



A comparison of hemagglutination inhibition and neutralization assays for characterizing immunity to influenza A

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Complete List of Authors:	Truelove, Shaun; Johns Hopkins Bloomberg School of Public Health, Epidemiology Zhu, Huachen; The University of Hong Kong, State Key Laboratory of Emerging Infectious Diseases Lessler, Justin; Johns Hopkins Bloomberg School of Public Health, Epidemiology Riley, Steven; School of Public Health, Imperial College, Ecology and Epidemiology of Infectious Disease Read, Jonathan; Institute of Infection and Global Health, University of Liverpool, Epidemiology and Public Health Wang, Shuying; Guangzhou No. 12 Hospital, Kwok, Kin; International Institute of Infection and Immunity, Shantou University Medical College, Guan, Yi; The University of Hong Kong, State Key Laboratory of Emerging Infectious Diseases; International Institute of Infection and Immunity, Shantou University Medical College, Jiang, Chao; Guangzhou No. 12 Hospital, Cummins, Derek; Johns Hopkins Bloomberg School of Public Health, Epidemiology
Keywords:	cross protection, epidemiology, hemagglutination inhibition tests, immunity, influenza, neutralization test

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3 **A comparison of hemagglutination inhibition and neutralization assays for characterizing**
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5 **immunity to influenza A**
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10 **Authors and Affiliations:**

11 Shaun Truelove¹, Huachen Zhu^{2,6}, Justin Lessler¹, Steven Riley³, Jonathan M. Read⁴, Shuying Wang⁵, Kin
12 On Kwok⁶, Yi Guan^{2,6}, Chao Qiang Jiang⁵, Derek A.T. Cummings¹
13

14
15
16 ¹Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland,
17 USA 21205.

18
19
20 ²State Key Laboratory of Emerging Infectious Diseases, The University of Hong Kong, Hong Kong SAR,
21 People's Republic of China.
22

23
24
25 ³School of Public Health, Imperial College, London, UK.

26
27 ⁴Department of Epidemiology and Public Health, Institute of Infection and Global Health, University of
28 Liverpool, Neston CH64 7TE, UK.
29

30
31 ⁵Guangzhou No. 12 Hospital Guangzhou, Guangdong 510620, China.

32
33
34 ⁶International Institute of Infection and Immunity, Shantou University Medical College, Shantou,
35 Guangdong, 515031, People's Republic of China.
36

37
38
39
40 Correspondence: Derek Cummings, derek.cummings@jhu.edu

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Competing financial interests

The authors declare no competing financial interests.

Previous presentation of results

None of these results have been presented previously in any meetings.

Authors' Contributions

Conceived and designed the study: ST JL DATC. Performed the analysis: ST JL DATC. Contributed reagents/materials/analysis tools: HZ JL SR JMR SW KOK YG CQJ DATC. Wrote the manuscript: ST DATC. Contributed edits and critical review of the manuscript: ST HZ JL SR JMR SW KOK YG CQJ DATC. Designed the empirical study and collected the data and serum samples: HZ JL SR JMR SW KOK YG CQJ DATC.

Summary

Background

Serum antibody to influenza can be used to identify past exposure and measure current immune status.

The two most common methods for measuring this are the hemagglutination inhibition assay (HI) and the viral neutralization assay (NT), which not been systematically compared for a large number of influenza viruses.

Methods

151 study participants from near Guangzhou, China were enrolled in 2009 and provided serum. HI and NT assays were performed for 12 historic and recently circulating strains of influenza A. We compared titers using Spearman correlation and fit models to predict NT using HI results.

Results

We observed high positive mean correlation between HI and NT assays (Spearman's rank correlation, $\rho=0.86$) across all strains. Correlation was highest within subtypes and within close proximity in time.

Overall, an HI=1:20 corresponded to NT=1:10, and HI=1:40 corresponded to NT=1:20. Linear regression of $\log(\text{NT})$ on $\log(\text{HI})$ was statistically significant, with age modifying this relationship. Strain-specific AUCs indicated good accuracy (>80%) for predicting NT with HI.

Conclusions

While we found no exact equivalence between assays, we found high overall correspondence of titers between NT and HI assays for influenza. We also found that the equivalence between the assays changed with age. These findings support generalized comparison of results between assays and give further support for use of the hemagglutination inhibition assay over the more resource intensive viral neutralization assay, though attention should be given to understanding the effect of age on the results of these assays.

Keywords: cross protection, epidemiology, hemagglutination inhibition test, immunity, influenza, neutralization test

Introduction

Accurate measurement of individuals' pathogen exposure history is an essential tool for understanding risk factors of infection and population scale patterns of transmission. Determined through a variety of methods, the presence of antibodies in sera is considered the gold standard method to estimate past exposure to pathogens. Two of the most common methods for measuring serum antibody to influenza are the hemagglutination inhibition assay (HI) and the viral neutralization assay (NT).¹ Though both tests serve as measures of antibody concentration in sera, they have important differences in how they are conducted and how they measure immunity. The HI test, which is fast and relatively easy to perform, is considered to be easily standardized and reproducible across laboratories. However, only the effect of antibodies on the hemagglutination process is measured, and the endpoint is only a correlate of the ability of antibodies to inhibit viral reproduction in host cells.^{2,3} In contrast, NT titers directly measure inhibition of viral pathogenic activity, regardless of the mode of this inhibition. Although NT is intuitively more appealing because it more closely mirrors the infection process *in vivo*, it is more time consuming and expensive, and considered harder to standardize across laboratories.^{2,3}

Despite the widespread usage of these two methods, there have been few formal comparative studies of these measures. In a 2007 study by Stephenson et al., HI and NT tests were performed in 11 laboratories to investigate reproducibility of each assay for detection of anti-H3N2 influenza antibodies. They found significantly higher variation in NT results between laboratories than in HI results, yet better discrimination among NT and generally limited correlation between the tests.² In a follow-up study of anti-H1N1pdm antibodies, significant correlation between HI and NT was found, yet the conversion factors between laboratories varied significantly. Furthermore, NT titers were both significantly higher and significantly more variable than HI titers.³

The difference in reliability between labs with these two assays is a direct result of how they are measured. Hemagglutination inhibition and viral neutralization assays assess the level of functional

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3 immunity to a virus in a similar manner, both using serial dilution of sera applied to a fixed amount of
4 virus to determine at which titer of sera the virus is effectively inhibited. The difference is in the biologic
5 mechanism used as an indicator for inhibition. The HI assay utilizes the natural process of viral
6 hemagglutination, a process in which a lattice forms by binding of viruses to red blood cells; this process
7 is blocked when sufficient antibody with affinity to the virus is present. A serum HI titer of ≥ 40 is
8 assumed to indicate a 50% reduction in susceptibility compared with an individual with undetectable
9 titer.⁴⁻⁶ The NT assay, in contrast, measures cytopathic effects of the virus, the invading and killing of
10 cells, through plaque formation. Again, the antibodies in the sample serum are tested for their ability to
11 block this activity. Results are expressed as the dilution at which the number of plaques is reduced by
12 some percentage compared to controls, typically 50%.⁷

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27 The viral neutralization test is valued for its high sensitivity and specificity. The viral neutralization assay
28 has been found to be higher than microneutralization test (MNT), microneutralization fluorescent-
29 antibody test (MFA), and HI, though some have indicated similar sensitivity and specificity between HI
30 and NT tests for certain viruses, including influenza A H1N1 2009.^{2,5,8,9} Additionally, it has been found to
31 be more strain-specific than HI for seasonal and H5N1 viruses, and HI tests have been found to be
32 insensitive for the detection of human antibody responses to avian hemagglutinin, especially when intact
33 virus is present.^{2,6} According to Gross and Davis, the neutralization test “appears to detect lower levels of
34 viral antibody than does the HI test”, a difference that “may be related to the high serum concentrations
35 and the additional viral antigens detected by NT”.¹⁰ It is, however, a laborious and time-consuming
36 procedure, making it less suitable for testing large numbers of samples.¹¹ Disadvantages of NT include its
37 difficulty level and time required to perform, the need for live virus, and that technical aspects of the
38 assay can affect titers.^{3,8} However, the major disadvantage of it has been poor reproducibility between
39 labs.^{2,3}

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3 In addition to low sensitivity, in particular as compared with radioimmunoassay (RIA) and enzyme-linked
4 immunosorbent assay (ELISA), disadvantages of HI include subjectivity of results interpretation and
5 reliability issues with relation to freshness of reagents. For both tests, immunity measurement is not exact,
6 but rather based on titer cut-points, and the end point of both assays requires visual inspection. Other than
7 cost, ease, and reduced variability, a major advantage of HI over NT for measurement of seasonal
8 influenza immunity is that HI does not require cytopathy, which does not always occur for each influenza
9 virus in this assay.⁷
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20 HI and NT assays have been utilized for years to investigate influenza immunity, though only a few
21 studies have directly compared the assays' influenza antibody detection capabilities, and most of these
22 studies evaluated vaccine-derived immunity.^{2,3,12} Here we compare HI and NT antibody titers from a
23 sample of individuals from Guangdong province, China, in order to formally compare the performance of
24 HI and NT titers for measuring naturally-derived immunity to twelve historic and recently circulating
25 strains of influenza A. These twelve strains are seasonal influenza strains of both H3N2 and H1N1
26 subtypes that have been or are in broad circulation since 1968. Additionally, we will attempt to determine
27 an equivalence factor between HI and NT titers for direct translation and comparison of results from both
28 assays.
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42 **Methods**

43 *Sample Collection*

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45 Sera samples were collected from 151 study participants from randomly selected households in five study
46 locations in a transect extending to the northeast of Guangzhou, China from 8 July 2009 to 21 September
47 2009, as described in Lessler et al.¹³ All study participants were administered informed consent, and a
48 single blood sample was collected in a 5mL non-heparin containing vacuum tube from each. Sera were
49 extracted and split at Guangzhou Hospital, and testing and storage were done at Shantou University.
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Laboratory Testing

Hemagglutination inhibition (HI) and neutralization (NT) assays were performed for 12 historic and recently circulating strains of influenza A: nine H3N2 strains (A/Hong Kong/1/1968, A/Victoria/3/1975, A/Bangkok/1/1979, A/Beijing/353/1989, A/Wuhan/359/1995, A/Fujian/411/2002, A/Shantou/90/2003, A/Shantou/806/2005, and A/Shantou/904/2008), two previous seasonal H1N1 strains (A/Shantou/104/2005 and A/Shantou/92/2009), and a 2009 pandemic H1N1 strain (A/California/07/2009). Laboratory tests were performed as described in Lessler et al.¹³ In brief, the 50% tissue culture infectious dose (TCID₅₀) for each virus was determined on Madin-Darby Canine Kidney cells (MDCK). The neutralization concentration was considered to be the reciprocal of the highest dilution of sera at which 50% of wells were infected (calculated using the method of Reed and Muench).¹⁴ Sera were thawed, treated with a receptor-destroying enzyme (RDE) to remove non-specific inhibitors, then heat inactivated at 56°C for 30 minutes. RDE pretreated sera were absorbed with Turkey red blood cells before being used in HI or NT tests, to remove substances causing nonspecific agglutination. Antibody titer was determined by testing serial two-fold dilutions from 1:10 to 1:1280 in duplicate, resolving uncertain results by additional quadruplicate tests. Positive and negative control sera were also tested. HI assay were conducted with 0.5% turkey erythrocytes using four hemagglutination units. NT tests were carried out by mixing serially diluted sera with 100 TCID₅₀ of MDCK cell adapted viruses of each strain, incubated for one hour at 37°C and added to a MDCK cell monolayer. Cytopathic effect was read and hemagglutination assays performed to detect the presence of viral replication three days after inoculation. The highest dilution with complete protection of the cell monolayer in >2 quadruplicate wells was considered to be the NT titer. Both assays were performed in the same laboratory within the same week of each of other by an overlapping study team. Samples undergoing each assay were handled and processed identically, undergoing same sequence of freezing and thawing before testing. The full protocol is described in the WHO Manual on Animal Influenza Diagnosis and Surveillance.¹⁵

Statistical Analysis

HI and NT titers were compared using Spearman correlation between $\log_2(\text{HI}/5)$ and $\log_2(\text{NT}/5)$ titers on the 12 reference strains. We refer to titer values as $\log(\text{HI})$ and $\log(\text{NT})$ for the remainder of this manuscript. Cross correlation between strains was calculated by the same method.

We examined the ability of one assay to predict status based on the other. We treated detectable NT titers as the gold standard (i.e., NT titer $\geq 1:20$) to calculate receiver-operator characteristic (ROC) curves using different cutoffs for “positivity” for equivalent HI titers to predict status. Linear regression models were fit to predict $\log(\text{NT})$ titer using $\log(\text{HI})$ titer. All statistical analyses were conducted in R Statistical Software, version 3.0 (www.cran.org).

Results

One hundred fifty-one study participants were included in this analysis. The median age among them was 45 years (SD=18.75; range=7-81), with 7.3% aged <15 years (11/151). 54.3% of participants were male (82/151). Among all participants, 66.2% (100/151) had never received an influenza vaccination, with only 7 (4.7%) receiving vaccines during the current or previous years, 6 (4.0%) within the last 2-5 years, and 19 (12.6%) >5 years prior; 19 (12.6%) were unsure or unknown.

Antigenic testing of sera from the 151 pilot study participants for twelve strains of influenza A virus (shown in Figure 1) indicated a high positive mean correlation between hemagglutination inhibition and neutralization assays (Spearman’s rank correlation, $\rho=0.86$) (Figure 2), and both H3N2- and H1N1-specific correlations were found to be high ($\rho=0.84$; $\rho=0.83$) (Figure 2). Strain-specific correlation varied by year and serotype, with the highest correlation observed for A/Fujian/411/2002 (H3N2) ($\rho=0.92$) and the lowest observed correlation observed for A/California/07/2009 (H1N1) ($\rho=0.48$) (Figure 3). Two strains of H1N1, A/Shantou/92/2009 and A/California/07/2009, had very low titer results

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3 overall (Table 1), resulting in low correlation coefficients. Cross-reactivity correlation was highest
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5 between strains of the same subtype and in close proximity in time (Supplemental Figure 2). The highest
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7 correlations were found between A/Shantou/806/2005 and A/Shantou/90/2003 ($\rho=0.83$) and
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9 A/Shantou/806/2005 and A/Shantou/904/2008 ($\rho=0.82$) for HI, and between A/Fujian/411/2002 and
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11 A/Shantou/90/2003 ($\rho=0.88$) for NT.

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16 Linear regression of $\log(\text{NT})$ on $\log(\text{HI})$ values indicated a statistically significant association between the
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18 values. The resulting model was $\log(\text{NT}) \text{ titer} = 0.0007 + 0.9733 \log(\text{HI})$, with β_1 p -value $< .0001$. Separate
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20 models for H3N2 and H1N1 were nearly identical, with $\beta_1=0.9684$ (SE = 0.017) and $\beta_1=0.9782$
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22 (SE=.0225). When added to the model, age was found to have a statistically significant association with
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24 the relation between $\log(\text{NT})$ values and $\log(\text{HI})$ values. The resulting model, $\log(\text{NT}) = 0.2542 + 0.9663$
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26 $\log(\text{HI}) - 0.0055 \text{ age}$, indicates a 0.0055 reduction in $\log(\text{NT})$ for every year of age, adjusting for $\log(\text{HI})$.
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28 HI of $\geq 1:40$ and NT of $\geq 1:20$ are commonly used as thresholds for indication of immunity, signifying a
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30 50% reduction in the risk of contracting influenza¹⁶. Among all of the assays performed, 44.0% and
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32 52.3% measured HI titers $\geq 1:40$ and NT titers $\geq 1:20$, respectively (Table 1), indicating, based on these
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34 standard thresholds, that there was protective immunity to about half of the influenza strains performed
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36 among all study participants. Strain-specific HI $\geq 1:40$ results ranged from 0.7% for A/California/07/2009
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38 to 80.8% for A/Fujian/411/2002, and NT $\geq 1:20$ results ranged from 1.3% for A/California/07/2009 to
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40 84.8% for A/Wuhan/359/1995. Among participants aged < 15 years, all (11/11) were found to have at
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42 least one HI titer $\geq 1:40$ and at least one NT titer $\geq 1:20$, whereas among adults (≥ 15 years), 2.9% (4/140)
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44 had no HI titers $\geq 1:40$ and only 1/140 had no NT $\geq 1:20$.

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51 We determined HI titer thresholds that predicted NT titer status using multiple titer threshold and
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53 determined which ones maximized sensitivity and specificity to predict NT status. Optimal thresholds
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55 were found to vary by serotype and strain. Overall, an HI titer threshold of 1:20 corresponded to a NT
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57 titer of 1:10, and an HI titer of 1:40 corresponded to a NT titer of 1:20 (Table 2). These titer thresholds
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3 varied for H3N2 and H1N1 serotypes, with higher titer thresholds for H3N2 (1:40 and 1:40) and lower
4 titer thresholds for H1N1 (1:10 and 1:20). Strain-specific HI titer thresholds varied from 1:10 to 1:80
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6 corresponding to NT titers of 1:10 and 1:20. We observed a mean bias between log(HI) and log(NT) of
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8 0.06 for all strains (Supplemental Figure 3). However, the plot of log(NT) versus bias demonstrated a
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10 significant negative slope of 0.23 ($P < .0001$), with the greatest magnitude in bias at high NT titers. Figure
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14 2 shows this with log(NT) titer being lower than log(HI) titer at low titers, but higher at titers
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16 (Supplemental Figure 3, Figure 2).
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21 ROC curves comparing results of NT and HI assays indicated similar findings as the correlation tests
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23 (Figure 4/Table 2). Area under the curve (AUC) statistics indicated excellent accuracy of the HI test, as
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25 compared with NT as the gold standard, on all titer levels for both overall (all serotypes and years
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27 combined) and serotype-specific analysis (Table 2). Strain-specific AUCs indicated at least good (>0.80)
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29 accuracy for HI tests at NT gold standard titers of 1:10 and 1:20 on all strains. A/Shantou/92/2009
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31 (H1N1) and A/California/07/2009 (H1N1) strains, which had the lowest correlation coefficients,
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33 produced AUC values of 84.6% and 99.5% for NT titers of 1:10 and 93.5% and 99.5% for NT titers of
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35 1:20, respectively.
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40 Discussion

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42 The primary goal of this study was to gain a better understanding of the direct comparability between
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44 hemagglutination inhibition and neutralization assay results for determining level of influenza immunity.
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46 Overall, we found that correlation between HI and NT titers was high for all influenza, as well as within
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48 serotypes and among most specific influenza strains. While we were unable to determine a consistent
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50 equivalence factor between HI and NT titers for influenza, we did confirm previous findings that HI titers
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52 were consistently and significantly higher than NT titers.^{12,17} Furthermore, we found age to be
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54 significantly associated with the equivalence between HI and NT indicating that the difference between
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56 NT titers and HI titers reduced with increasing age.
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5 The usefulness of a generalizable equivalence factor between NT and HI tests is high, allowing for
6 comparison across studies, better use and understanding of variable levels of immunity, and increased
7 support for use of HI over NT. Although we did not identify a consistent equivalence factor for influenza,
8 we did confirm the findings of previous studies that, while HI and NT titers were similar within
9 individuals overall, NT titers were generally lower than HI titers for both H3N2 and H1N1 influenza
10 serotypes.^{12,17} Complicating the comparison between HI and NT titers is our novel finding of the
11 significant negative effect of age on the equivalence between HI and NT. While this effect was modest,
12 resulting in a reduction in the equivalent NT titer by about 4% for every 10 years of age, because of the
13 standard use of titer cut points (i.e. 1:20, 1:40, 1:80), this can result in full titer level differences between
14 the two assays. This finding supports previous findings that titer equivalences between HI and NT for
15 adults might not be the same for children.¹⁸ Furthermore, this points to the potential existence of an
16 underlying biological mechanism of waning immunity or modified immune response as people age that
17 differs between the HI and NT assays. It is not clear whether this effect is truly a factor of biological age
18 or rather a factor of time since initial immunological challenge since these were confounded in our study.
19 However, this finding further exemplifies the challenges of directly comparing HI and NT titer results for
20 understanding influenza immunity. More research is need to understand these differences by age and how
21 this might impact our understanding of immunity and vaccination.
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44 Our results confirm previous practices of HI titer of 1:40 corresponding with a gold standard of NT =
45 1:20 for influenza overall and for H3N2 influenza.¹⁷ Titer threshold equivalence testing for H1N1
46 influenza titers, which was determined to be 1:20 and 1:20, was limited due to minimal titer results for
47 H1N1 influenza strains. However, strain-specific HI titer thresholds at which specificity and sensitivity
48 were maximized for gold standard NT \geq 1:20 varied between 1:20 and 1:80. As previously found by
49 Stephenson et al., producing a single effective equivalence factor, particularly one between an HI titer of
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3 1:40 and a specific NT titer, was not possible for this study due to dependence on the virus-serum
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5 combination and strain-specific variation, as well as age.¹²
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10 An important limitation to determining the equivalence factor in this study was the use of discrete titer
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12 thresholds (i.e. 1:20, 1:40, 1:80, etc.) and lack of absolute titer data. It is possible, for example, that an
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14 equivalence factor found to be 2.0 (NT=1:20 vs. HI=1:40) could correspond to a true equivalence factor
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16 closer to 1.0 if the NT mean absolute titer for this group is 1:38.
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20 Both tests are widely used, and very few studies have directly compared their results. While NT remains
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22 the gold standard, HI offers several advantages that make a better understanding of how it correlates with
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24 NT needed. Here, we find overall correspondence between results, but because of the dependence of
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26 virus-serum interactions and an association between age and the HI-NT mapping, no consistent model
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28 that can be used across all viruses. With the broad consistency of results between HI and NT, the
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30 substantially increase in resources required to conduct NT coupled with the consistency challenges when
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32 testing at multiple laboratories, the HI titer might be a superior assay, depending on the specific aim of
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34 study.
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Figure 1

Neutralization assay (NT) (left) and hemagglutination inhibition assay (HI) (right) titers for each of the 151 participants in the study plotted by rank of age (oldest at top). Color indicates the titer measured by each assay. Strains are indicated on the x-axis of each figure.

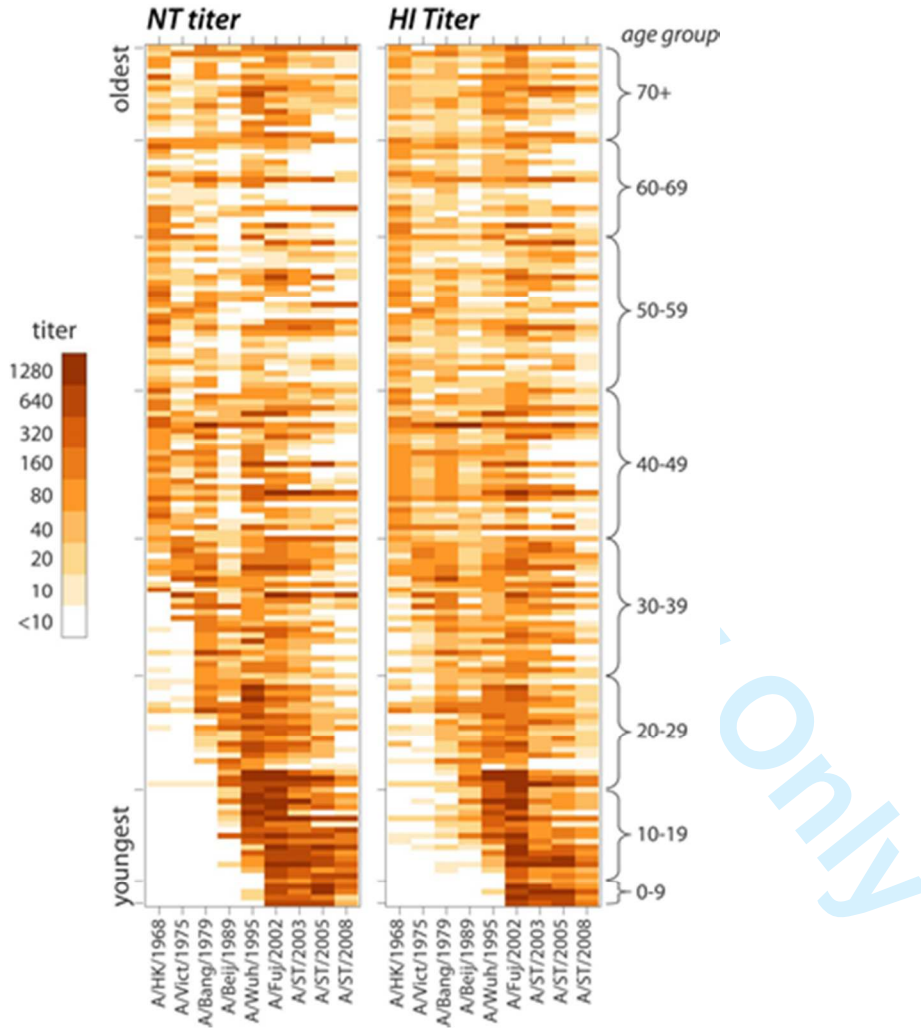


Figure 2. Correlation of HI and NT titers for all influenza A strains, H3N2 strains, and H1N1 strains.

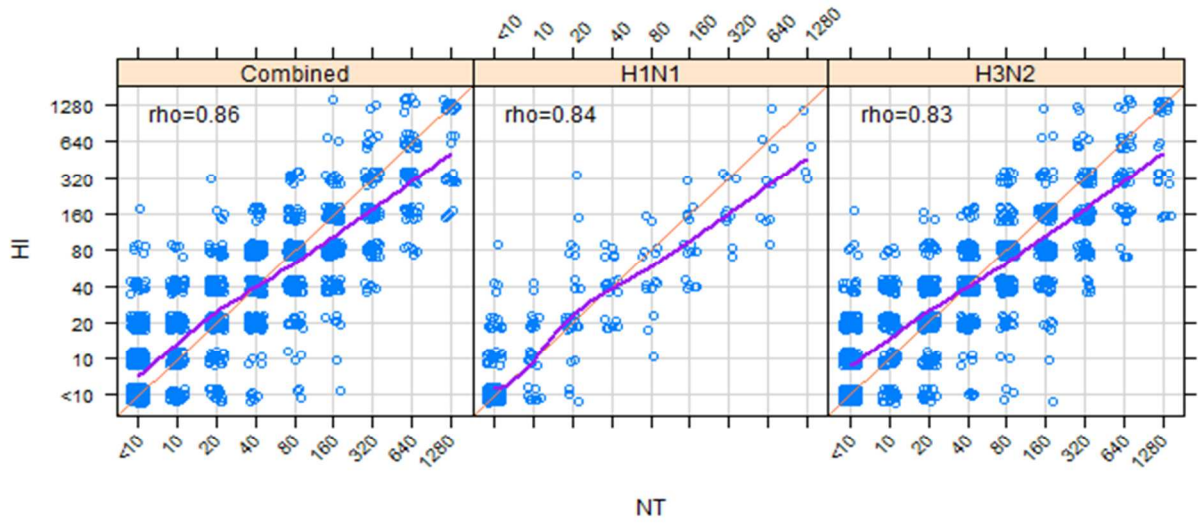


Figure 3. Correlations of HI and NT titers by influenza A strain.

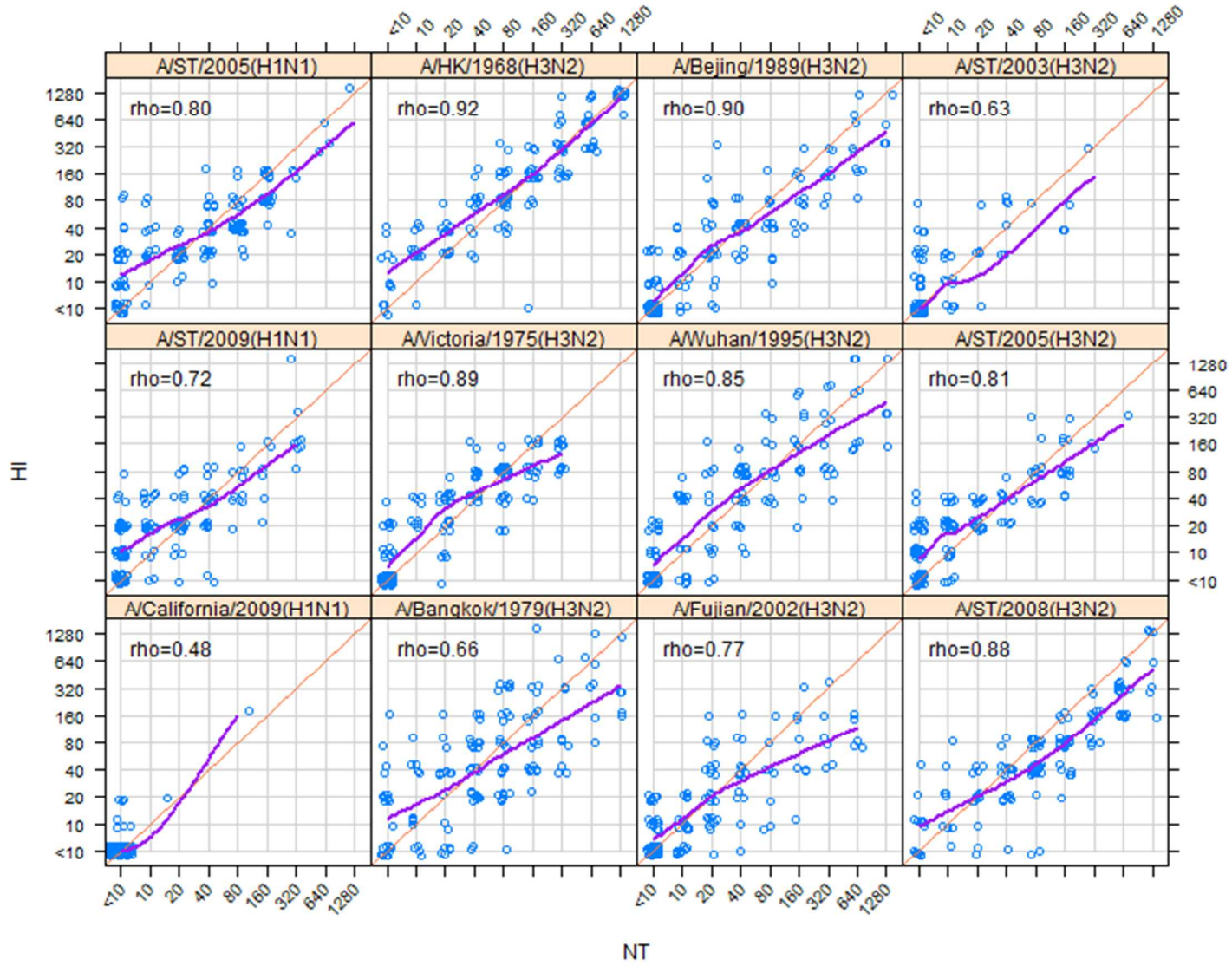
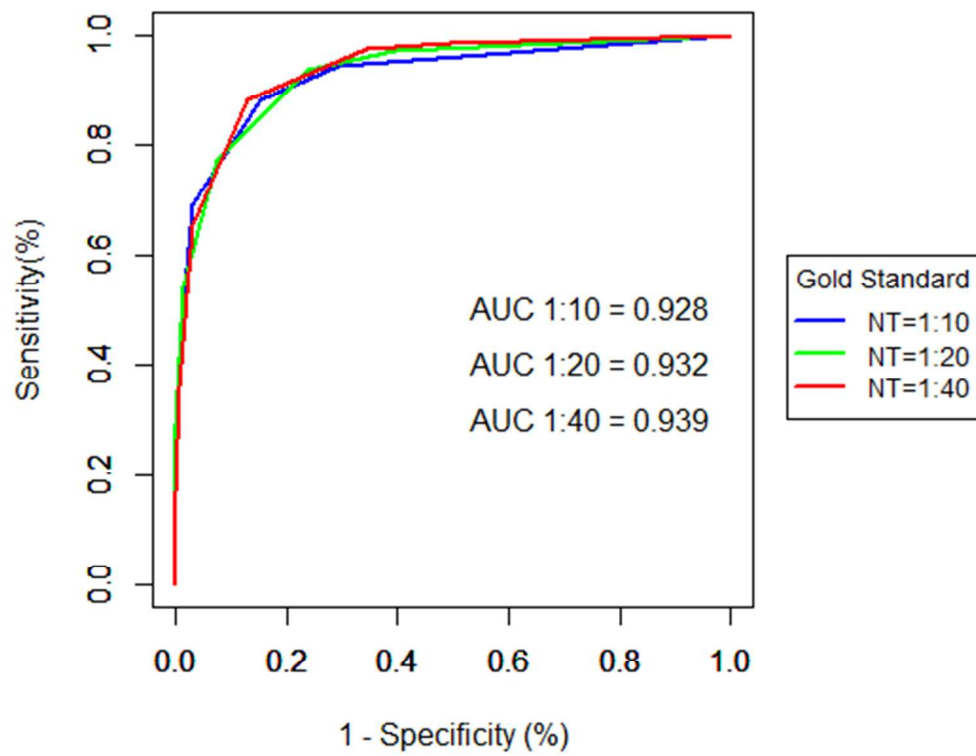
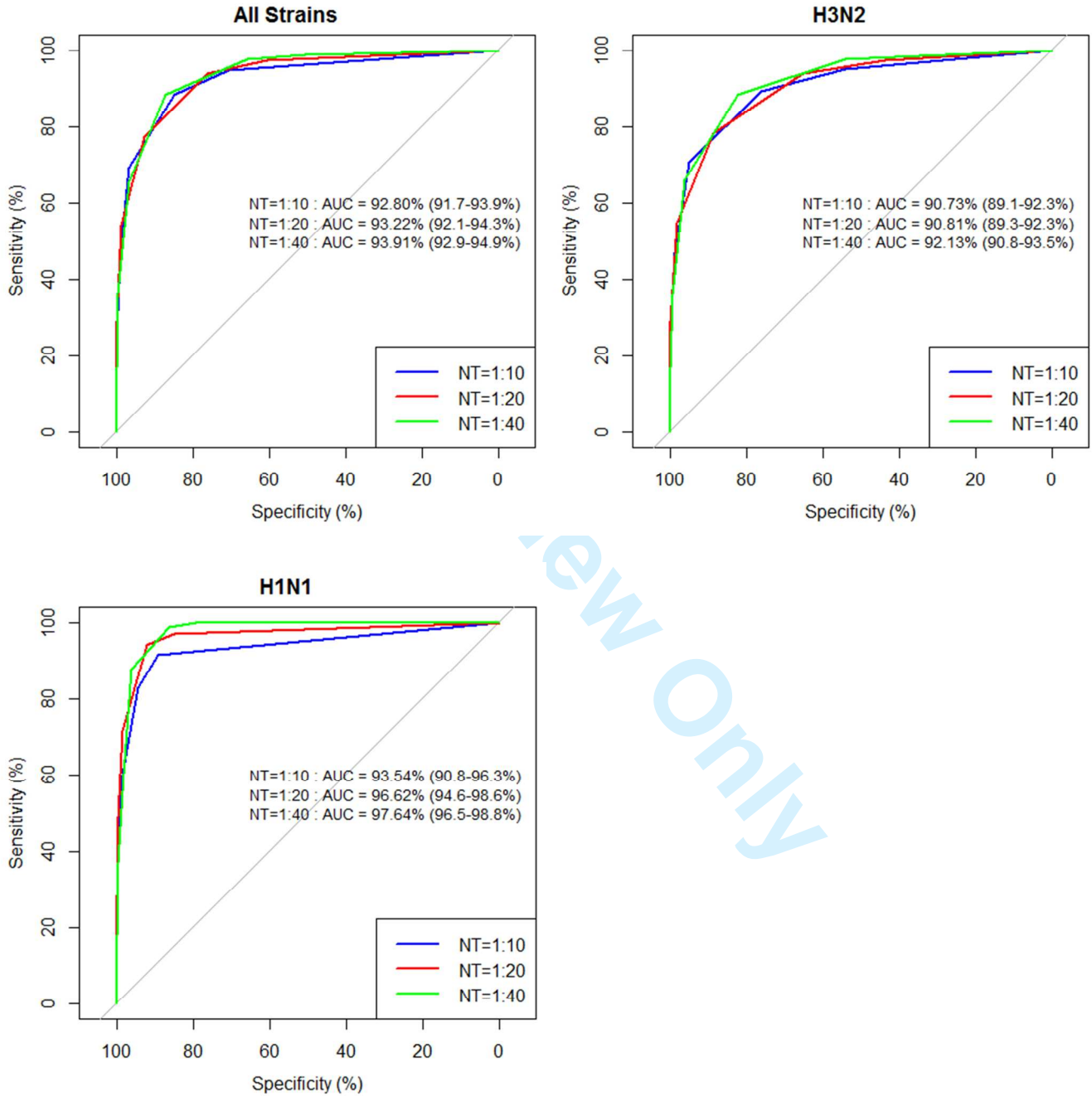


Figure 4. Sensitivity and specificity of predicting NT titer of 1:10, 1:20, and 1:40 using HI data for all influenza strains.

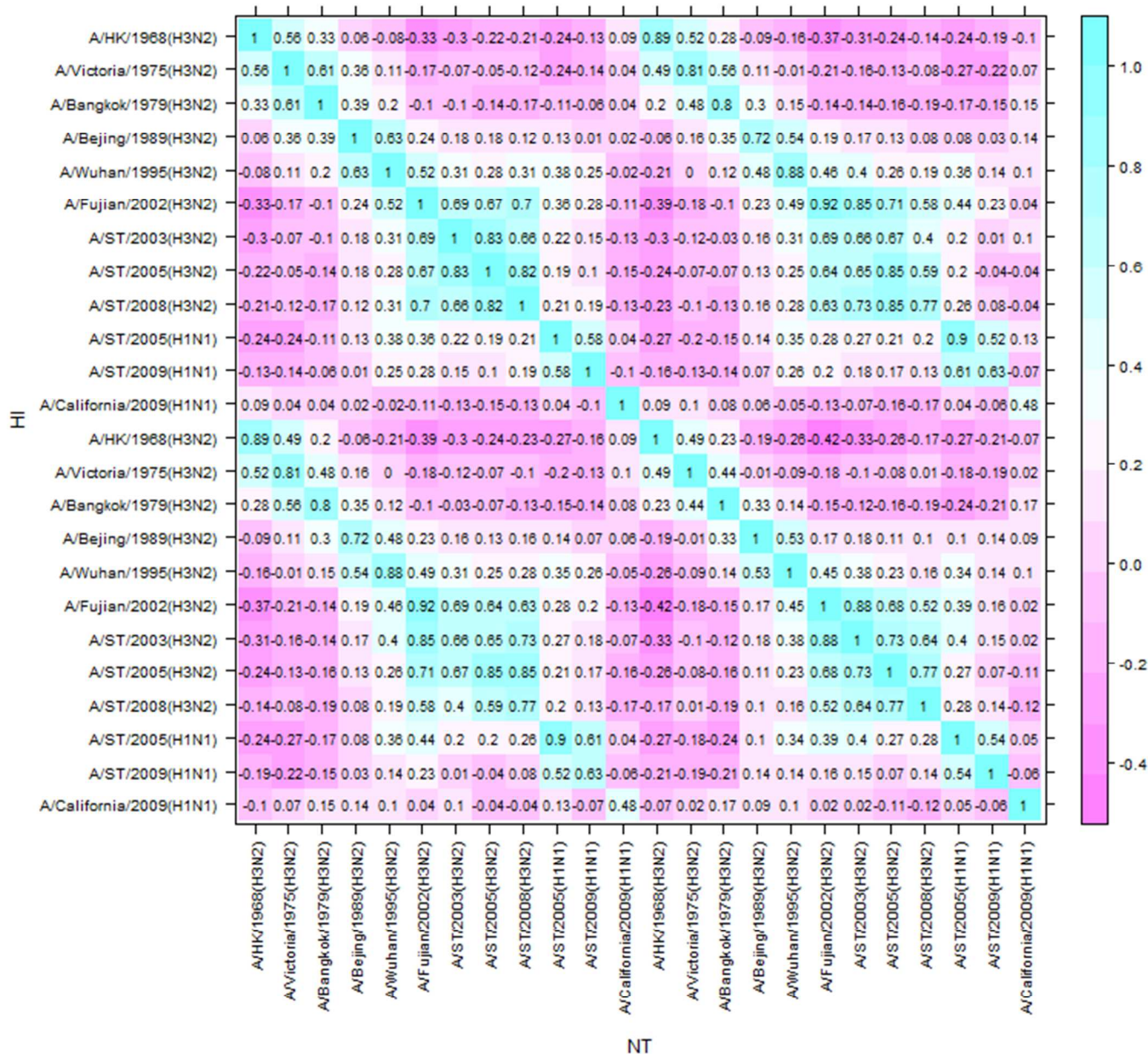


Supplemental Figure 1. ROC showing sensitivity and specificity of predicting NT status using HI titer for different thresholds of NT titer. (a) Results for all strains and multiple NT thresholds of 1:10, 1:20, and 1:40 as outcome, (b) results for H3N2, and (c) results for H1N1.



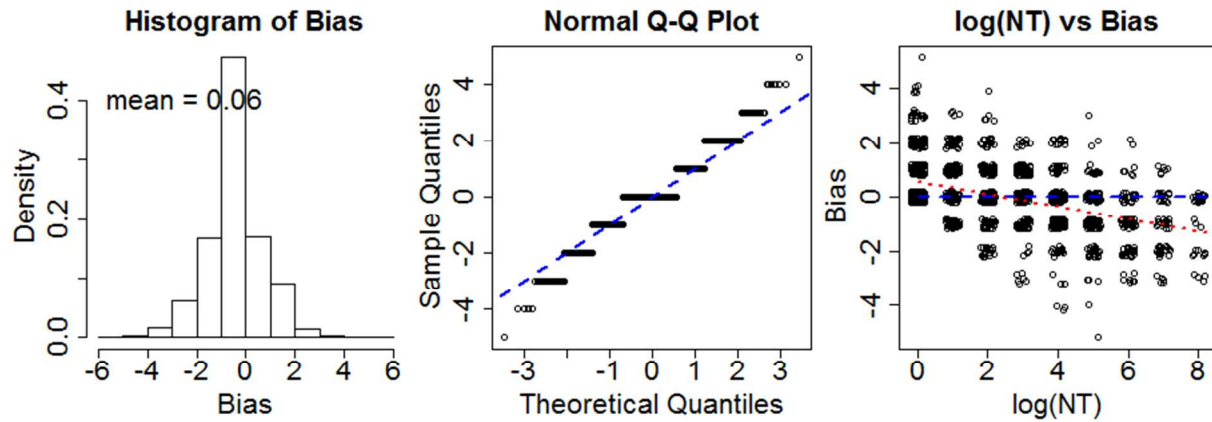
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Supplemental Figure 2. Cross reactivity correlations by NT and HI. Colors indicate the strength of correlation according to the legend on the right side. The upper right triangle of the matrix is identical to the lower left.

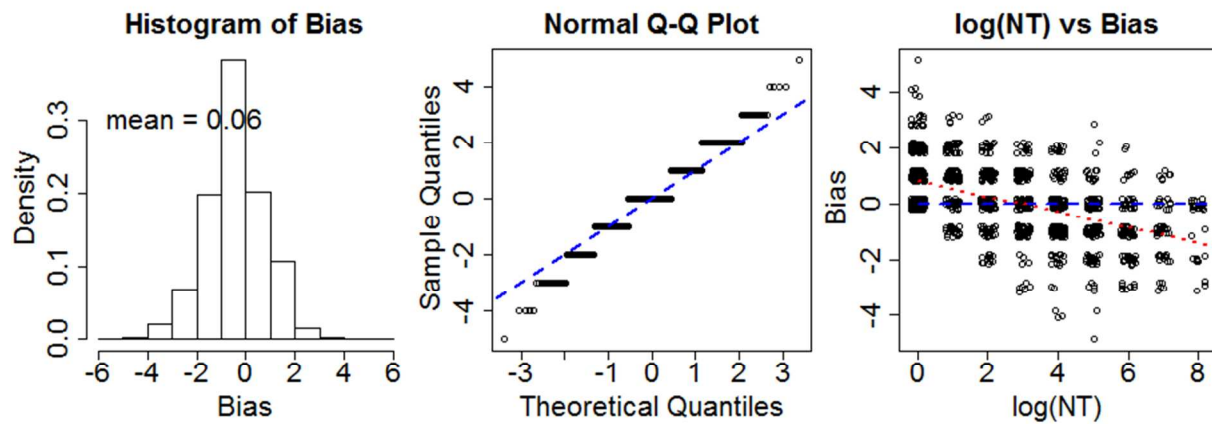


Supplemental Figure 3. Bias between NT and HI for (a) all strains, (b) H3N2, and (c) H1N1. Each panel shows a histogram of the bias ($\log(\text{HI}) - \log(\text{NT})$) for each set of strains, a normal quantile-quantile plot of $\log(\text{HI})$ versus $\log(\text{NT})$, and a plot of the bias versus $\log(\text{NT})$.

A. All Strains



B. H3N2



C. H1N1

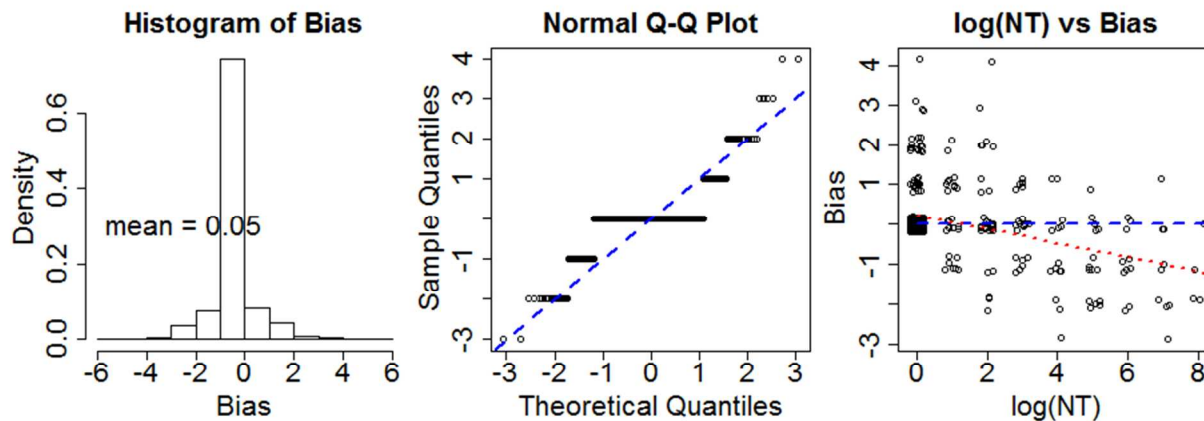


Table 1. HI and NT median titers and proportions of titers equal to or greater than the current gold standards of both for all twelve recently circulating influenza strains.

Strain	HI titers		NT titers	
	Median Titer	Proportion titers \geq 1:40 (%)	Median Titer	Proportion titer \geq 1:20 (%)
A/Hong Kong/1/1968	1:40	55.00	1:20	59.60
A/Victoria/3/1975	1:40	31.10	1:10	36.40
A/Bangkok/1/1979	1:40	56.30	1:40	67.50
A/Beijing/353/1989	1:20	35.80	1:10	47.70
A/Wuhan/359/1995	1:40	69.50	1:80	84.80
A/Fujian/411/2002	1:80	80.80	1:80	83.40
A/Shantou/90/2003	1:40	62.30	1:40	75.50

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A/Shantou/806/2005	1:40	57.00	1:40	60.30
A/Shantou/904/2008	1:10	28.50	1:10	45.00
A/Shantou/104/2005	1:20	42.40	1:20	55.00
A/Shantou/92/2009	<1:10	9.30	<1:10	11.30
A/California/07/2009	<1:10	0.70	<1:10	1.30
Total	-	44.00	-	52.30

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Table 2. ROC results of comparing NT titers cutoffs with HI titers for all twelve influenza strains, H3N2 strains, and H1N1 strains.

NT titer cutoff	All Strains		H3N2		H1N1	
	AUC (95% CI)	HI threshold maximizing sens/spec	AUC (95% CI)	HI threshold maximizing sens/spec	AUC (95% CI)	HI threshold maximizing sens/spec
10	92.8 (91.7-93.9)	20	90.7 (89.1-92.3)	40	93.5 (90.8-96.3)	10
20	93.2 (92.1-94.3)	40	90.8 (89.3-92.3)	40	96.6 (94.6-98.6)	20
40	93.9 (92.9-94.9)	40	92.1 (90.8-93.5)	40	97.6 (96.5-98.8)	20
80	93.5 (92.4-94.6)	40	91.7 (90.3-93.1)	40	97.6 (96.2-98.9)	40
160	94.0 (92.8-95.1)	80	92.3 (90.8-93.8)	80	98.3 (97.3-99.3)	40
320	95.3 (94.2-96.3)	80	93.9 (92.4-95.3)	160	98.9 (98.1-99.7)	80
640	96.8 (95.8-97.8)	160	96.1 (94.8-97.4)	160	98.8 (97.6-99.9)	80

Supplemental Table 1. ROC results comparing NT titer cutoffs of 1:10 and 1:20 with HI titers for each individual influenza strain.

Strain	NT \geq 1:10		NT \geq 1:20	
	HI threshold maximizing sens/spec	AUC (95% CI)	HI threshold maximizing sens/spec	AUC (95% CI)
A/Hong Kong/1/1968	1: (and below)20	97.5 (95.5-99.6)	40	95.5 (92.7-98.3)
A/Victoria/3/1975	20	89.6 (84.8-94.5)	20	93.3 (90.0-96.6)
A/Bangkok/1/1979	40	88.2 (81.8-94.7)	40	85.5 (79.2-91.8)
A/Beijing/353/1989	20	84.9 (78.9-91.0)	40	83.5 (77.4-89.6)
A/Wuhan/359/1995	20	92.8 (87.1-98.4)	40	90.0 (83.4-96.5)
A/Fujian/411/2002	80	94.2 (89.6-98.8)	80	94.8 (91.6-97.9)
A/Shantou/90/2003	40	80.6 (71.9-89.3)	40	82.4 (75.2-89.5)
A/Shantou/806/2005	40	93.2 (89.6-96.9)	40	93.2 (89.5-96.8)
A/Shantou/904/2008	10	85.9 (80.4-91.4)	20	91.8 (87.6-95.9)
A/Shantou/104/2005	20	95.2 (92.2-98.3)	20	95.6 (92.7-98.5)
A/Shantou/92/2009	10	84.6 (76.4-92.8)	20	93.5 (86.6-100)

A/California/07/2009	20	99.5 (98.4-100)	20	99.5 (98.4-100)
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