NRC·CNRC

PAN-HA MONOCLONAL ANTIBODIES

For the quantification of Influenza

HIGHLIGHTS

Influenza type A and B are responsible for seasonal flu epidemics. Influenza is classified based on the viral surface proteins hemagglutinin (HA) and neuraminidase (NA). Currently, there are 18 known HA subtypes; point mutations in any HA subtype or antigenic shift can produce the conditions necessary for an influenza pandemic.

Vaccines are produced every year to mitigate the potential negative effects of influenza. Working with regulatory agencies, the production and distribution of antibodies necessary to quantify new vaccine lots can take up to 16 weeks, which can cause significant delays for vaccine production.

The Single Radial Immunodiffusion (SRID) assay is the only accepted quantification method, and requires specific standardized antibodies against each strain of influenza. The usage of antibodies with broad-specificity to influenza could yield fast and accurate quantification through alternative methods, potentially accelerating vaccine production. Indeed, the vaccine development process could be advanced through the use of pan-HA monoclonal antibodies (mAbs), while the associated accumulation of data could prompt replacement of SRID by a more efficient assay to speed up annual vaccine distribution.

To address this need, the NRC has generated two monoclonal antibodies showing specificity against 40 strains of influenza A and B, encompassing HA subtypes H1 to H13. When combined, they form a pan-HA mAb cocktail that greatly reduces the need for strain-specific antibodies, which could thereby accelerate the annual vaccine production process.

TECHNOLOGY TRANSFER

- Commercial exploitation licence
- R&D agreement for development
- Market application

- Replace strain-specific antibodies to accelerate the development of yearly vaccine lots.
- Use in alternative quantification methods to SRID, which is limited by the availability of reagents.
- Applicable to Western blot, dot-blot, ELISA and other immunoassays using denatured antigens.

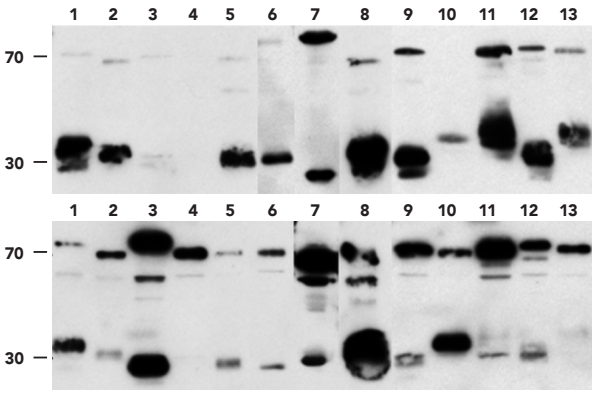

Figure 1: Binding of mAb 11H12 to 13 subtypes of influenza. Molecular markers corresponding to HA2 at 30kDa and HA1 at 70kDa are indicated. Full names of the virus strains corresponding to each lane are shown in the table below.

LANE	SUBTYPE	STRAIN
1	A/H1N1	A/Puerto Rico/8/34
2	A/H2N2	A/Singapore/1/57
3	A/H3N2	A/New York/55/01
4	A/H4N6	A/Duck/Czechoslovakia/56
5	A/H5N9	A/Turkey/Wisconsin/68
6	A/H6N2	A/Turkey/Massachusetts/3740/65
7	A/H7N7	A/Equine/Prague/1/56
8	A/H8N4	A/Turkey/Ontario/6118/68
9	A/H9N2	A/Turkey/Wisconsin/1/66
10	A/H10N8	A/Quail/Italy/1117/65
11	A/H11N6	A/Duck/England/56
12	A/H12N6	A/Duck/Wisconsin/480/79
13	A/H13N6	A/Gull/Maryland/704/77

- Could be immobilized onto a surface or linked to a cargo molecule for novel quantification methods, diagnostic methods, or therapeutic applications.
- Use as a full-length IgG, Fv, scFv, Fab, F(ab')2 fragment, or part of a chimeric molecule.
- Use for detection and quantification.

HOW IT WORKS

A highly-conserved peptide sequence among influenza strains and subtypes was used to generate mouse hybridomas. Following screening, monoclonal antibodies 10A9 and 11H12 were selected for their broad reactivity amongst tested influenza strains, and were thus sequenced. The NRC's proprietary CHO cell line was transfected to generate stable pools and cell lines. The stable pools were then thawed, expanded and induced with cumate to start production of the mAb under predetermined conditions. Large quantities of mAbs could be produced by the use of large-scale bioreactors, thus providing high-quality monoclonal pan-HA antibodies to ensure a consistent production system to support industrial needs.

The purified antibodies show no degradation after 12 months, and binding affinity remains unchanged after three freeze-thaw cycles. Immunoassay results show that 11H12 binds preferentially to influenza A group 1 while 10A9 binds preferentially to group 2. Tested influenza type B strains were recognized by either one of the antibodies. Combination of the two antibodies generated a complementary cocktail that allows detection of a broad spectrum of influenza strains and types.

BENEFITS

- Reproducible quantification of Influenza strains A and B.
- Avoid the need of strain-specific anti-HA antibodies.
- Effectively detects full-length HA and peptides in three (3) conformations: monomers, dimers, and oligomers.
- 10A9 and 11H12 mAb both recognize a highly-conserved region of HA with a different, but complementary, binding profile.
- 10A9 and 11H12 mAb can be used as a cocktail for pan-HA detection, or alone based on specific needs.

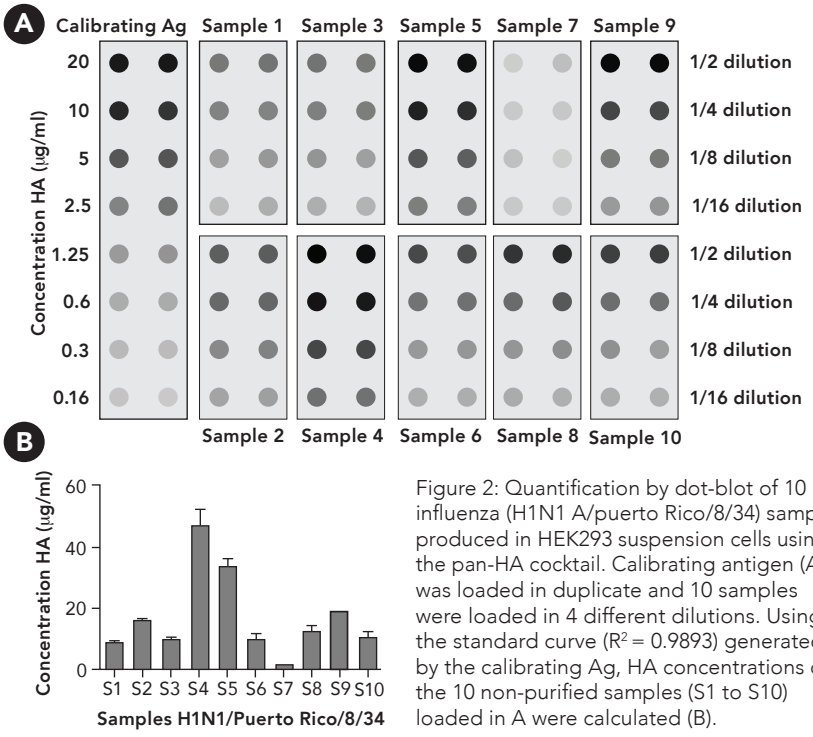

Figure 2: Quantification by dot-blot of 10 influenza (H1N1 A/puerto Rico/8/34) samples produced in HEK293 suspension cells using the pan-HA cocktail. Calibrating antigen (Ag) was loaded in duplicate and 10 samples were loaded in 4 different dilutions. Using the standard curve ($R^2 = 0.9893$) generated by the calibrating Ag, HA concentrations of the 10 non-purified samples (S1 to S10) loaded in A were calculated (B).

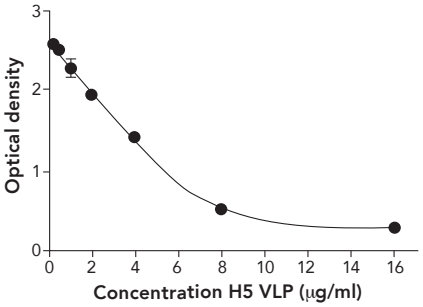

Figure 3: A standard curve was generated with H5 VLP in a competitive ELISA. The 10A9 antibody at a concentration of 800ng/mL was mixed with different concentrations of H5 VLP and added to a coated plate. A four parametre logistic regression was performed to fit th curve.

- Detection of recombinant HA (rHA), virus-like particles (VLPs), purified viruses or crude samples.
- Recognition of HA originating from different production platforms: mammalian cells, insect cells, avian cells, eggs, and plants.
- Stable pools of CHO cells readily-available for efficient production of both mAbs.
- Production process can be scaled up to bioreactors to address industrial needs.

PATENTS

WO 2018/138681 A1

REFERENCE

Manceur et al, Generation of monoclonal pan-hemagglutinin antibodies for the quantification of multiple strains of influenza (2017) PLoS ONE 12(6):e0180314

CONTACT

Daniel Desmarteaux,
Client Relationship Leader
Research centre or program
514-496-5300
Daniel.Desmarteaux@cnrc-nrc.gc.ca

http://www.canada.ca/nrc-human-health-therapeutics

PDF: Cat. No. NR16-320/2020E-PDF
ISBN 978-0-660-34836-0
062020 • Également disponible en français