

## **New methods to dissect antibody responses against influenza**

Influenza pseudotypes have been successfully used to undertake serological assays for pharma, and will hopefully contribute eventually towards the licensure of influenza vaccines, at least as adjunct assay. We have shown that pseudotypes can be used to quantify globular head-focused neutralizing antibody responses elicited by a pre-pandemic H5N1 vaccine and that these correlated significantly with those measured by HI, SRH and microneutralization. Pseudotype neutralization assays for influenza have been shown to be exquisitely sensitive for the measurement of responses to the HA stalk which is one of the primary targets of many 'Universal' vaccine approaches. The responses directed against the stalk can be separated from those against the head by making use of a hybrid HA pseudotype. One such virus that we have created has an HA stalk derived from the 2009 pandemic H1N1 strain, and an HA globular head derived from an H11 strain which is found in wild birds. Humans have negligible serological reactivity to H11 so if a neutralization assay is undertaken with this hybrid HA pseudotype, one can readily measure the response against the HA stalk only. The traditional HI assay (for which a correlate of immunity exists) used by the regulators only measures responses against the globular HA head and thus is not fit for purpose for the licensing of many new 'Universal' vaccines. HA stalk serological assays, such as those based on pseudotypes or ELISA will no doubt gain prominence as many of these new vaccines move down the clinical pipeline. Recently we have developed a pseudotype-based ELLA (PV-ELLA) which allows the quantification of antibody responses against neuraminidase (NA). This assay innovatively uses NA-only pseudotypes as a source of NA. The availability of this PV-ELLA to the R&D community is instrumental as it means that ELLA assays can be performed without requiring access to Reverse Genetic (RG) viruses which often have IP attached. Also, there are moves towards standardizing the amount of NA in recombinant vaccines, making PV-ELLA assays increasingly important.

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