

## Comparison of Hemagglutination Inhibition and Mass Spectrometric Immunoassays to Investigate the Antigenicity of the Influenza Virus

Alexander B Schwahn, Bethny Morrissey and Kevin M Downard

School of Molecular and Microbial Biosciences, The University of Sydney, 2006, Australia  
E-mail: aschwahn@usyd.edu.au

### Introduction

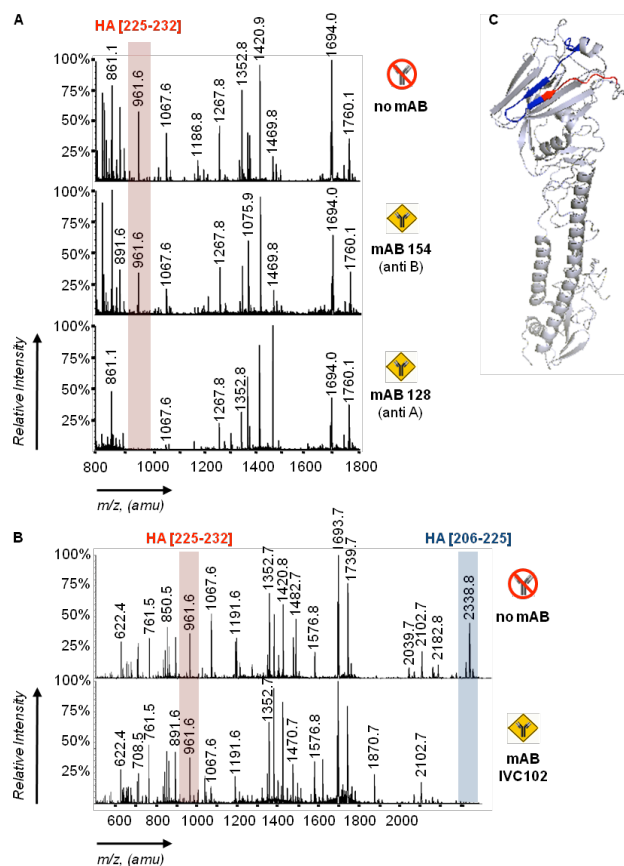
Random accumulation of mutations in the influenza surface proteins hemagglutinin (HA) and neuraminidase (NA) together with the reassortment of gene segments of two different virus strains, alters the antigenicity of these viral proteins and enables the virus to elude detection and evade the host's immune response. The first process is referred to as antigenic drift; the latter is termed antigenic shift [1].

HA binds to sialic acid containing surface receptors of the host cell resulting in an attachment of the virus particle to the cell and its subsequent internalization by membrane fusion [2]. Attachment of influenza virions to Red Blood Cells (RBCs) likewise results in cross-linking of the cells at a certain virus titre which impedes cell sedimentation. This observation is termed virus-mediated hemagglutination. Hemagglutination is inhibited if binding of an antibody to hemagglutinin prevents virus attachment to RBCs. The inhibition of hemagglutination (HI) by antisera or monoclonal antibodies (mABs), specific against certain virus subtypes, is utilized in the HI assay to determine the serotype of an unknown virus isolate [3].

While this serological approach forms the basis of present day screening of the virus, it is not without limitations. The HI assay provides no molecular detail or information about the structural changes that underlie antigenic drift or shift. To overcome this, a mass spectrometric (MS) immunoassay was developed and successfully applied to characterise both the primary structure and antigenicity of the influenza virus in a single step [4-6]. This immunoassay is based on a comparison of mass spectra, obtained for whole virus [4] or electrophoretically separated HA antigen [5, 6], before and after treatment with monoclonal antibodies. The mass map provides a survey of the structure specific to the protein and similarity of the viral antigen to known strains while the use of different mABs, reactive against specific HA isoforms, enables the immunoassay to assess the antigenicity of a strain and localize specific epitopes. This report compares the application of the MS-based immunoassay [5] and the HI assay to characterize the antigenicity of influenza type A strain New Caledonia/20/99 (H1N1).

### Results and Discussion

**Mass Spectrometric Immunoassay:** Gel-recovered hemagglutinin was digested with trypsin and a proportion was treated with monoclonal antibody mAB 128 or mAB IVC102, respectively, both specific for influenza A subtype H1N1. A treatment with anti influenza B



**Fig. 1.** MALDI mass spectra of tryptic digested hemagglutinin of influenza A/New Caledonia/20/99 before and after treatment with (A) anti influenza A mAB 128, anti influenza B mAB 154 or (B) anti influenza A mAB IVC102. (C) Position of peptide HA[206-225] and HA[225-232] relative to H1 hemagglutinin of A/Puerto Rico/8/34.

mAB 154 was used as a negative control. MALDI mass spectra of the treated and untreated (no mAB) samples were recorded and evaluated for suppression of discrete ion signals upon the addition of mAB. Comparison of these spectra reveals a significant and specific reduction in the ion signals at  $m/z$  961.6 (Fig. 1A) and  $m/z$  2338.8 (Fig. 1B) in the mass spectra of the HA samples treated with either mAB 128 or mAB IVC102, respectively. In contrast, the relative areas of all other ion signals remain unaffected by mAB treatment. Furthermore suppression of  $m/z$  961.6 only occurs upon treatment with mAB 128 and not with mAB 154 (Fig. 1A) or mAB IVC102 (Fig. 1B). The ions at  $m/z$  961.6 and  $m/z$  2338.8 correlate to tryptic cleavage

products compromising amino acid residues 225-232 or 206-225 of the A/New Caledonia/20/99 hemagglutinin antigen, respectively. The sequences of these peptides were confirmed by MALDI-MS/MS. Sequence alignment with H1 hemagglutinin of strain A/Puerto Rico/8/34, whose structure has been solved [7], further revealed that both peptides are surface accessible and are located consecutively atop of the HA1 subunit of hemagglutinin (Fig. 1C).

**Hemagglutination Inhibition assay:** The HI assay was used to analyze the ability of mAB IVC102 and mAB 127 to inhibit the agglutination of RBCs, mediated by influenza strain A/New Caledonia/20/99 (note: mAB 127 and mAB 128 possess equal reactivity in HI assays; data not shown). Figure 2 demonstrates that the hemagglutination, caused by a standardized amount of influenza A/New Caledonia/20/99, is specifically inhibited in the presence of mAB 127 in the concentration range of  $c_{mAB} \geq 0.8 \text{ ng} \cdot \mu\text{L}^{-1}$ . This effect is highly specific since mAB 127 cannot inhibit the hemagglutination caused by comparable titres of influenza strains belonging to the H3N2 subtype or the B-type of the virus (Fig. 2A and data not shown).

However, despite the unambiguous binding results of the MS-based immunoassay, mAB IVC102 fails to inhibit the A/New Caledonia/20/99-mediated hemagglutination of RBCs even if its concentration is raised above  $1 \mu\text{g} \cdot \mu\text{L}^{-1}$  (Fig. 2B). This result was unexpected and the reasons for it unclear and currently under investigation. Binding of mAB IVC102 to hemagglutinin of A/New Caledonia/20/99 was verified and confirmed by western blot analysis (data not shown).

**Conclusions:** This work [9] together with previously published data [4-6, 8] demonstrates that the antigenicity of the influenza virus can be assessed by the use of MALDI-MS. The mass spectrometric immunoassay is highly specific and able to distinguish between the different types and subtypes of the influenza virus. Moreover, it is able to discriminate between different epitopes within the same antigen. Like the HI assay, the mass spectrometric assay can also be conducted using isolated virus particles [4]; however, monoclonal antibodies over antisera are required. A further benefit of the MS-based approach is that it can generally be applied to study any protein-protein interactions which survive MALDI deposition and MS analysis.

The HI assay is also highly specific and discriminative against different influenza types and subtypes but cannot provide any molecular detail about the antigenicity of different influenza strains. It can be performed with either antisera or monoclonal antibodies and allows the latter to be preselected in terms of their ability to interfere with cell attachment of the influenza virus.

Each assay provides complementary capabilities and both are beneficial for antigenic surveillance of the influenza virus.

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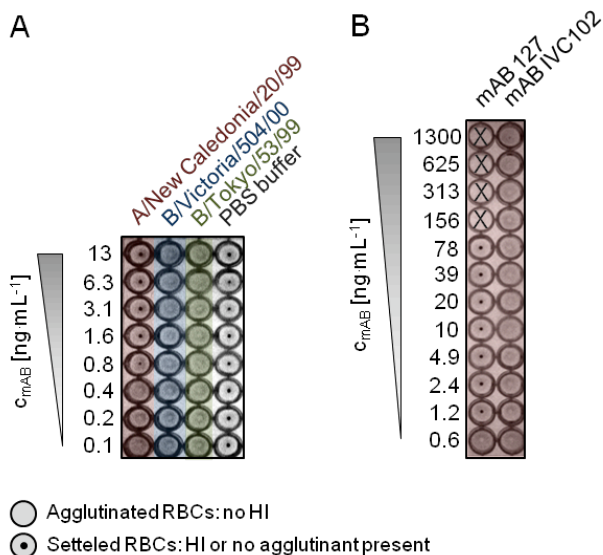


Fig. 2. HI assay of influenza strains against serial dilutions of (A) mAB 127 to assess inhibition of hemagglutination of a 1% RBC suspension containing 2-4 HA units of the indicated influenza strains. (B) HI assay of influenza A/New Caledonia/20/99 against serial dilutions of either mAB 127 or mAB IVC102. X = not utilized