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Consensus 2 day MN assay for laboratory comparison of A(H1N1)pdm09 virus –

Developed by the CONSISE Laboratory Working Group (based on Rowe et al 1999 J Clin Microbiol 37(4):937; WHO Global Influenza Surveillance Network. Manual for the laboratory diagnosis and virological surveillance of influenza. WHO 2011. http://whqlibdoc.who.int/publications/2011/9789241548090_eng.pdf

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	100$TCID_{50}$	-
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
F. Endpoint estimation		
Endpoint determination		Viral antigen detection by ELISA using anti-nucleoprotein antibody (clone)
Endpoint calculation method	50% neutralization	