

Poster: V-448f

Implications of Cell Type in Influenza A/CA/7/2009 H1N1 HAI Assay

G. Defang¹, T. Rehman³, R. Dewar³, J Metcalf², D. Ewing¹, J. Danko¹, T. Kochel¹, T. Luke¹, T. Burgess¹, J. Beigel^{2,3}



1) Naval Medical Research Center, Silver Spring, MD, 2) National Institute of Allergy and Infectious Diseases, Bethesda, MD, 3) SAIC Inc., Frederick, MD



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Introduction

In 2009, a novel swine-origin influenza A strain caused a worldwide pandemic with over 18,000 related deaths. The NIH and Department of Defense (DoD) are participating in a multicenter effort to collect high titer anti-influenza plasma to be used in a randomized, multicenter study to explore the effectiveness of convalescent plasma therapy as an alternate treatment modality for severe influenza disease.

In performing the screening hemagglutinin-inhibition (HAI) for this study, discrepancies were noted among the HAIs performed in different laboratories, both following the CDC HAI protocol, one using an egg-derived A/CA/7/2009 X-179A (vaccine strain) virus antigen and the other a MDCK cell culture-derived A/CA/7/2009 wild type (wt) antigen.

Methods

Enrolled subjects and previously collected units of plasma at the NIH and DoD were screened for the presence of high anti-influenza titers (HAI titer ≥ 160). Samples from 31 subjects were tested by HAI assay using an egg-derived A/CA/7/2009 X-179A and MDCK cell culture-derived A/CA/7/2009 wt virus antigen.

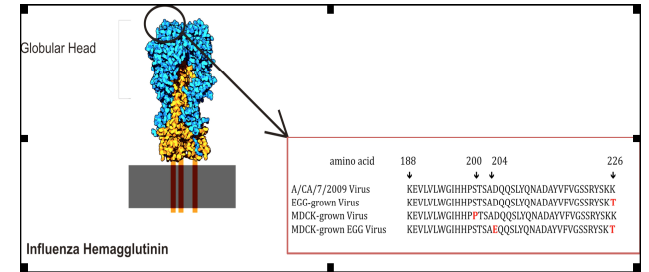
Hemagglutinin (HA) region of both virus antigens were amplified using HA1/2 specific primers and sequence analysis was performed using MegAlign program from DNASTar.

Results

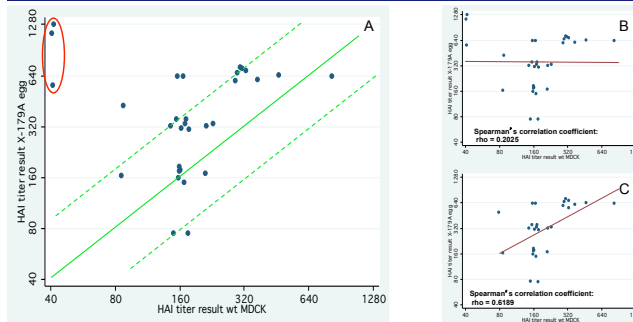
HAI titers achieved using MDCK-grown A/CA/7/2009 wt antigen had substantial differences from titers obtained using egg-grown A/CA/7/2009 X-179A antigen, with up to 6 fold differences observed in some cases. When corrected for strain differences, MDCK-grown A/CA/7/2009 antigen still resulted in substantial differences in HAI titers from that achieved with egg-grown A/CA/7/2009 X-179A. Importantly, egg-derived virus grown in MDCK cells assumed the MDCK-derived virus phenotype. We identified amino acid (aa) differences between the MDCK- and egg-derived viruses around the Sb site in the HA globular head region. The aa differences and/or the presumed differences in HA glycosylation in egg- and MDCK-derived viruses may explain the discrepancy in the results and are being investigated.

Amino acid changes in the globular head of A/CA/7/2009 HA following growth in eggs and MDCK cells

Hemagglutinin (HA) gene sequences of egg-grown, MDCK-grown, and MDCK-grown egg viruses were aligned with the consensus sequence of A/CA/7/2009 to identify any differences that could account for the observed differences in HAI results. Egg-grown virus was shown to contain an amino acid change (K226T) outside of the antigenic and receptor binding sites. The MDCK-grown virus (S200P) and the MDCK-grown egg virus (D204E) both contained an amino acid change around the Sb antigenic region; one of four defined antigenic sites of the H1 HA molecule. Amino acid changes in and around the antigenic sites have the potential to disrupt the conformational integrity of these antibody recognition epitopes.



HAI A/CA/7/2009 Wild Type (wt) grown in MDCK versus X-179A Grown in Egg



The HAI between the two methods generally correlated well. 81% of results were within 1 dilution (dashed green line) with the egg grown antigen usually resulting in a slightly higher HAI (A). However, in 10% (3/31) (red circle) there were marked differences (A). The spearman coefficient with and without these 3 samples are provided in graphs B and C.

HAI wt MDCK versus X-179A Egg versus MN egg

Given the discrepant results, 8 samples were tested against the microneutralization assay. In those samples with large discrepancies between the HAI assays, the MN was found to correlate with the HAI with virus grown in egg.

HAI X-179A egg	HAI wt MDCK	MN X-179A egg
640	905	>2560
640	453	1280
320	160	160
160	160	640
640	320	>2560
640	320	>2560
1280	40	>2560
1280	40	>2560

Conclusion

The methodology used to derive the influenza virus antigen (A/CA/7/2009) for the HAI assay, either from egg or MDCK cells, can affect the results.

The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government. The study protocol was approved by the Infectious Disease Clinical Research Program (IDCRP) Institutional Review Board in compliance with all applicable Federal regulations governing the protection of human subjects. This work was supported in part by GEIS 1_204_10. This research was supported in part by the National Institute of Allergy and Infectious Diseases. Funded in part by NCI Contract No. HHSN26120080001E.