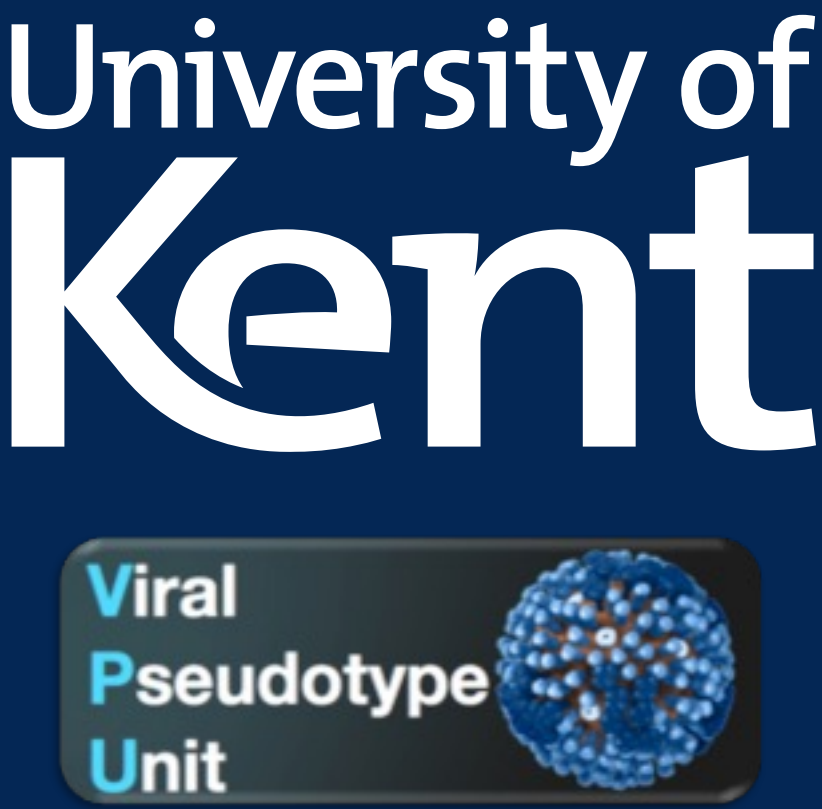


AN INTEGRATED SEROLOGICAL PLATFORM FOR IMMUNOGENICITY READOUTS AND THE ELUCIDATION OF POTENTIAL CORRELATES OF PROTECTION FOR INFLUENZA HAEMAGGLUTININ- AND NEURAMINIDASE-BASED VACCINES

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Introduction

With the rapid expansion of new universal influenza vaccine technologies, there is an acute need to match this with a holistic evaluation of immunogenicity against multiple viral targets (HA head, HA stalk and NA), subtypes and strains, and to define new correlates of protection.

Methods

A best-in-class influenza hemagglutinin (HA) pseudotype library encompassing Influenza A subtypes HA1-18, Influenza B (Vic/Yam), Influenza C and Influenza D has been developed using lentiviral vector platform technologies and co-expressed proteases (Fig 1 and 2).

These viruses have been evaluated in influenza pseudotype microneutralization (pMN) assays with post-vaccine sera and HA1/HA2-directed mAbs (HA2-specific responses are delineated using HA pseudotypes bearing immunologically unreactive heads (H11) with a mismatched stalk (MethodsX 2016)). In parallel lentiviral vectors pseudotyped with an avian H11 HA and the NA of all influenza A (N1–N9) subtypes and influenza B (Vic/Yam) were produced and used in a pseudotype-based lectin assay, pELLA (Fig 3). An open-source R Shiny web application and R library (AutoPlate) was developed to accelerate data analysis of dose-response curve immunoassays, pMN, pMN-HA2 and NA making use of established international standards (Fig 6 and 7).

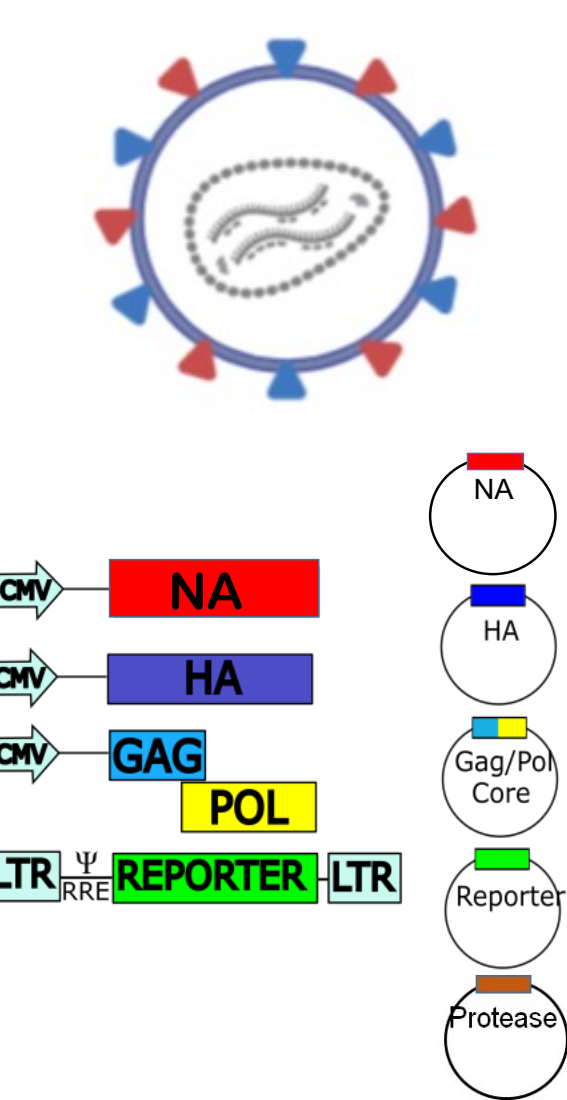


Figure 1: Lentiviral pseudotype production of influenza by plasmid co-transfection. Influenza pseudotypes require the addition of a protease plasmid for cleavage of the HA. The NA can be provided either as a plasmid or exogenously (as bacterial NA) the day after transfection.

Conclusions

- A best-in-class influenza (genera A-D) library is available for serological, therapeutics and vaccine evaluation studies, and includes both lentiviral and VSV core pseudotypes with a choice of reporters (luciferase or eGFP).
- Pseudotype virus neutralisation data analysis and inhibition curve graphics can be streamlined using **AutoPlate** web-based software (inhibition curves and IC50s): (<https://philpalmer.shinyapps.io/AutoPlate/>)
- For any enquires regarding access and collaboration, please contact n.temperton@kent.ac.uk. Note: a subset of the HA expressing plasmids will be available via the newly established MRC PPU & CVR Influenza Toolkit (<https://www.influenza.bio/>). The authors declare no conflicts of interest.



Results

The pMN and pELLA are highly sensitive and specific for detecting virus-specific neutralizing antibodies against influenza virus HA (HA1 and/or HA2) and NA and can be used to safely assess antibody functionality in vitro, and in high-throughput systems (Fig 4 and 5). We demonstrate their utility in detecting serum responses to infection and vaccination with the ability to evaluate cross-subtype neutralizing responses elicited by specific vaccinating antigens. Additionally, we demonstrate their sensitivity for the study of broadly neutralizing mAbs including CR9114 and CD6. Using the assembled HA and NA response data sets we show that AutoPlate improves on available analysis software in terms of ease of use, flexibility, speed and standardisation (Fig 6 and 7).

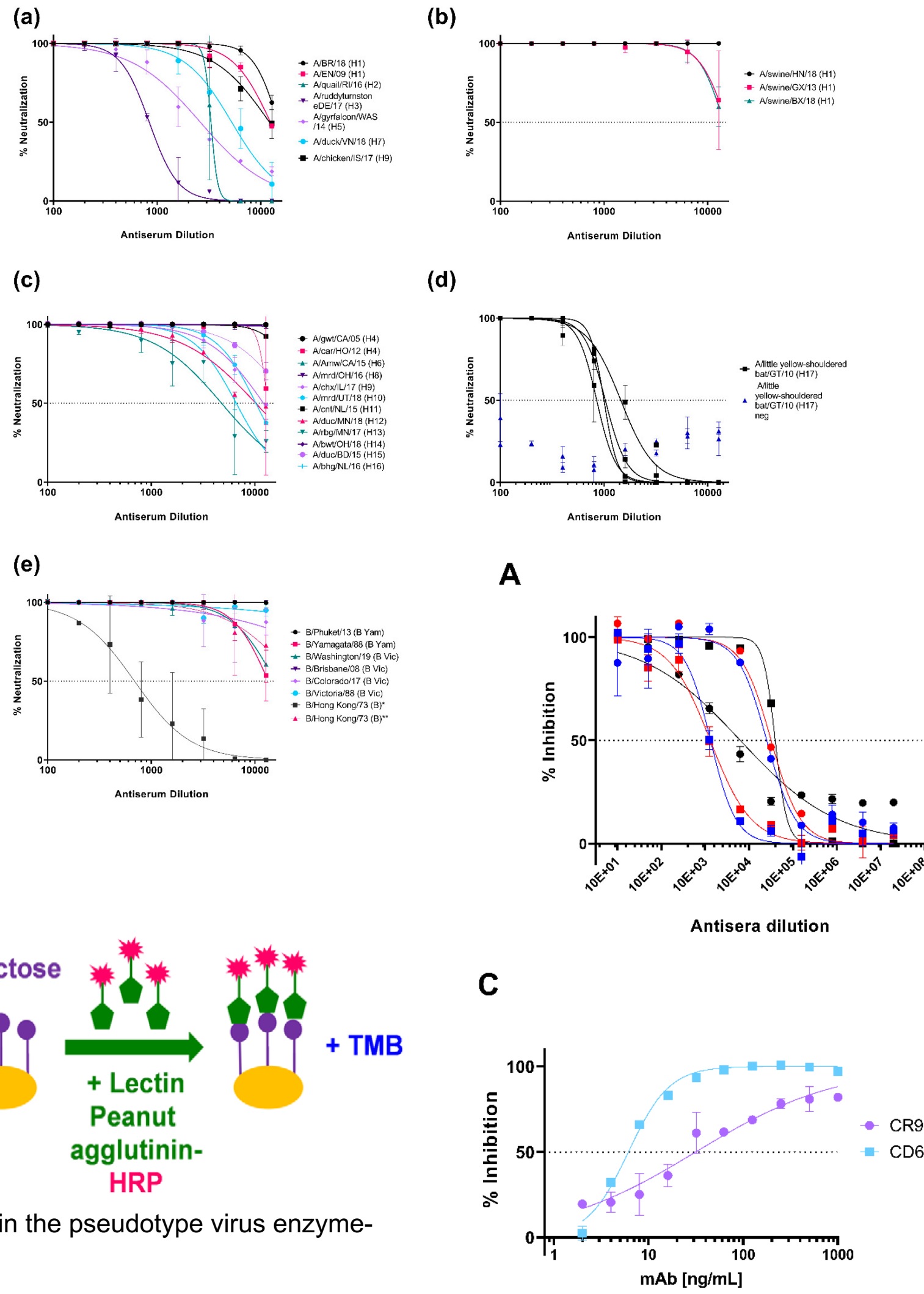


Figure 4: Neutralization of influenza pseudotypes by reference antisera and bat sera from influenza surveillance. (a) Neutralization of representative IAV subtypes that have previously caused infection in humans (H1, H2, H3, H5, H7, and H9). (b) Neutralization of pseudotypes representing IAV isolated from swine (H1). (c) Neutralization of pseudotypes that are representative of IAV found in avian populations (H4, H6, H8, H9, H10, H11, H12, H13, H14, H15, and H16). (d) Neutralization of H17 PV (A/little yellow-shouldered bat/Guatemala/060/2010) by bat sera from bat surveillance sampling in Nigeria as provided by APHA. (e) Neutralization of IBV pseudotypes that have caused human infection (B/Yamagata-like and B/Victoria-like viruses and presplit IBV).

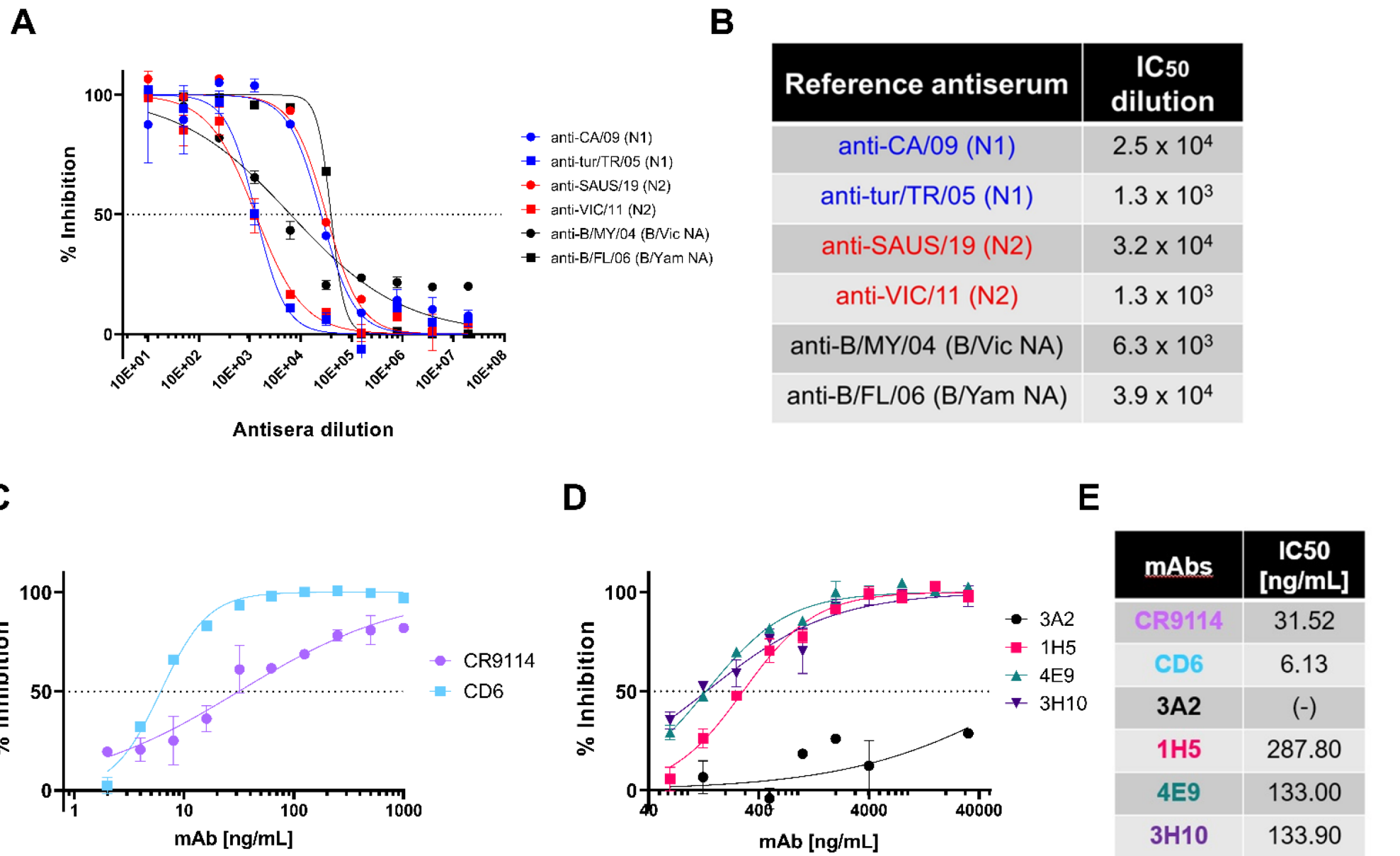


Figure 5: In vitro inhibition of NA pseudotypes by antisera and monoclonal antibodies.

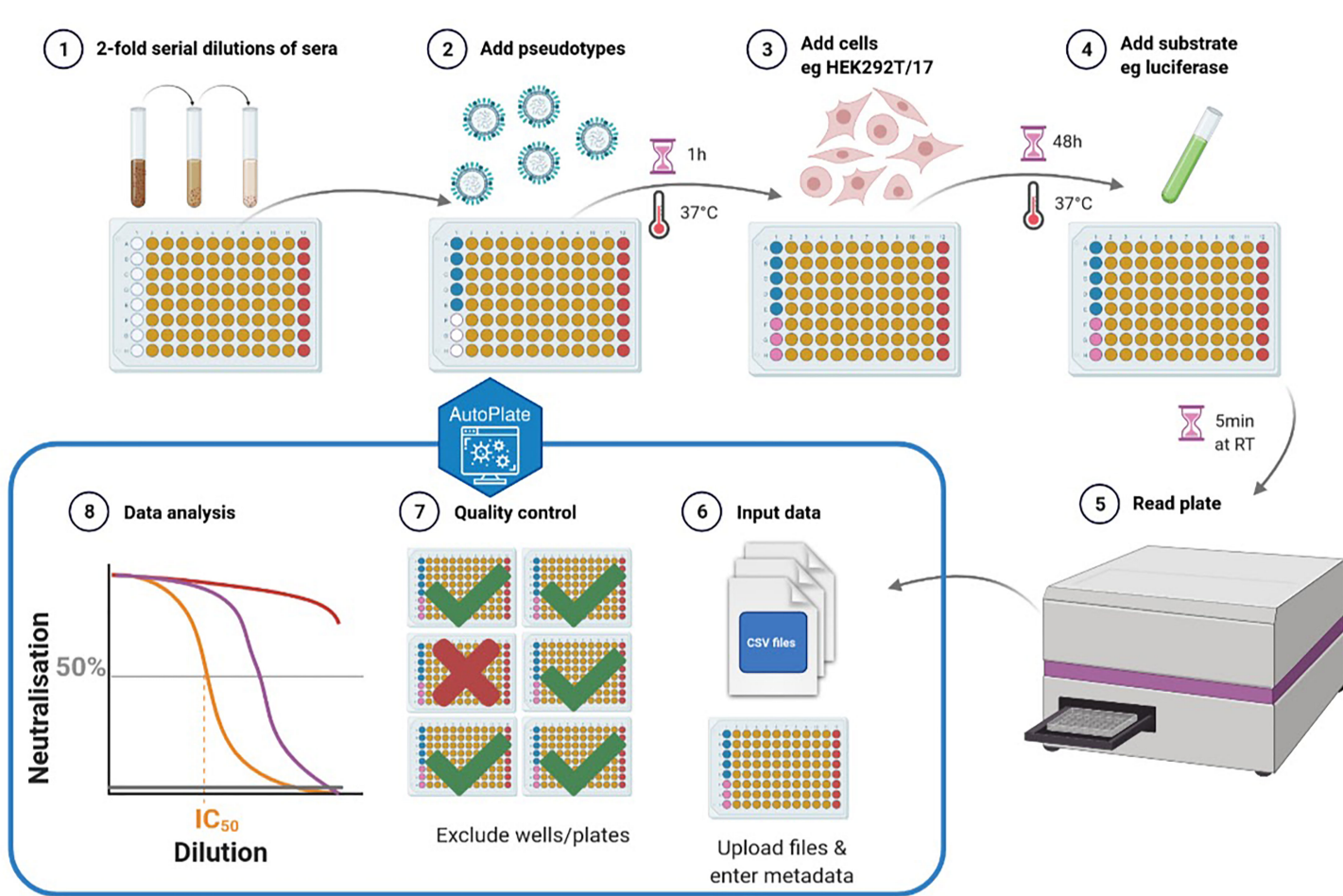


Figure 6: pMN neutralisation assay and AutoPlate computational analysis

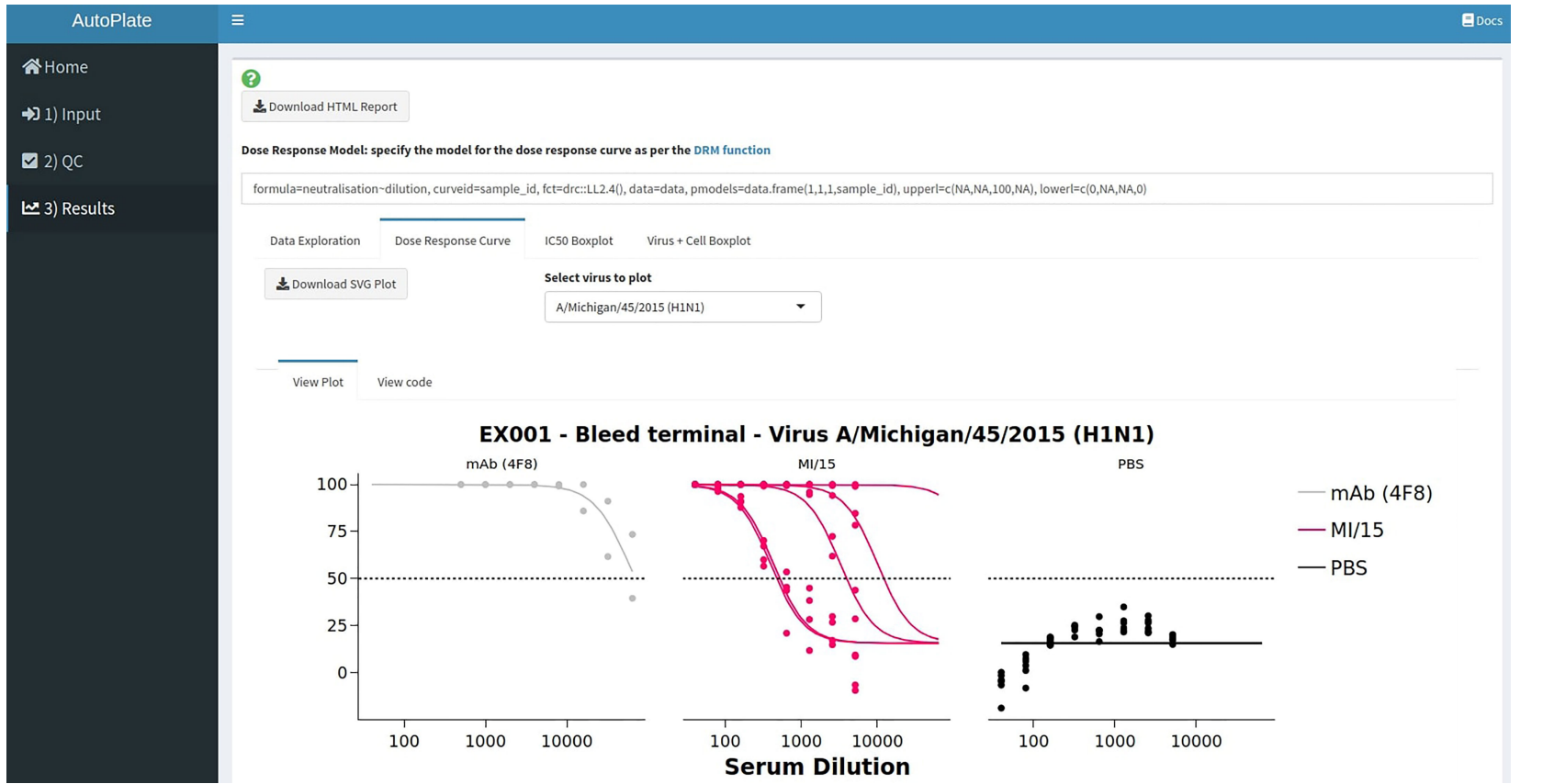


Figure 7: AutoPlate output of pMN data

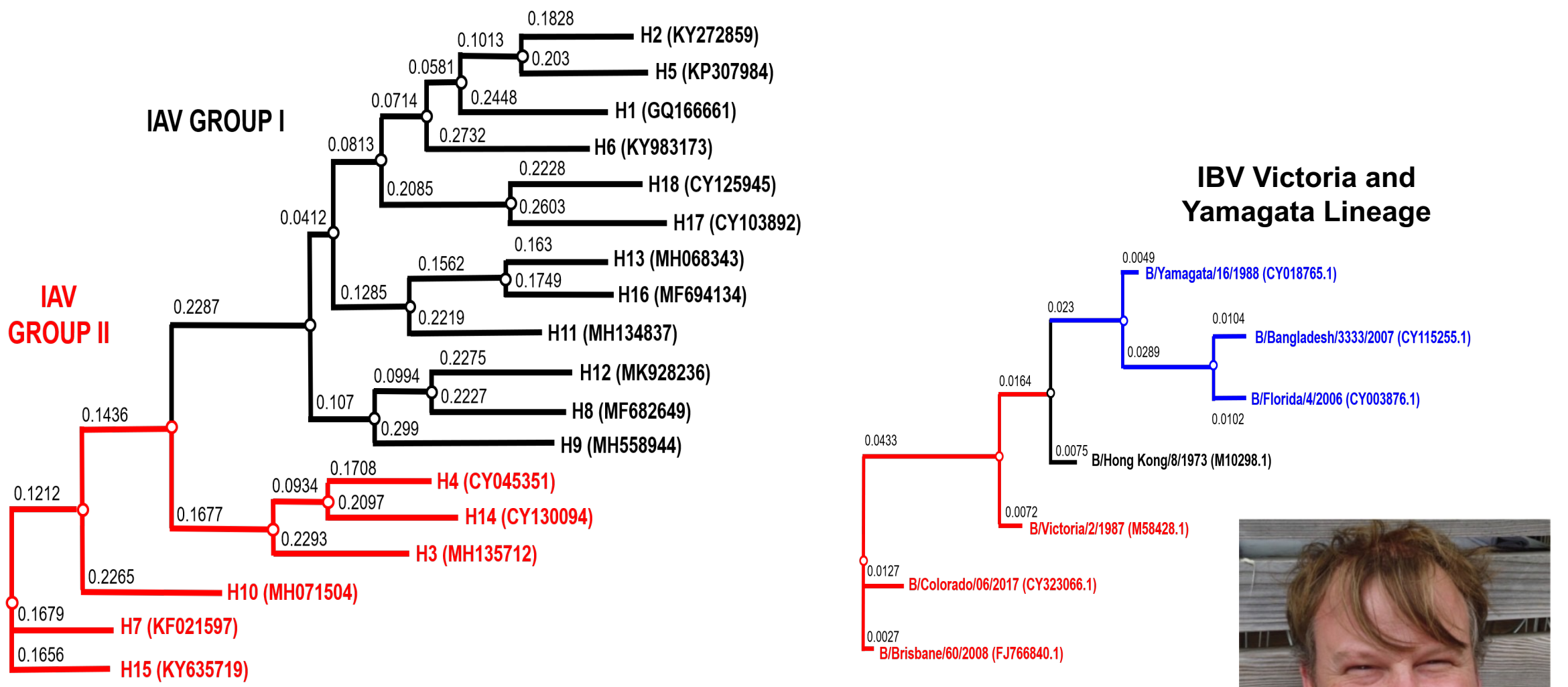


Figure 2: Phylogenetic tree of representative influenza A and B HA from the established library. Influenza A Group (IAV) I HA pseudotypes are shown in black, IAV Group II in red. Influenza B lineage B/Yamagata are shown in blue and B/Victoria in red. Tree was constructed with PhyML.

