



Improving the antigen yield of candidate influenza vaccine viruses through the use of chimaeric haemagglutinin genes

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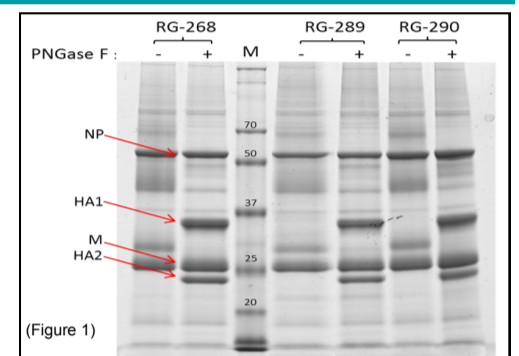
INTRODUCTION

The antigen yield of Candidate Vaccine Viruses (CVVs), generated by classical reassortment or reverse genetics, is variable and unpredictable. Based on previous results (1,2), we attempted to increase the yield of the haemagglutinin (HA) by generating viruses with chimaeric HAs that have the ecto-domain of the HA of the respective wild type (wt) virus and the signal peptide, trans-membrane region/cytoplasmic tail as well as non-coding regions of the HA of the high-yield donor virus PR8. Viruses with chimaeric HA genes derived from different subtypes were generated and analysed for HA yield.

MATERIALS AND METHODS

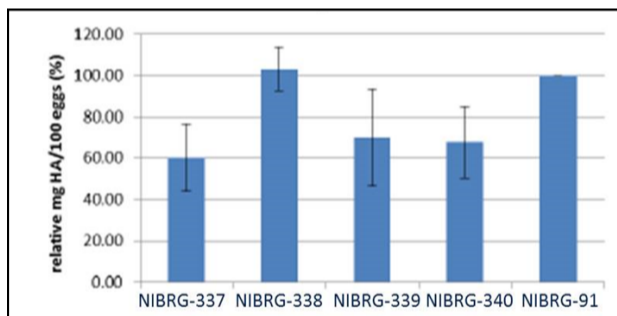
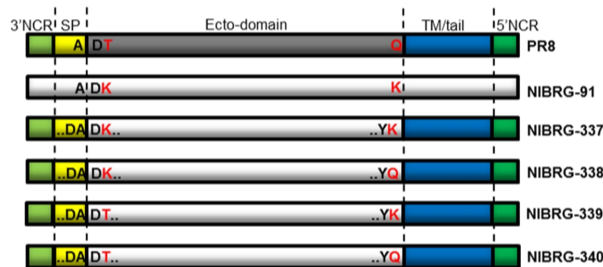
- Viruses were generated by reverse genetics in Vero cells.
- For yield assessment, viruses were grown in 3 bulks of 30 eggs each. Infected allantoic fluid was harvested 72 hours post-infection and the virus was purified on a sucrose gradient and concentrated.
- Total protein yield was measured using the Bicinchoninic acid (BCA) assay.
- HA content, denoting the proportion of HA in total viral protein, was estimated using densitometry on SDS PAGE gels. Virus concentrates were deglycosylated with PNGase-F and four major viral protein bands (indicated by red arrows in Figure 1.) were considered for the HA content assessment. The formula used for the calculation is:
- Results are shown as the yield relative (\pm standard error) to that of the comparator virus from NIBSC's library of CVVs for pandemic preparedness.

$$\%HA \text{ (HA content)} = \frac{HA1 + HA2}{NP+HA1+M+HA2}$$

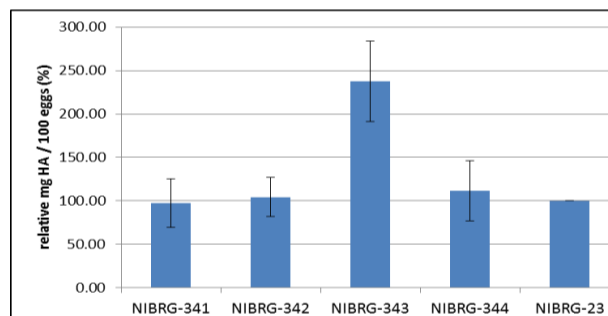
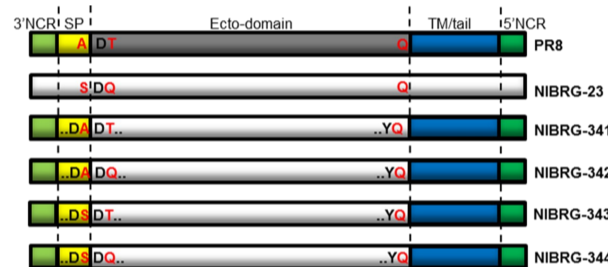


RESULTS

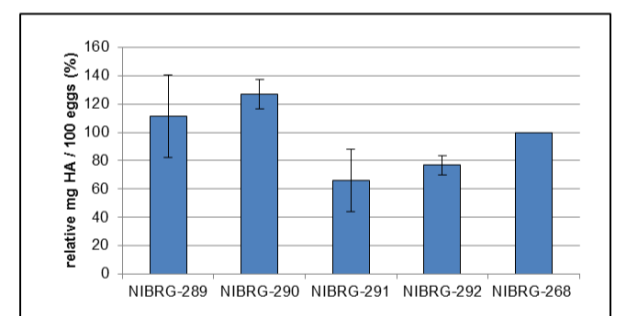
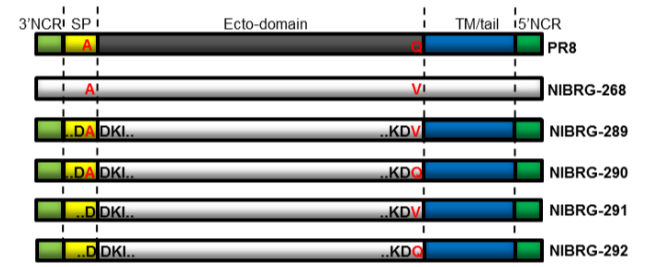
A/chicken/Hong Kong/G9/97 (H9N2) NIBRG-91



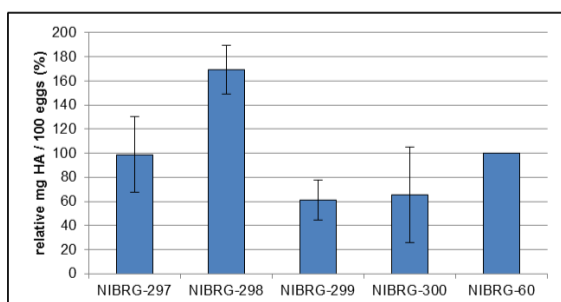
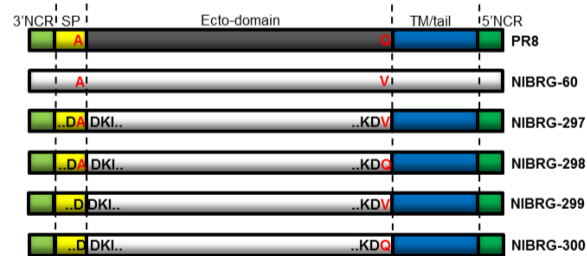
A/turkey/Turkey/1/2005 (H5N1) NIBRG-23



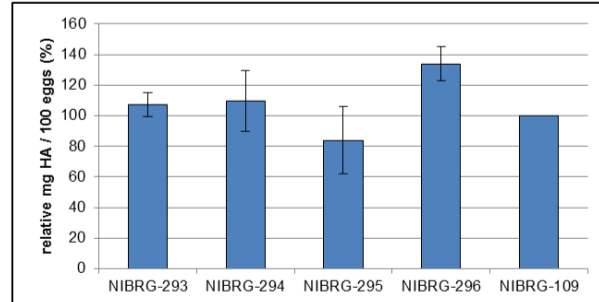
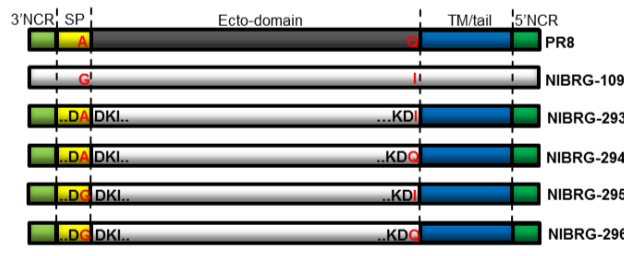
A/Anhui/1/2013 (H7N9) NIBRG-298



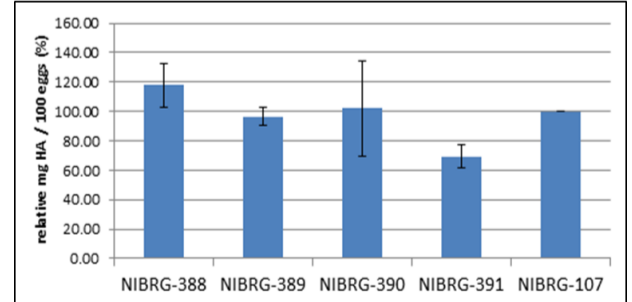
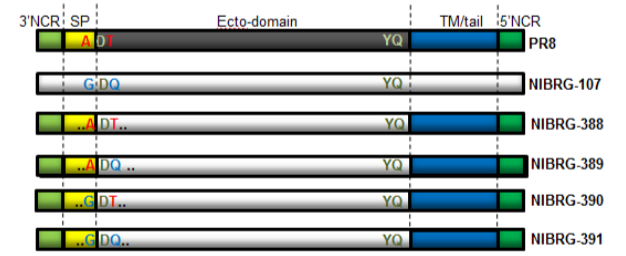
A/mallard/Netherlands/12/2000 (H7N3) NIBRG-60



A/New York/107/2003 (H7N2) NIBRG-109



A/mallard/England/727/2006 (H2N3) NIBRG-107



CONCLUSIONS

- Chimaeric HA genes can have a positive effect on the yield for most of the strains tested so far.
- Chimaeric structures with as much PR8 sequences as possible are generally the most effective.
- The extent of yield improvement is strain specific.
- The increase of HA yield can relate to either higher HA content or better total protein yield or an improvement of both parameters.
- The choice of the amino acids at the junctions between the ecto-domain and the signal peptide or the trans-membrane region plays an important role.

ACKNOWLEDGEMENTS AND REFERENCES

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- (1) Harvey R et al. *Vaccine* 2010; 28: 8008-8014.
(2) Harvey R et al. *Journal of Virology* 2011; 85: 6086-6090.

