PAN-HA ANTIBODIES FOR INFLUENZA DETECTION AND QUANTIFICATION

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The influenza virus imposes a heavy burden for society in terms of health and economy. Influenza is an elusive enveloped virus due to antigenic shift and drift of two surface proteins: neuraminidase (NA) and hemagglutinin (HA). As a result, new strains emerge every year which require seasonal vaccination for protection. Furthermore, large vaccine quantities are urgently needed in case of pandemics. Theoretically, vaccines against a new strain can be manufactured in as little as three weeks with certain platforms and technologies. However, vaccine quantification and release are still relying on the use of the Single Radial Immunodiffusion (SRID) assay using a strain-specific antibody to calculate HA concentration. This is a major limitation because it can take up to three months to generate the reagents necessary to run the SRID assay, including the strain-specific antibody. Hence, one of the major hurdles in the process of influenza vaccine production is the quantification of HA which is critical to establish proper dosing.

To circumvent the need for strain-specific antibodies, we have produced two monoclonal antibodies (F211-11H12-3 and F211-10A9-2) against a highly conserved peptide sequence found within the HA molecule (1). Multiple strains belonging to 13 different influenza A subtypes, as well as 6 strains belonging to B lineages were detected by Western blot and dot blot. Overall, mAb F211-11H12-3 recognizes preferentially influenza A subtype 1, while the mAb F211-10A9-2 has a higher affinity for influenza A subtype 2. Therefore, all strains tested could be detected when both mAb are combined and used as a cocktail. Next, we performed quantitative dot blots by generating a standard curve ranging from 160ng/ml to 20µg/ml HA. This method is simple, easy to implement and highly reproducible. In-process samples as well as purified material can be quantified by dot blot after denaturation with urea. Even though the SRID is the only assay approved by regulatory agencies, quantitative dot blots can be used during manufacturing to optimize and monitor the production process. Finally, ELISA is widely used for quantification and preliminary data demonstrates that samples can be quantified with the pan-HA mAbs.

In conclusion, a pan-HA antibody cocktail was generated against a highly conserved peptide sequence of influenza. Viruses produced in eggs and mammalian cells from 40 different strains were detected by Western blot. Reproducible quantification was achieved by dot blot using the two mAbs and an appropriate calibrating standard. The combination of pan-HA antibodies with an immunoassay such as the dot blot assay could accelerate process development and help establish new generation quantification methods for influenza. As the field is looking for flexible and versatile solutions to shift away from the SRID assay and strain-specific antibodies, the development of broad-spectrum antibodies offers a long-awaited alternative.

1)   Chun et al, Universal antibodies and their applications to the quantitative determination of virtually all subtypes of the influenza A viral hemagglutinins, Vaccine (26), pp 6068-6076, 2008.