Sensitivity and Specificity of Serologic Assays for the Detection of Human Infection with 2009 Pandemic H1N1 Virus in U.S. Populations

Vic Veguilla1, Kathy Hancock1, Jarad Schiffer2, Paul Gargiullo1, Xiuhua Lu1, Darbi Aranio2, Alicia Branch1, Libo Dong3, Crystal Holiday1, Feng Liu1, Evelene Steward-Clark2, Hong Sun1, Byron Tsang1, David Wang1, Melissa Whaley2, Yaohui Bai1, Li Cronin2, Peter Browning1, Hanan Dababneh2, Heather Noland1, Leilani Thomas1, Lydia Foster1, Conrad P. Quinn2, Stephen D. Soroka2, Jacqueline M. Katz1*

1 Influenza Division, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, 2 Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, 3 Chinese National Influenza Center, State Key Laboratory for Infectious Disease Prevention and Control, National Institute for Viral Disease Control and Prevention, China CDC, Xuanwu District Beijing, China.

Running title: 2009 H1N1 serum antibody response characterization.

*Corresponding author: Jacqueline M. Katz

Influenza Division MS G-16
1600 Clifton Rd. NE
Atlanta, GA 30333 USA
Abbreviations: pandemic H1N1, 2009 H1N1; real-time reverse-transcription polymerase chain reaction, rRT-PCR; microneutralization, MN; hemagglutination-inhibition, HI; post-symptom onset, pso; receptor-destroying enzyme, RDE

Competing interests statement
All authors declare that they have no competing financial interests.

Sources of financial support
This study was fully supported by the Centers for Disease Control and Prevention.
The work has not been submitted for consideration for publication elsewhere.
Swine origin 2009 H1N1 influenza virus has spread globally to cause the first influenza pandemic of the 21st century. Serological studies can improve our understanding of the extent of human infection and risk factors associated with transmission of this pandemic virus. The “gold standard” for serodiagnosis of human influenza infection is the detection of seroconversion between acute and convalescent stage samples. However, timing of seroepidemiologic investigations often precludes collection of truly acute phase sera, requiring development of serologic criteria for evaluating convalescent phase sera that optimize detection of true positives and true negatives. To guide seroepidemiologic investigations into the spread of the novel 2009 pandemic H1N1 virus, we characterized serum antibody responses to 2009 H1N1 virus in 87 individuals with confirmed viral infection and 227 non-exposed U.S. individuals using microneutralization (MN) and hemagglutination-inhibition (HI) assays. Sensitivity and specificity were determined for each assay alone, and in combination, for detection of 2009 H1N1-specific antibodies in convalescent sera. Although the HI assay was more specific for detecting antibody to 2009 H1N1, the MN was more sensitive, particularly for detecting low titer seroconversions. A combination of titers (MN ≥40 and HI ≥20) provided highest sensitivity (90%) and specificity (96%) for individuals aged < 60 years and 92% specificity for adults aged ≥60 years for detection of serologically confirmed 2009 H1N1 infections in U.S. populations during the first pandemic waves. These studies provide an approach to optimize timely serologic investigations for future pandemics or outbreaks of novel influenza viruses among humans.
INTRODUCTION

Since first emerging among humans in North America in the spring of 2009, the swine origin 2009 H1N1 influenza virus has spread globally to cause the first influenza pandemic in over 40 years (2, 8, 28). Estimating the total number of pandemic H1N1 (2009 H1N1) infected persons is challenging since estimates based on virologic laboratory confirmation, and even disease surveillance, vastly underestimate the true number of infected persons (7, 20, 21). Serological studies can provide a better understanding of the extent of human infection with 2009 H1N1 in different settings (1, 19). In particular, seroepidemiologic studies can assess risk factors for infection and rates of transmission in defined populations by linking detection of serum antibody responses as retrospective evidence of infection with information on illness, demographics, and behavioral factors. Because such studies focus on confirmation of infections at the individual level, rather than infection rates within a population, they require development of serologic criteria that optimize detection of true positives and true negatives.

Although detection of seroconversion, a 4-fold or greater rise in influenza antibody titer, between acute and convalescent phase sera remains the optimal serodiagnostic approach, the timing of serologic investigations often precludes collection of baseline sera over which to detect seroconversion. In such situations, it may be possible to develop
criteria for seropositivity based on a single convalescent serum, if the virus hemagglutinin is sufficiently novel with respect to seasonal influenza viruses. The 2009 H1N1 is antigenically and genetically distinct from seasonal H1N1 viruses that have circulated in the last 60 years (11). Nevertheless, studies in Europe and the U.S demonstrated that prior to the 2009 pandemic, approximately 20-30% of adults 60 or 65 years and older possessed serum antibody cross-reactive with 2009 H1N1 (13, 20, 22).

The hemagglutination-inhibition (HI) has long been used to detect serologic responses to influenza infection or vaccination. An HI titer of ≥40 is associated with a 50% or greater reduction in the risk of influenza infection or disease in susceptible populations (9, 14). More recently, virus neutralization or microneutralization (MN) assays have also been used, because they detect functional neutralizing antibodies, and in some cases, offer greater sensitivity than traditional HI assays for the detection of antibodies following influenza infection or vaccination, particularly with novel influenza A viruses, such as avian-origin H5N1 viruses (12, 24). However, there is only limited data on the comparative sensitivity of these serological assays for the diagnosis of infection with 2009 H1N1 (4, 6). In this study, we aim to bridge this knowledge gap and describe an approach to compare the relative sensitivity and specificity of the MN and HI assay against a novel virus such as 2009 H1N1. We establish seropositivity criteria for convalescent sera to identify influenza infected persons against 2009 H1N1 virus and discuss the relative value of either assay for different serologic purposes.

METHODS
Sera collection.

Sera (n = 162) were collected from 87 U.S. residents (aged 3 months to 80 years), with 2009 H1N1 infection confirmed by real-time reverse-transcription polymerase chain reaction (rRT-PCR) during April-August, 2009. The proportion of confirmed cases by age group was: < 10 years, 8%; 10-19 years, 30%; 20-29 years, 48%; 30-39 years, 8%, 40-59 years, 5%, >60 years, 1%. These included sera collected at a single time point [n=12; 7 to 23 days post-symptom onset (pso)] or paired sera from 75 individuals collected 1 to 77 days pso (Figure 1). A total of 227 sera from U.S. residents aged 6 months to 88 years were used for the specificity analysis; 168 sera were collected prior to the circulation of the 2009 H1N1 virus as previously described (13) and 59 sera were from individuals with rRT-PCR confirmed seasonal H1N1 virus infection in the 2008-2009 season collected 3 to 6 months pso (Figure 1). The collection and testing of serum samples at CDC was considered to be a public health, non-research activity that was exempt from human-subjects review.

Serological procedures.

For the HI assay, sera were first treated with receptor-destroying enzyme (RDE; Denke-Seiken, Japan) followed by heat inactivation at 56°C for 30 minutes. Sera containing nonspecific agglutinins were preadsorbed with turkey erythrocytes. For the MN assay, sera were first heat inactivated at 56°C for 30 minutes. Sera were tested by HI assay using 0.5% turkey erythrocytes and MN assay according to previously published procedures and using A/Mexico/4108/2009, an A/California/7/2009-like 2009 H1N1 virus, which was propagated in 10 to 11 day old embryonated chicken eggs (13, 18, 24). For both assays, serial two fold dilutions of serum (1:10 to 1:1280) were tested in duplicate. HI or
MN titers were expressed as the reciprocal of the highest dilution of serum that gave complete hemagglutination or 50% neutralization, respectively.

**Linear regression model and statistical analyses.**

Linear regression models using antibody titers from all rRT-PCR confirmed 2009 H1N1 sera (Figure 1) were performed to estimate the correlation between serum antibody titers measured by MN and HI assays and to determine the predicted 2009 H1N1 MN titers corresponding to 2009 H1N1 HI titers. HI titers (ranging from 5 to 640) and MN titers were transformed to log$_2$. To best determine the relationship between HI and MN titers and to observe the proportion of variation in the MN titers that can be explained by HI titers, the following independent variables were included in the model: log$_2$ HI titer, log$_2$ HI titer$^2$, and log$_2$ HI titer$^3$. An age variable was not included in the model as the majority of the rRT-PCR confirmed sampled population had ages that ranged between 10 and 29 years. Using a backward elimination, only variables that were statistically significant (p ≤ 0.05) were kept in the model.

The percent sensitivity and specificity achieved at different individual and combination titer cutoffs were determined using only sera collected 15 or more days pso (Figure 1) from 2009 H1N1 rRT-PCR confirmed cases (n=79) and sera belonging to non-exposed populations (n=227), respectively. The significance of geometric mean titers and seroconversion rates were determined by ANOVA and Chi-Squared tests, respectively. P-values of less than 0.05 were considered significant. SAS® V9.1 software (SAS Institute Inc., Cary, NC) was used for all statistical analysis.
RESULTS

Correlation and linear regression analysis of serological tests.

Sera from 87 confirmed cases were tested by MN and HI assays for the presence of antibody against 2009 H1N1 influenza virus (Figure 2). A strong positive correlation (Spearman’s rank correlation, $r = 0.84$) was noted between HI and MN titers. The final linear regression model, which included only statistically significant variables ($p \leq 0.05$), was as follows: $\log_2$ MN titer $= 0.3761 + 1.196 \log_2$ HI titer. The assumptions of linearity and homogeneity of variance were met as residual plots showed neither a linear nor an outward/inward curvature pattern (data not shown). As shown in Table 1, the nearest predicted discrete MN titer was generally 2-fold higher for HI titers $\leq 160$ and 4-fold higher for HI titers of 320 and 640.

Kinetics of antibody response analysis.

Figure 3 shows the kinetics of antibody response in all rRT-PCR confirmed cases grouped in 7 day interval time points to depict the rising titer trend over time. The proportion of individuals with HI titers of $\geq 40$, a titer threshold generally associated with a 50% reduction in the risk of influenza illness in susceptible populations (14), increased from 2% to 26% after the first week of infection and rose to 71 and 76% for sera collected either between 15-21 or $\geq 22$ days pso, respectively. We also assessed the proportion of individuals that achieved a MN titer of $\geq 40$ (the corresponding HI titer of $\geq 20$, as predicted by our linear regression model). Only 48% of individuals with sera collected between 8-14 days pso achieved these titers, whereas greater than 90% of individuals achieved these titers in sera collected 15 or more days pso. As expected, the difference between the
geometric mean titers for sera collected less than 15 days pso (MN=15, HI=9; n=69) and sera collected during convalescent phase or ≥15 days pso (MN=203, HI=61; n=93) was statistically significant for both assays (p < 0.01). Nevertheless, there was no significant difference in the geometric mean titers of sera collected 15-21 days pso (MN=160, HI=66; n=21), sera collected 22-28 days pso (MN=247, HI=59; n=50), and sera collected >28 days pso (MN=163, HI=61; n=22). Among 55 individuals for whom well-timed paired sera were available, seroconversions were detected more often by MN (91%) than by HI (84%) assay. Furthermore, seroconversion detections were statistically higher (p < 0.05) by the MN than by the HI assay, when data were stratified to include only paired sera with acute sample titers of 20 or less by both assays. Among these data, the MN detected 100% (41/41) of the seroconversions while the HI detected only 85% (35/41).

**Sensitivities and specificities of 2009 H1N1-specific serological tests.**

To establish serologic criteria for detection of 2009 H1N1 infection in convalescent sera, we performed a sensitivity (using sera from 79 rRT-PCR confirmed cases < 60 years of age and collected 15 days or more pso; see Figure 1) and specificity (using sera from non exposed population) analyses for MN and HI assays. As shown in Table 2, the titer cutoff value that provided the highest sensitivity was a MN titer of ≥40 (94%) or an HI titer of ≥20 (92%). Since the majority (86%) of our rRT-PCR confirmed cases were < 30 years of age, it was not possible to stratify the sensitivity results by age or estimate sensitivity in adults ≥60 years of age.

Because pre-pandemic cross-reactive antibody to the 2009 H1N1 virus has been demonstrated particularly in older adults (13, 16, 17, 20, 22), we compared the specificity
for detection of 2009 H1N1 antibody in different age groups. For individuals < 60 years of age, the MN titer cutoff which gave optimal sensitivity (≥40) was only 83% specific, whereas the comparable HI titer cutoff (≥20) gave 91% specificity. The reduced specificity of the MN assay among these individuals was primarily due to the lower specificity (61%) observed in adults 40-59 years of age. The MN assay was also less specific than the HI among adults ≥60 years of age; the specificity of a MN titer of ≥40 or an HI titer of ≥20 was 59% or 92%, respectively. Overall, for those < 60 years of age, a 2-fold increase in cutoff titer (MN titer of ≥80 or HI titer ≥40), considerably improved the specificity, but substantially reduced the sensitivity to unacceptable levels (80 or 75%, respectively).

We next assessed whether a combination of MN and HI titers could maximize sensitivity and specificity (Table 2). Combining a MN titer of ≥40 and an HI titer of ≥20 resulted in a sensitivity of 90% and a specificity of 96% for all ages < 60 years and a specificity of 92% for the ≥60 years age group. Although, combinations using higher titer cutoffs modestly improved specificity, the sensitivity dropped to ≤75%. These results suggest that seropositivity criteria based on a combination of serologic titers can provide maximal sensitivity and specificity for the detection of 2009 H1N1-specific antibody in individuals < 60 years and specificity comparable to the HI alone in those ≥60 years of age.

**DISCUSSION**

The 2009 pandemic has highlighted the need for timely studies to investigate the extent of age-specific human infection after multiple pandemic waves in different geographic
regions (19). Such studies can estimate total numbers of infection upon which to base more accurate estimates of rates of severe or fatal disease. They may also provide policy makers with a better understanding of the proportion of susceptible persons remaining in populations in order to predict better the public health impact of successive pandemic waves. To better understand the relative benefits of the HI versus the MN assay for the detection of 2009 H1N1 virus infected persons, we characterized serum antibody responses to 2009 H1N1 virus in rRT-PCR confirmed cases, using both serologic assays, and confirmed the titers obtained by either assay were highly correlated. Furthermore, using an additional set of sera from non-2009 H1N1 exposed populations, we assessed the relative sensitivity and specificity of both assays. Although the HI assay alone was more specific for detecting antibody to 2009 H1N1, the MN was significantly more sensitive for detecting low titer seroconversions. Finally, based on our sensitivity and specificity analysis, we identified a combination of threshold titers (MN $\geq 40$ and HI $\geq 20$) that provided highest sensitivity and specificity to identify 2009 H1N1 infected persons < 60 years of age and high specificity for adults aged $\geq 60$ years using only convalescent sera, and in the absence of demonstrable seroconversion in paired sera. These criteria have been used to facilitate the analyses of multiple seroepidemiologic investigations conducted in the U.S. during the first wave of the 2009 pandemic.

The kinetics of antibody responses in 2009 H1N1 rRT-PCR cases confirmed that the optimal timing of acute serum collection is within one week of symptom onset. Although 90% of cases achieved thresholds titers for seropositivity (MN titer of $\geq 40$ and HI $\geq 20$) by day 15 pso, sera collected 22-28 days pso had the highest geometric mean titer, suggesting
that this remains the optimal timeframe for the collection of convalescent phase sera. These results are consistent with those of Miller et al (20) and Hung et al (15) who reported that 11% of 2009 H1N1 confirmed cases in England failed to develop HI antibody titers of ≥32 and 10% of confirmed cases in Hong Kong failed to develop neutralizing antibody titers of ≥40. Similar to our results, Hung et al (15) also found that the MN assay detected a higher seroconversion rate (89%) compared to the HI assay (82%).

The HI assay detects antibodies that bind near the receptor binding site of the viral HA blocking the interaction of HA with sialic acid receptors on erythrocytes and inhibiting their agglutination. Virus neutralization assays such as the MN, detect antibodies that neutralize the virus by inhibiting viral entry and/or replication in mammalian cells including antibodies recognizing epitopes within the stem region of HA which block membrane fusion and which are conserved among viruses of different influenza A subtypes (26). Detection of cross-reactive antibodies to the stem region could consequently lower the specificity of the MN assay, particularly in the adult and older adult population, whom presumably have had a greater exposure to different influenza A viruses throughout their lifetime.

Several studies have used an HI titer of ≥40 as a marker of infection with or immunity to 2009 H1N1 (20, 23). This is a reasonable approach for large-volume seroprevalence and seroincidence studies, and for optimal rapidity of reporting results following successive pandemic waves. However, our data suggest that this titer threshold may underestimate the numbers of 2009 H1N1-infected individuals. On the other hand, due to its lower specificity in adults aged 40 and over, the use of the MN assay alone may overestimate
2009 H1N1 infections in this age group for U.S. populations. Where resources permit and particularly when studies seek to identify 2009 H1N1 infected individuals rather than population rates, our results suggest that the use of both assays and the combination titer achievements provide optimal sensitivity and specificity. However, it should be noted that while providing a sensitive and specific serologic marker for infection, the combination of titer achievements cannot be correlated with a level of protection against the pandemic virus. Furthermore, once a pandemic virus becomes seasonal, as is now the case for the 2009 H1N1 virus (10), serologic confirmation of human infection will once again require detection of seroconversion by either assay, the gold standard for all influenza serodiagnosis.

Our study had several limitations. The age distribution of our rRT-PCR confirmed cases, differed from that of 2009 H1N1 cases based on national estimates (21); age groups 0-14 years were under-represented, and the 5-24 year age group was over-represented. In addition, we were unable to estimate the sensitivity of the assays and titer cutoffs for adults ≥60 years of age. Furthermore because individuals 80 years and older, exhibit high frequencies of serum antibodies that cross-react with 2009 H1N1, due to structural similarities that exist between the HA molecules of 2009 H1N1 and 1918-like influenza viruses, seropositivity criteria developed here cannot be applied to this age group (16, 17, 20, 29). Efforts to discriminate preexisting serum cross-reactive antibody from 2009 H1N1 infection induced antibodies in this age group are ongoing in our laboratory.

Studies from China and Singapore found little evidence of pre-existing 2009 H1N1 virus cross-reactive antibodies in older adults, even those ≥80 years of age, suggesting that
there are geographic or other factors that contribute to the presence of pre-existing antibody in human populations (3, 5, 27). These findings support the need for laboratories undertaking seroepidemiologic or seroprevalence studies for detection of antibody against novel viruses to individually evaluate pre-pandemic age-specific prevalence of cross-reactive antibody in local populations. Furthermore, due to inter-laboratory assay variation, caution should be exercised in directly using the seropositivity criteria developed here to identify 2009 H1N1 infected persons based on serologic data from other laboratories (25).

In summary, we have demonstrated an approach whereby serologic criteria can be developed to identify human infections using only convalescent sera when novel influenza viruses first emerge to infect humans. Assessing the relative sensitivity and specificity of serologic assays is an important component for establishing threshold titers used to estimate the extent of seropositivity among populations after the first pandemic waves. We believe these studies may provide a strategy to assist with timely serologic investigations for future pandemics or outbreaks of novel influenza viruses among humans.
FIGURE LEGENDS

Figure 1. Sera included and excluded in analyses.

Figure 2. Linear regression and correlation between log$_2$ Microneutralization (MN) titers and log$_2$ Hemagglutination-inhibition (HI) titers.

Figure 3. Kinetics of antibody response in pandemic H1N1 rRT-PCR confirmed cases. Hemagglutination-inhibition (HI) and microneutralization (MN) titers are grouped by number of days post-symptom onset based on seven day intervals for 162 serum samples from 87 cases. The number of sera samples collected per seven day intervals post-symptom onset were: ≤ 7 days, 46 sera; 8-14 days, 23 sera; 15-21 days, 21 sera; ≥ 22 days, 72 sera. Individual MN (●) and HI (○) titers as well as proportion of individuals that achieved a MN titer of ≥40 or the corresponding HI titer of ≥20 are shown. The lines indicate titers of 20, 40, and 80.

ACKNOWLEDGEMENTS

The authors thank Drs. Edward Belongia and Patrick Blair, and State and CDC epidemiologists and laboratory staff who contributed sera used in this study. We thank Dr. Xiyan Xu and Amanda Balish for reagents and technical assistance, respectively. Drs. George Carlone and Sandra Steiner for allowing their staff to assist with the pandemic response and Dr. Claudia Pappas for graphical assistance.

The findings and conclusions in this report are those of the authors and do not
necessarily represent the views of the Centers for Disease Control and Prevention.

REFERENCES

Virological analysis of fatal influenza cases in the United Kingdom during the early wave of influenza in winter 2010/11. Euro Surveill 16.


Table 1: Predicted 2009 H1N1 Microneutralization (MN) titers corresponding to 2009 H1N1 Hemagglutination-inhibition (HI) titers using linear regression model.

<table>
<thead>
<tr>
<th>HI Titer</th>
<th>Predicted MN Titer</th>
<th>Discrete Predicted MN Titer</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>9</td>
<td>5</td>
<td>43</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>20</td>
<td>47</td>
<td>40</td>
<td>13</td>
</tr>
<tr>
<td>40</td>
<td>107</td>
<td>80</td>
<td>19</td>
</tr>
<tr>
<td>80</td>
<td>245</td>
<td>160</td>
<td>15</td>
</tr>
<tr>
<td>160</td>
<td>560</td>
<td>320</td>
<td>11</td>
</tr>
<tr>
<td>320</td>
<td>1282</td>
<td>1280</td>
<td>3</td>
</tr>
<tr>
<td>640</td>
<td>2932</td>
<td>2560</td>
<td>4</td>
</tr>
</tbody>
</table>
Table 2. Microneutralization (MN) and Hemagglutination-inhibition (HI) assays sensitivity and specificity summary.

<table>
<thead>
<tr>
<th>Group</th>
<th>Sensitivity (Confirmed Cases)</th>
<th>Specificity (All Non-Exposed Individuals)</th>
<th>Specificity (Ages 0-39)</th>
<th>Specificity (Ages 40-59)</th>
<th>Specificity (Ages ≥ 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>≥ 40 % (95% CI)</td>
<td>≥ 20 % (95% CI)</td>
<td>MN titer ≥ 40 % (95% CI)</td>
<td>MN titer ≥ 80 % (95% CI)</td>
</tr>
<tr>
<td>Ages &lt; 60</td>
<td>79</td>
<td>94 (85 - 98)</td>
<td>92 (84 - 97)</td>
<td>90 (81 - 95)</td>
<td>96 (92 - 98)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80 (69 - 88)</td>
<td>75 (63 - 84)</td>
<td>75 (63 - 84)</td>
<td>98 (91 - 99)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>91 (92 - 98)</td>
<td>98 (91 - 100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>91 (92 - 98)</td>
<td>99 (96 - 100)</td>
</tr>
<tr>
<td></td>
<td>176</td>
<td>83 (76 - 88)</td>
<td>91 (86 - 95)</td>
<td>96 (92 - 99)</td>
<td>97 (94 - 100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>94 (89 - 97)</td>
<td>97 (93 - 99)</td>
<td>98 (93 - 99)</td>
<td>98 (94 - 100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>91 (92 - 99)</td>
<td>98 (91 - 100)</td>
</tr>
<tr>
<td></td>
<td>127</td>
<td>91 (85 - 95)</td>
<td>91 (84 - 95)</td>
<td>91 (82 - 98)</td>
<td>94 (82 - 98)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>96 (91 - 99)</td>
<td>96 (91 - 99)</td>
<td>100 (91 - 100)</td>
<td>100 (91 - 100)</td>
</tr>
<tr>
<td></td>
<td>49</td>
<td>61 (46 - 74)</td>
<td>94 (82 - 98)</td>
<td>94 (82 - 98)</td>
<td>100 (91 - 100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>88 (75 - 95)</td>
<td>100 (91 - 100)</td>
<td>100 (91 - 100)</td>
<td>100 (91 - 100)</td>
</tr>
<tr>
<td></td>
<td>51</td>
<td>59 (44 - 72)</td>
<td>92 (80 - 97)</td>
<td>92 (80 - 97)</td>
<td>94 (83 - 98)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>84 (71 - 93)</td>
<td>94 (83 - 98)</td>
<td>94 (83 - 98)</td>
<td>94 (83 - 98)</td>
</tr>
</tbody>
</table>

* Confidence Interval
Sera collected for study = 162
rRT-PCR confirmed patients = 87
(75 paired collections and 12 single time point collections)

Linear Regression Analysis
Kinetics of Antibody Response Analysis

Age > 60 yrs = 1 patient
(sera = 2)

Sera collected for study = 160
rRT-PCR confirmed patients = 86
(74 paired collections and 12 single time point collections)

Single time point collection sera drawn
<15 days post symptom onset = 6 patients
(sera = 6)

Convalescent sera from paired collections drawn
<15 days post symptom onset = 1 patient
(sera = 2)

79 patients/sera included in analysis
(73 convalescent sera from paired collection and
6 single time point collections)

Sensitivity Analysis

Sera collected for study = 227
Non 2009 H1N1 exposed people = 227
(227 single time point collections)

Age ≥ 60 yrs = 51 non 2009 H1N1 exposed people
(sera = 51)

Specificity Analysis

Age < 60 yrs = 176 non 2009 H1N1 exposed people
(sera = 176)

Specificity Analysis

Figure 1
Figure 2

\[ \log_2 MN \text{ titer} = 0.3761 + 1.196 \log_2 HI \text{ titer} \]

\[ r = 0.84 \]
Figure 3

- Days Post-Symptom Onset
- Sera Titors (log₂)
- MN ≥ 40: 7% 48% 95% 93%
- HI ≥ 20: 4% 48% 95% 92%