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Consensus 3 day HA Consensus MN Assay for laboratory comparison of of A(H1N1)pdm09 virus - developed by the CONSISE Laboratory Working Group

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	50 µl
Virus/serum mix incubation		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 ºC, 37 ºC, 5% CO_2
# of sample replicates		Replicates
F. Endpoint estimation		
Endpoint determination		turkey/guinea pig RBC agglutination, CPE
Endpoint calculation method	50% neutralization,	