International laboratory comparison of influenza microneutralization assays for A(H1N1)pdm09, A(H3N2), and A(H5N1) influenza viruses by CONSISE

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2 **An international laboratory comparison of influenza microneutralisation assay**

3 **protocols for A(H1N1)pdm09, A(H3N2) and A(H5N1) influenza A viruses by CONSISE**

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25

Abstract – 205 words (250 allowed)

The Microneutralisation assay is commonly used to detect antibodies to influenza virus and multiple protocols are used worldwide. These protocols vary in the incubation time of the assay as well as in the order of specific steps and even within protocols there are often further adjustments in individual laboratories. The impact these protocol variations have on influenza serology data is unclear. Thus a laboratory comparison of the 2-day ELISA and 3-day hemagglutination (HA) microneutralisation (MN) protocols, using A(H1N1)pdm09, A(H3N2) and A(H5N1) viruses, was performed by the CONSISE Laboratory Working Group. Individual laboratories performed both assay protocols, on multiple occasions, using different serum panels. Thirteen laboratories from around the world participated. Within each laboratory, serum sample titres for each assay protocol were compared to determine the sensitivity of each assay and between replicates to assess the reproducibility of each protocol, for each laboratory. There was good correlation between the results obtained using the two assay protocols in most laboratories, indicating these assays may be interchangeable for detecting antibodies to the influenza A viruses included in this study. Importantly, participating laboratories have aligned their methodology to the CONSISE Consensus 2-day ELISA and 3-day HA MN assay protocols to enable better correlation of these assays in the future.

Keywords: influenza; serology; CONSISE; microneutralisation assay; pandemic; A(H5N1); A(H1N1)pdm09; A(H3N2)

48 **Introduction**

49 Following infection with influenza viruses, most people develop antibodies specific to
50 the infecting virus that can be measured by serological assays. These antibodies can be
51 detected in the majority of people 2-3 weeks after symptom onset and persist for months (1,
52 3, 7, 13). Thus serology can confirm past infection in the absence of clinical symptoms or
53 virological data, detecting most symptomatic and asymptomatic infections(6).

54 In 2011, an international partnership termed 'CONSISE', the Consortium for the
55 Standardization of Influenza Seroepidemiology, was created out of a need identified during
56 the 2009 pandemic, for timely seroepidemiological data to better estimate pandemic virus
57 infection severity and attack rates and to inform policy decisions. CONSISE is comprised of
58 individuals from various organisations, with free membership. The activities of CONSISE are
59 performed by two inter-linked Working Groups, Laboratory and Epidemiology, and a
60 Steering Committee. The focus of the Laboratory Working Group is to improve serological
61 assay comparability and standardisation through consensus assay development, comparative
62 laboratory testing and quality assurance (12)(www. https://consise.tghn.org).

63 The main serological assays to detect antibodies to influenza virus are the
64 Hemagglutination Inhibition (HI) assay and the Microneutralisation (MN) assay. The HI
65 assay detects antibodies that block the influenza virus hemagglutinin binding to sialic acid-
66 linked residues on red blood cells (RBC), whilst the MN assay detects functional antibodies
67 primarily directed towards the hemagglutinin that prevent infection of cells in tissue culture
68 (reviewed in (5, 17)). There are various forms of the MN assay used in laboratories around
69 the world, such as the 2-day ELISA protocol (8, 17), 3-day hemagglutination (HA) protocol
70 (16) and 7-day HA protocol(4, 9). For the purposes of seroepidemiology, the shorter
71 protocols of 2 and 3 days are preferred. The 2- and 3-day MN assays measure antibodies to
72 hemagglutinin, yet differ in preparation of cell monolayers for infection as well as detection

of virus infection. Cells are plated with the virus:serum mixture for the 2 day MN assay, whilst a pre-formed cell monolayer is used for the 3-day MN assay. The 2-day MN assay detects nucleoprotein in infected cells (8), whilst the 3-day assay measures hemagglutinating virus in the culture medium or CPE in the cell monolayer. Although there have been some direct comparisons between serological assays performed by multiple laboratories (9, 10, 14, 15), the impact of various MN assay protocols on the determination of serological titres is unknown. Therefore, the aim of this study was to assess the intra-laboratory variability and sensitivity of the 2-day ELISA MN assay and the 3-day HA MN assay for detecting antibodies to A(H1N1)pdm09 virus and, as an extension, A(H3N2) and A(H5N1) influenza viruses. The study was performed by the CONSISE Laboratory Working Group members (see Appendix).

Methods

Reagents used in study

Laboratories were required to supply their own reagents, virus stocks, MDCK cell lines and appropriate cell culture media for the study. Wildtype or reassortant viruses were used: the A(H1N1)pdm09 strains were antigenically similar to the A/California/7/2009 vaccine strain; the A(H3N2) strains were antigenicity similar to the A/Perth/16/2009 or A/Victoria/361/2011 vaccine strains. A representative A(H5N1) virus from a clade that was recognised by the laboratory's serum panel was used. Serum panels contained approximately 10 test samples (sera or plasma), comprising low, medium and high titre antibody levels. Sera were from seroepidemiology studies, vaccine studies and ferret sera (to obtain high titre serum in some laboratories), and were supplied by each participating laboratory.

Development of consensus 2-day ELISA and 3-day MN protocols

Parameters and variables for the 2-day ELISA (17) and the 3-day HA (11, 16) MN assays were listed. Laboratories within CONSISE shared their protocols for either, or both, MN assays and listed their preferred variables for each parameter identified. Data were collected anonymously, collated and used to develop the consensus protocols.

Consensus 2-day ELISA and 3-day HA MN assays

The 2-day ELISA MN assay was to be performed as described by (8, 17) whilst the 3-day HA MN assay was to be performed as described by (11, 16). Laboratories were required to use the specified parameters listed in the CONSISE consensus protocols (**Tables 1** and **2**). Cell culture conditions, virus and serum panels varied between laboratories. The reciprocal of the highest dilution whereby 50% infection was prevented was recorded as the titre for each serum sample.

Design of study – laboratory assay comparison

Individuals who are members of CONSISE were invited to participate in the experimental laboratory comparative study. Thirteen laboratories agreed and were assigned a code letter from A-M, not representing the order of listing of participants in the Appendix. Eleven laboratories took part in the initial A(H1N1)pdm09 study (Labs A-K), seven in the A(H3N2) study (Labs A, C, D, F, I, K, L) and three in the A(H5N1) study (Labs H, L, M). Overall twelve laboratories provided data that could be included in the analyses (Labs A-L). Each laboratory was required to assay A(H1N1)pdm09, A(H3N2) or A(H5N1) antibody levels in their panel of sera on at least three separate occasions using both the CONSISE consensus MN assay protocols: 2-day ELISA and 3-day HA.

123

124 **Statistical analysis**

125 All analyses were based on the titres reported by the participants. To enable comparison of
126 assays for each laboratory, the geometric mean titre (GMT) was calculated across runs and
127 replicates to give a single value for each sample for each MN assay method. To calculate the
128 overall ratios between the assays for each laboratory, the ratio of the 3-day titre (detected by
129 HA or cytopathic effect (CPE)) to the 2-day ELISA MN titre was calculated for each sample.
130 The GMT was then calculated for all samples in the serum panel for each laboratory. For the
131 purpose of calculations, negative titres reported as <10 were substituted by '5', while high
132 titres reported as ≥ were assigned the next 2-fold titre, e.g.. '≥1280' was assigned '2560'.
133 Correlations in results between assay methods for the panels of serum samples were
134 calculated using Spearman rank correlations.

135

136 **Results**

137

138 **Development of Consensus protocols for the MN assays**

139 We assessed the similarities between methodology used in ten laboratories for the 2-day
140 ELISA and the 3-day HA MN assays. Parameters were highly consistent between
141 laboratories for the 2-day ELISA MN assay method, and closely followed published methods
142 (8, 17). There was less consistency between the 3-day HA MN assay methods, particularly in
143 number of sample replicates performed and determination of the endpoint titre (50 % or
144 100% neutralisation). There was variability in both assays for cell culture conditions (data not
145 shown). To facilitate greater comparability between laboratories, we developed consensus
146 protocols for the 2-day ELISA and 3-day HA MN assays by discussion and agreement at
147 CONSISE meetings ((2) (**Tables 1** and **2**)). Parameters were classified as either required or

recommended, based on their importance in the interpretation of the assay titres. Required parameters included serum dilutions and reporting of sample titres, assay incubation times and endpoint calculation methods. Recommended parameters listed appropriate variables for use.

Data received

A(H1N1)pdm09 MN assays: Ten laboratories returned data for both assays. ELISA was used for detection of all 2-day MN assays. For the 3-day MN assay, 7 laboratories used HA only as the detection method (turkey or guinea pig RBC were used), 2 laboratories used CPE only and 2 laboratories used both HA and CPE detection methods. Both HA and CPE detection methods were assessed. Three laboratories performed multiple additional detection methods for one or both of the MN assays, but these data have not been included in the analysis. An eleventh laboratory did not perform the 2-day ELISA MN assay and returned data for only the 3-day HA MN assay (laboratory K). This laboratory shared a serum panel with laboratory F which performed both assays. The 3-day HA MN assay titres from laboratory K were compared with the 2-day ELISA MN assay titres from laboratory F. Laboratories G and J performed each assay twice, rather than three times; laboratory I performed the 3-day MN assays twice.

A(H3N2) MN assays: Data were received from seven laboratories. All laboratories used ELISA for detection of the 2-day MN assay and HA (turkey or guinea pig RBC) for detection of the 3-day HA MN assay and two laboratories also sent corresponding titres detected by CPE. Laboratory K only performed the 3-day HA MN assay and shared a serum panel with laboratory F.

A(H5N1) MN assays: Data were received from three laboratories. The results from laboratory M were negative for all of the serum samples for all tests. No further analysis was possible for this laboratory. Laboratories H and L used horse or goose RBC to read out the 3-day HA MN assay.

Reproducibility within laboratories: comparison of replicate tests

Laboratories performed an internal comparison of assay protocols using their own serum panels. The titres within each laboratory for each sample across replicate tests were compared. Detecting antibodies to A(H1N1)pdm09 virus, there was good reproducibility of the 2-day ELISA MN assays for the majority of the laboratories, with over 80% of the laboratories with replicate tests differing by ≤2-fold, whilst laboratories B and D had 10-30% of replicate titres differing by >2-fold (**Figure 1A**). No laboratories had replicate tests differing by >4-fold (**Figure 1B**). For the 3 day MN assays, the variability differed depending on the assay detection method. When detecting by HA, 4 laboratories had replicates differing by >4-fold, yet this was only for very few samples (one sample out of 10-12 samples, 8.3-10 %), whilst when detecting by CPE, two laboratories had replicate tests differing by >4-fold (two samples of 10 (20 %) and 7/12 samples (58 %)) (**Figure 1B**). Six laboratories had replicates differing by >2-fold by HA detection (7.5-40 %), whilst three laboratories had tests differing by >2-fold when detected by CPE (10-91.7 %) (**Figure 1A**). Laboratory A showed high variability between replicates when detecting the 3-day MN titres by CPE (91.7 %) as two replicate assays were comparable, whilst the third assay was inconsistent (data not shown). In seven laboratories (C, D, E, F, G, J, K), there were no replicates that differed by more than 2-fold.

For studies detecting antibodies to A(H3N2) and A(H5N1) viruses, reproducibility was also good, with three instances of replicates that differed by greater than 4-fold, though this

was for a small number of samples for each laboratory (10-20%). All other laboratories had replicates that differed by ≤4-fold (**Figure 1C** and **D**).

Relationship between 2-day ELISA MN assay and 3-day HA and CPE MN assays for test serum panels

Titres for individual test serum panels were compared for each laboratory between the two assays. Comparing the titres obtained using the 2-day ELISA and the 3-day HA MN assays for studies detecting antibodies to A(H1N1)pdm09 virus (**Figure 2A**), seven of the nine laboratories had individual correlation co-efficients that were above 0.9 (good) and two of the nine laboratories did not (**Table 3**). Two laboratories (G and H) had low correlation between the assays (0.580 and 0.638, respectively), as the 3-day MN HA assay gave narrow response ranges compared to the 2-day ELISA MN assay. Comparison of titres between the 2-day ELISA MN assay and the 3-day MN assay detected by CPE showed higher overall consistency between the assays (**Figure 2B**, **Table 3**).

The equivalent correlations were determined for comparison of assays detecting antibodies to A(H3N2) (**Figures 2C**, **D**) and A(H5N1) (**Figures 2E**, **F**). For assays detecting antibodies to A(H3N2) virus, the majority of laboratories obtained good correlation between the 3-day HA and the 2-day ELISA MN assays (range 0.865-0.966 (**Table 3**)). Laboratory L had a poor correlation with co-efficient of 0.439 as both of the assays gave a narrow titre range across the serum panel (<10-160). There were only two laboratories that had data detecting antibodies to A(H5N1) virus and they both showed reasonable correlation between the assays (**Table 3**). Laboratory L had much better correlation with the assays detecting antibodies to A(H5N1) virus than A(H3N2) virus.

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Overall relationship between assays for each laboratory

The ratio between titres for the 2-day ELISA and 3-day MN assays for the serum panels was calculated to assess whether a consistent relationship between the performances of assays could be observed for each laboratory (**Table 4**). Many of the ratios indicated average titres within a 2-fold range between methods (i.e. ratios between 0.5 and 2.0), representing reasonable agreement in assay sensitivity. For assays detecting antibodies to A(H1N1)pdm09, laboratories F and G had lower 3-day HA MN titres compared to their 2-day ELISA MN titres. For laboratory G, the 3-day HA MN assay gave negative or low titres for all serum panel samples. Laboratories I and K had much higher 3-day MN titres compared to the 2-day ELISA MN assay. The ratios between assays were different for the laboratories that also participated in the comparison studies detecting antibodies to A(H3N2) or A(H5N1). For the comparison assays detecting A(H3N2) antibodies, laboratories D and K also had much higher 3-day MN titres compared to the 2-day ELISA MN assay. For comparison assays detecting A(H5N1) antibodies, laboratory H had much higher 3-day HA MN assay titres compared to the 2-day ELISA MN assay titres (**Table 4**).

Analysis for bias within the study

Potential factors for bias were assessed. Although each laboratory had a preferred MN assay (2 day ELISA, 3 day HA or 3 day CPE, indicated in **Table 3**), overall this did not seem to affect the correlation between assays. However, for laboratories where the titres from the two assays did not correlate well (A(H1N1)pdm09 virus for laboratories G, H; A(H3N2) virus for laboratory L , **Table 3**) the 3-day MN assay showed less variation in titres (i.e. had less discriminating power) (Figure **2A** and **C**, respectively). Yet there was also variability in correlation for different viruses within the same laboratory (**Table 3**), indicating this effect may be virus specific or due to experience, as the studies were performed consecutively

(A(H1N1)pdm09, then A(H3N2)/A(H5N1)). However, for laboratories with markedly different overall titres between assays (laboratories F, H, I and K, **Table 4**) there was no relationship between the preferred assay and the titre achieved. Most laboratories used sera from adults (10 of 11) and wildtype influenza viruses (8 of 11); neither showed any effect on assay comparability. The ratio of HA/$TCID_{50}$ for the virus stock from each laboratory was assessed and there was no clear relationship, suggesting there was no bias due to the presence of interfering virus particles (HA titres range - 16-1280, median - 128; log_{10} $TCID_{50}$/ml range - 4.5-7, median - 6). Overall, there was no clear indication of major bias in the study.

Discussion

Upon emergence of a novel influenza virus, seroepidemiological data are critical in understanding the spread and attack rate of the virus to form the base of pandemic risk and severity assessments. Serology also can identify groups susceptible to a novel influenza virus in a population. Understanding the impact of different MN assay protocols would strengthen these estimates for policy decisions. Our comparison of MN assay methodology indicates that there is good correlation between the 2-day ELISA and 3-day HA MN assays for detection of antibodies to A(H1N1)pdm09 virus, in most laboratories. These findings were confirmed in an extension to this study with A(H3N2) and A(H5N1) viruses. Overall, there is potential for either assay to be used. Importantly, through participating in this study, laboratories have aligned their methodology to the CONSISE consensus assays described, harmonising protocols for the 2-day ELISA and 3-day HA MN assays internationally.

Our intra-laboratory assessment demonstrated the 2-day ELISA and 3-day HA MN assays were largely reproducible and comparable. The 2-day MN assay is read out by ELISA using spectrophotometry, which is objective, whilst the 3-day MN assay that is read out by HA and CPE requires more experience and training. In addition, as our study required

participating laboratories to perform the assays on three separate occasions, different preparations of RBC for the replicate 3-day HA MN assays were likely. As a two-fold range between titres for the same sample is considered acceptable for serological studies, the assays were overall highly reproducible on different days, with all laboratories having >97% of samples with titres within a four-fold difference for the 2-day ELISA, 92% for the 3-day HA and 92% for the 3-day CPE MN assays.

Importantly, in over half of the laboratories, there was very good correlation between the 2-day ELISA and 3-day HA MN assays when a panel of sera was tested. This suggests there is no inherent difference between the different assays, despite their different readouts. Thus there is no underlying scientific reason that the different MN assay formats cannot be compared when detecting antibodies to A(H1N1)pdm09, A(H3N2) or A(H5N1) viruses.

However, three laboratories did have poor correlation between the assays which may be related to experience in, and performance of, one particular method. Seven laboratories were experienced in the 2-day ELISA MN assay, whilst 6 laboratories were experienced in the 3-day HA MN assay before commencing this study. Poor correlation between assays was more likely in those laboratories inexperienced with the 3-day HA MN assay, indicating that training in HA or CPE readout might be required. From the MN assay comparison performed here, we anticipate that as laboratories gain experience in both assays, the correlation between the titres obtained for the 2-day ELISA and 3-day HA MN assays will improve. A mentoring system will be established in subsequent international comparison studies performed by CONSISE whereby laboratories who are learning an assay will be assisted by local 'experienced' laboratories. We anticipate that this collaborative assay development will encourage rapid and more comparable data between assays and laboratories in the future.

297 It is notable that although we were able to standardise many assay-specific variables in
298 this study, such as virus concentration, incubation times, serum dilution and end-point
299 determination, some factors are impossible to standardise. Cell culture conditions are
300 laboratory-specific and may differ by cell line, media supplements and incubation
301 temperatures. Cell culture conditions are often optimised for the variety of viruses and
302 experiments performed in a laboratory as well as the availability of reagents, thus cannot be
303 prescriptive.
304
305 A limitation of the present study is that test serum panels were not shared with each
306 laboratory. As our study compared assay protocols, rather than the performance of different
307 laboratories, this is acceptable. Thus the impact of using consensus assay protocols on inter-
308 laboratory variability could not be examined thoroughly. In a future study being planned by
309 the CONSISE Laboratory Working Group, shared serum panels will be tested for antibodies
310 to A(H1N1)pdm09 virus using consensus 2 day ELISA and 3-day MN Assays and a
311 consensus HI Assay in comparison with use of local assay protocols.
312
313
314

Appendix

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Figure Legends

Figure 1. Reproducibility within laboratories of serology assay results for assays detecting antibodies to A(H1N1)pdm09 (**A**, **B**) and A(H3N2) and A(H5N1) (**C**, **D**) viruses. Graphs show the proportion (%) of replicate assays differing by >2-fold (**A, C**) and >4-fold (**B, D**) for 2 day ELISA MN Assay (black bar), 3 day MN Assay detected by HA (white bar) and CPE (striped bar) for each participating laboratory for all sera. ND indicates where the assay or detection method was not performed.

Figure 2. Relationship between test sample titres for antibodies to A(H1N1)pdm09 (**A, B**), A(H3N2) (**C, D**) or A(H5N1) (**E, F**) viruses determined by 2-day ELISA MN Assay and 3 day MN Assay detected by HA (**A**, **C**, **E**) or CPE (**B**, **D**, **F**). Each laboratory is represented by a colour.

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Table 1. CONSISE Consensus 2-day ELISA MN Assay for detecting antibodies to A(H1N1)pdm09 virus (based on [1, 2])

Parameter	Required Parameter	Recommended parameter
A. Stock Virus preparation		
Cell substrate for virus growth		Day 10 embryonated eggs
Stock virus infectivity and method of determination		At least 10^6 $TCID_{50}$/ml, read by ELISA
Stock storage		Aliquots of bulk virus preparation
B. Sera preparation		
Storage of sera following receipt		-70 ºC, -20 ºC, 4 ºC, 1-2 freeze thaw cycles in testing laboratory
Pre-assay treatment of sera		Heat treatment 56 ºC for 30 min, undiluted in media
Initial sera dilution	1:10	-
Sample type		Sera only or plasma only
C. Virus preparation		
Final virus concentration per well	$100TCID_{50}$	-
Volume of virus solution added per sample	50 µl	-
Virus/serum mix incubation		1h at 37 ºC
Calculated starting sera dilution	1:10 excluding cell culture	-

	volume	
D. Cell preparation		
Preparation of cells		Cell suspension
Cell type used		MDCK ('Salisbury'), MDCK-SIAT1
Assay diluent/culture media		Coon's/Dulbecco's Modified Eagles with 1% BSA/FCS, laboratory preferred media
E. Assay set-up		
Incubation time of assay to endpoint reading	18 -22h	
Incubation conditions		35-37 ºC, 5% CO_2
# of sample replicates		Replicates preferred if available
F. Endpoint estimation		
Endpoint determination		Viral antigen detection by ELISA using anti-nucleoprotein antibody (clone)
Endpoint calculation method	50% neutralisation	

Table 2. CONSISE Consensus 3-day HA MN Assay for detecting antibodies to A(H1N1)pdm09 virus

Parameter	Required Parameter	Recommended parameter
A. Stock Virus preparation		
Cell substrate for virus growth		Day 10 embryonated eggs, MDCK cells, MDCK-SIAT1 cells
Stock virus infectivity and method of determination		At least 10^6 $TCID_{50}$/ml, read by RBC agglutination
Stock storage		Aliquots of bulk virus preparation
B. Sera preparation		
Storage of sera following receipt		-70 ºC, -20 ºC, 4 ºC, 1-2 freeze thaw cycles in testing laboratory
Pre-assay treatment of sera		Heat treatment 56 ºC for 30 min, undiluted in media
Initial sera dilution	1:10	-
Sample type		sera only or plasma only
C. Virus preparation		
Final virus concentration per well	100$TCID_{50}$	
Volume of virus solution added per sample/well		50 µl, 100 µl, 200µl
Virus/serum mix incubation		1h at 37 ºC
Virus/serum mix incubation on cell monolayer		1h at 37 ºC
Calculated starting sera dilution	1:10 excluding virus	-

	volume	
D. Cell preparation		
Preparation of cells		preformed monolayer
Cell type used		MDCK (ATCC), MDCK ('Salisbury'), MDCK-SIAT1
Assay diluent		Coon's/Dulbecco's Modified Eagles, with trypsin (1/2 µg/ml), laboratory preferred media
Cell infection media		Coon's/Dulbecco's Modified Eagles, with trypsin (1/2 µg/ml), laboratory preferred media
E. Assay set-up		
Incubation time of assay to endpoint reading	3 days	
Incubation conditions		35-37 ºC, 5% CO_2
# of sample replicates		Replicates preferred if available
F. Endpoint estimation		
Endpoint determination		turkey/guinea pig RBC agglutination, CPE
Endpoint calculation method	50% neutralisation	

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Table 3. Correlation of titres for test samples between assays by laboratory. For each laboratory, Spearman rank correlation coefficients were calculated to compare the 2-day ELISA MN against the 3-day HA or CPE MN assay.

Laboratory	Correlation of 2-day MN to 3-day MN						Preferred assay
	A(H1N1)pdm09		A(H3N2)		A(H5N1)		
	3-day HA	3-day CPE	3-day HA	3-day CPE	3-day HA	3-day CPE	
A	0.966	0.901	0.865				2 day
B	0.976						2 day
C	0.992		0.966				3 day
D		0.892	0.890	0.898			3 day
E	0.944						2 day
F	0.965		0.966				3 day
G	0.580						2 day
H	0.638	0.738			0.883	0.908	2 day
I	0.970		0.954	0.901			2 day
J		0.944					3 day
K[a]	0.931		0.942				3 day
L			0.439		0.833		both

[a]Titres for 2-day ELISA MN from laboratory F, as laboratories F and K shared serum panels.

Table 4. Ratios between 2-day ELISA MN Assay and 3-day MN Assay detected by HA and CPE.

	Average Ratio of 3-day MN titre to 2-day MN titre					
Laboratory	A(H1N1)pdm09		A(H3N2)		A(H5N1)	
	3-day HA	3-day CPE	3-day HA	3-day CPE	3-day HA	3-day CPE
A	2.4	1.5	1.9			
B	0.9					
C	0.8		1.5			
D		2.0	3.7	3.8		
E	1.0					
F	0.3		1.3			
G	0.1					
H	1.5	1.6			5.8	6.3
I	5.22		0.88	0.87		
J		1.2				
K[c]	2.4[a]		3.2			

1

L	1.1	0.4

[a]Titres for 2-day ELISA MN from laboratory F, as laboratories F and K shared serum panels.

Figure 1

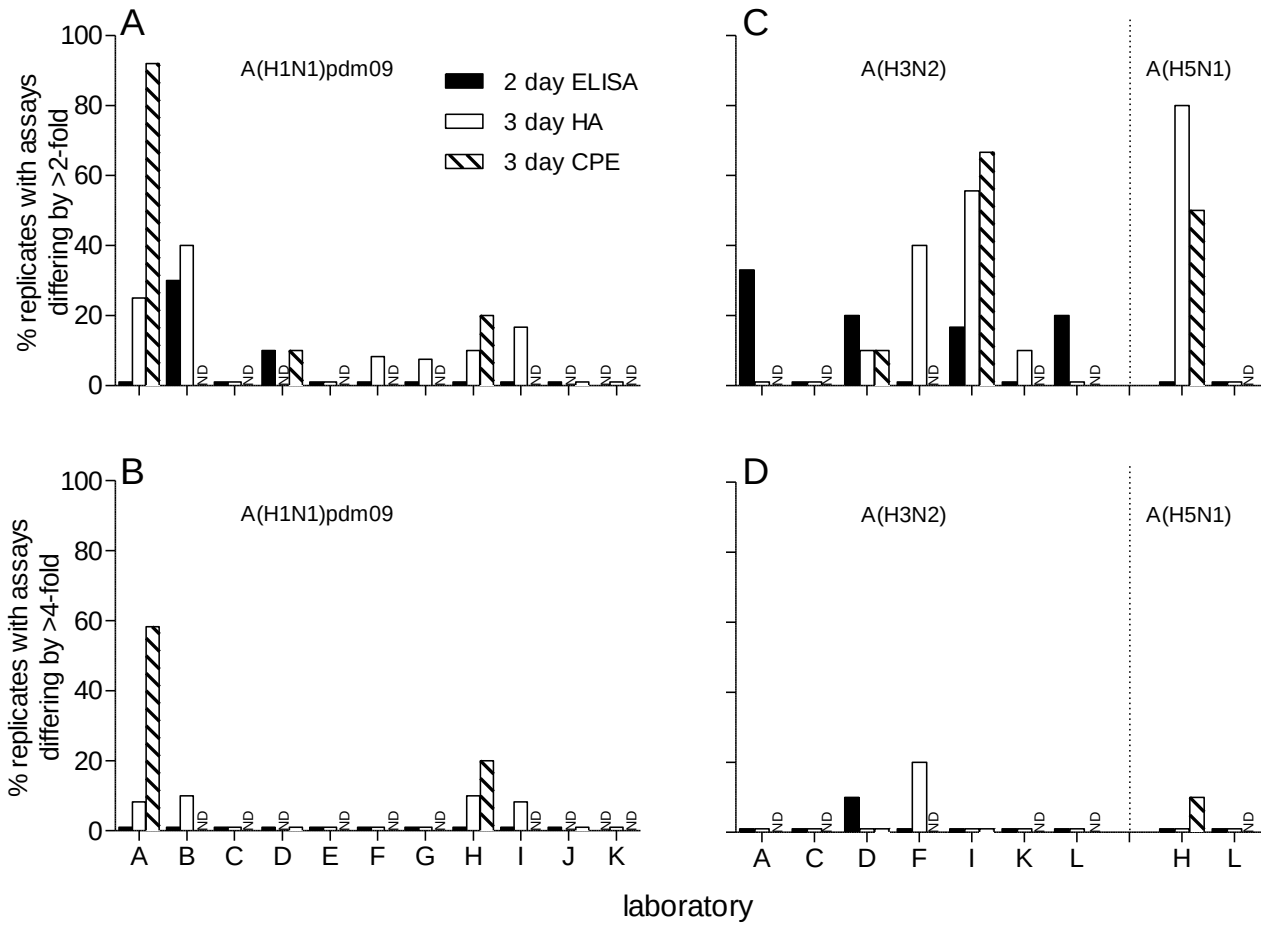

Figure 2