaffinity Capture and Isotope-Dilution Liquid Chromatography Tandem Mass Spectrometry

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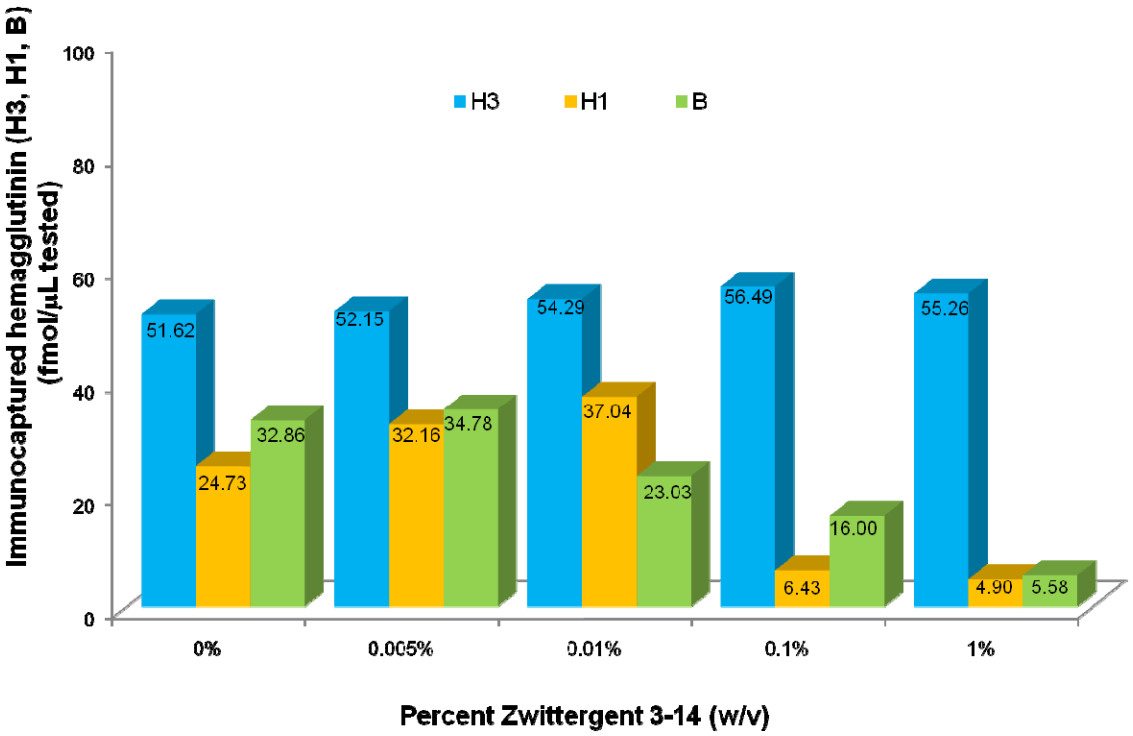

Figure S-1: Graph of immunocaptured HA (fmol/µL tested) versus percent Zwittergent 3-14 (w/v) concentration. Seasonal TIV samples were pretreated prior to pAb-H3 antibody capture and binding incubations were performed at five Zwittergent 3-14 concentrations both above and below the critical micelle concentration (CMC) of 0.011 % (w/v); 0.0%, 0.005%, 0.01%, 0.1% and 1%. The 0% Zwittergent corresponds to the PBS reference. Triplicate IC-IDMS digests were prepared at all Zwittergent 3-14 concentrations. No significant differences were observed in the amount of H3 HA specifically bound to the pAb-H3 when compared to the PBS reference (mean = 53.97 fmol/µL, Stdev = 2.06, (n = 5)). Non-specific H1 HA and B HA binding was observed in all PBS and Zwittergent-treated samples, however at the higher concentrations of Zwittergent 3-14, a significant drop in non-specific interactions was observed.

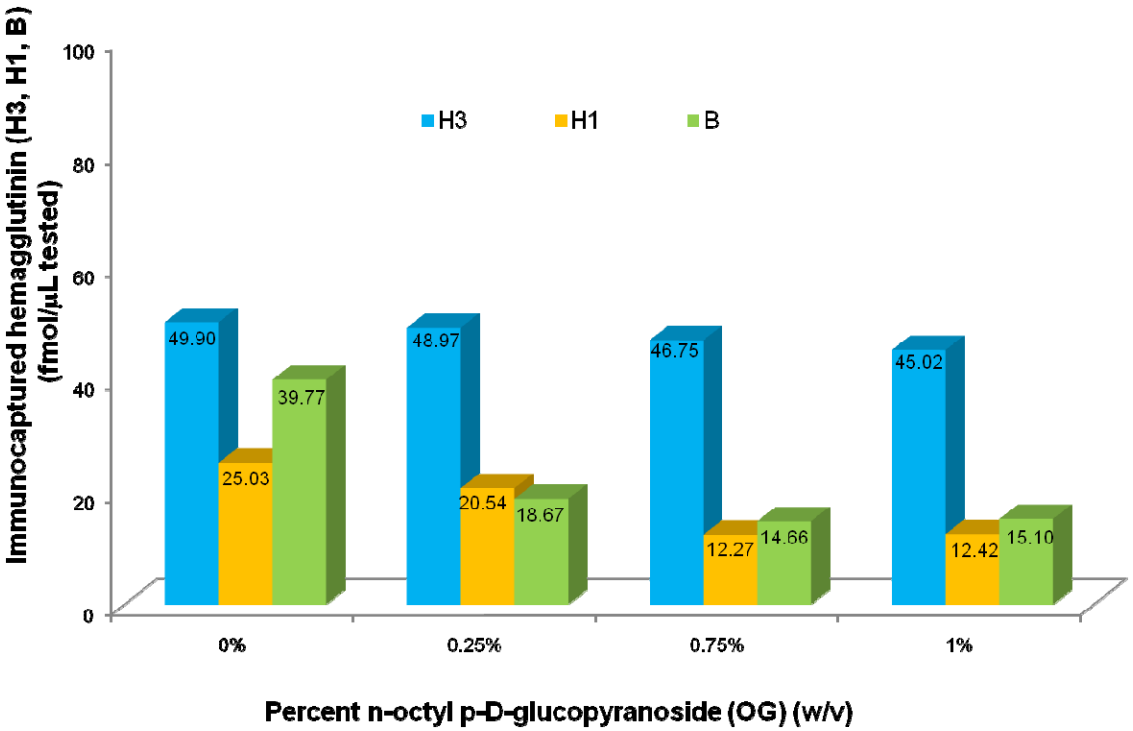

Figure S-2: Graph of immunocaptured HA (fmol/µL tested) versus percent n-octyl p-D-glucopyranoside (OG) (w/v) concentration. Seasonal TIV samples were pretreated prior to antibody capture and binding incubations were performed at four OG concentrations both above and below the critical micelle concentration (CMC) of 0.73 % (w/v); 0.0%, 0.25%, 0.75%, and 1%. The 0% OG corresponds to the PBS reference. Triplicate IC-IDMS digests were prepared at all OG concentrations. No significant differences were observed in the amount of H3 HA specifically bound to the pAb-H3 when compared to the PBS reference (mean = 47.66 fmol/µL, Stdev = 2.20, (n = 4)). Non-specific H1 HA and B HA binding was observed in all PBS and OG-treated samples. At all OG concentrations non-specific binding was reduced, with the greatest reduction observed at the highest percent OG (1%).

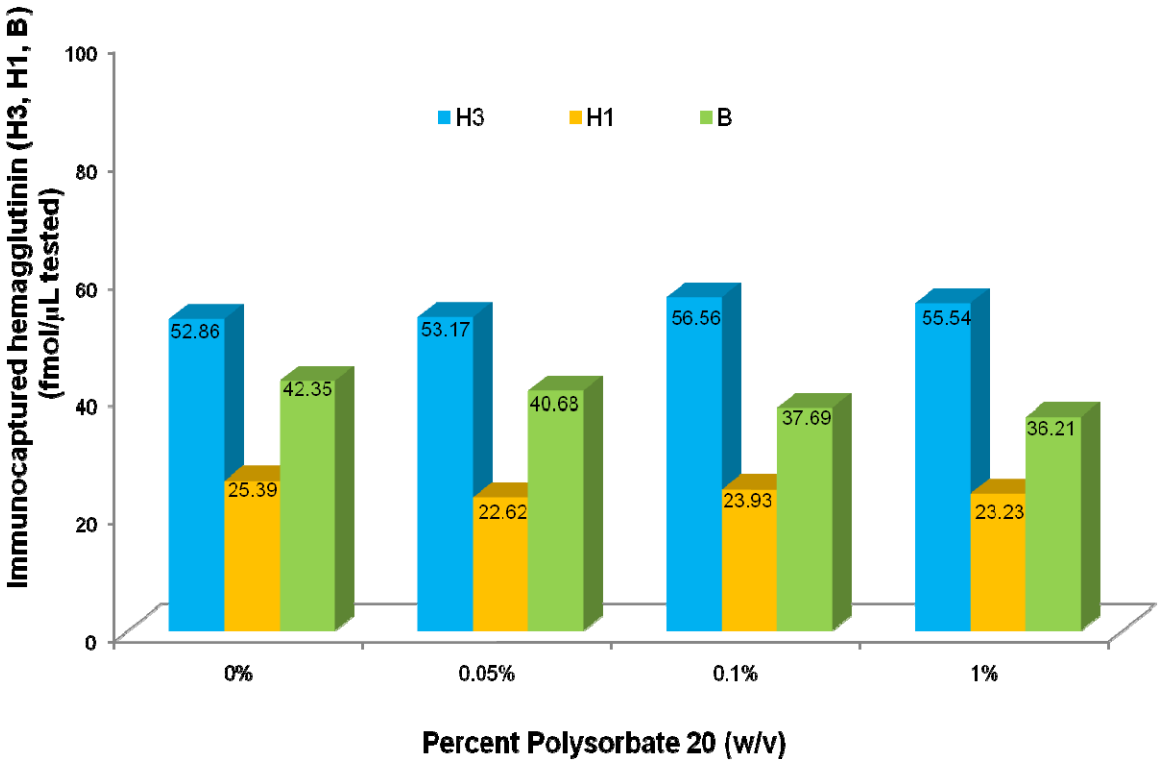

Figure S-3: Graph of immunocaptured HA (fmol/µL tested) versus percent Polysorbate 20 (Tween 20) (w/v) concentration. Seasonal TIV sample binding incubations were in PBS. Following antibody capture, experimental wash procedures were performed at four Tween 20 concentrations both above and below the critical micelle concentration (CMC) of 0.07 % (w/v); 0.0%, 0.05%, 0.1%, and 1%. The 0% Tween 20 corresponds to the PBS reference wash buffer. Triplicate IC-IDMS digests were prepared at all Tween 20 concentrations. No significant differences were observed in the amount of H3 HA specifically bound to the pAb-H3 when compared to the PBS reference wash buffer (mean = 54.46 fmol/µL, Stdev = 1.80, (n = 4)). Non-specific H1 HA and B HA binding was observed in all TIV samples washed with PBS and Tween 20 post-antibody capture. At the Tween 20 concentrations tested, addition of Tween 20 in the wash buffer did not appear to affect the pAb-H3 non-specific interactions (H1 HA mean = 23.79 fmol/µL, Stdev = 1.19, (n = 4); B HA mean = 39.23 fmol/µL, Stdev = 2.79) and no measureable differences were observed.