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A miniaturized glycan microarray assays for assessing avidity and specificity of influenza A virus hemagglutinins

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Abstract:

Influenza A Viruses (IAV) hemagglutinins specifically recognize sialic acids on the cell surface as functional receptors to gain entry into cells. IAVs originate from wild waterfowl, but adapt in order to cross the species barrier to poultry, swine, horses and humans. Avian viruses recognize sialic acid attached to a penultimate galactose by a α2–3 linkage (avian type receptors) whereas human viruses preferentially recognize sialic acid with a α2–6 linkage (human type receptors). To monitor if avian viruses are adapting to human type receptors, several methods can be used. Glycan microarrays with a diverse library of synthetic sialosides are increasingly used to evaluate receptor specificity. However this technique is not used for measuring avidities. Measurement of avidity is typically done looking at the binding of serially diluted hemagglutinin or virus to glycans adsorbed to conventional polypropylene 96 well plates. In this assay glycans with α2–3 or α2–6 sialic acids are coupled to biotin and adsorbed to streptavidin plates, or are coupled to polyacrylamide (PAA) which directly adsorb to the plastic. Here we have significantly miniaturized this assay by directly printing PAA-linked sialosides and their non PAA-linked counterparts in a micro well glycan array consisting of 48 arrays on a single glass slide. This allows a complete assay to run 6 glycan binding proteins with 8 dilutions toward 6 different glycans including two non-sialylated controls in six replicates on a single glass slide. Effectively running 18× 96-well plates per slide. This glycan array assay decreases consumption of compounds and biological with an efficiency increase of 5.4×10ˆ5.

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