

Consensus HI assay for laboratory comparison of A(H1N1)pdm09 virus – Developed by CONSISE Laboratory Working Group.

Reference: 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 pages 43-59.

^aCalculation for RBC preparations using haemocytometer is described in WHO Manual above

^bFurther protocols are available from WHO CC for Reference and Research on Influenza, VIDRL, Australia

	Parameter	Required parameters	Recommended parameters
RBC Preparation	RBC species used	turkey	
	RBC washing		Initial resuspension in PBS plus two washes in PBS
	RBC storage		Store for approx. 1 week but monitor appearance ie discard if RBC begin to lyse
	Method for RBC cell estimation		Estimate packed cells by haematocrit or by haemocytometer ^a
	Final concentration of RBCs for use in assay	0.5 % turkey hematocrit, 4x10 ⁷ cells/ml	
	Pooling of RBC from multiple animals		Choice of pooling or taken from single source. Pre-screening recommended of all new turkeys for non-specific agglutinins and sensitivity to the HA of cell grown virus ^b
Serum preparation	<i>V. cholerae</i> Receptor Destroying Enzyme (RDE)	Denka Seiken C. Ltd , WHO Influenza Reagent Kit (from Denka Seiken) or Sigma-Aldrich	Evaluate RDE by recipient laboratory ^b
	RDE:serum ratio for serum treatment	3 volumes RDE to 1 volume sera	
	Incubation conditions	37 °C in water bath or incubator 16-20 h	
	RDE inactivation	Heat inactivate at 56 °C in water bath for 30 min. Allow to cool, then add 6 volumes PBS or 0.85% NaCl	
	Final serum dilution	1:10	
	Detection of nonspecific agglutinins in treated sera	Performed before HI assay	
	Treatment of sera to remove non-specific agglutinins	20 volumes RDE-treated sera incubated with 1 volume packed RBC for 1 h at 4 °C (if necessary)	
Control sera		Run positive and negative control sera in each assay	
Haemagglutination conditions	Turkey RBC – concentration	0.5%	
	Turkey RBC – type of microtitre well	V	
	Turkey RBC - incubation time at RT after RBC addition	30 min at room temperature (18-25 °C)	
	Complete haemagglutination definition	RBC in suspension after RBC control has settled completely	
	Incomplete haemagglutination definition	Some RBC in suspension after RBC control has settled completely	

	No haemagglutination definition	RBC settled completely: turkey RBC 'run' or form 'tear drop' when tilted	
	Haemagglutination end-point	Highest dilution of virus that causes complete haemagglutination	
	Haemagglutination titre	Reciprocal of haemagglutination end-point	
HI Assay preparation	Volume RDE-treated sera added each well	25 µl	
	Dilution of sera across/down microtitre	2-fold	
	Sera diluted down or across microtitre plate		Either down or across (ie 1:10-1:20480) dependent on study design
	Diluent for sera dilution		PBS
	Final antigen concentration	4 HAU per 25 µl	
	Volume antigen per well	25 µl	
	Amount of antigen per well	4 HAU	
	Mixing protocol		Laboratory shaker or thorough manual agitation
	Incubation conditions of virus/serum	At least 15 min at room temperature (18-25 °C)	
	Volume RBC added per well	50 µl	
	Incubation conditions of virus/serum/RBC	30 min at room temperature (18-25 °C)	
	Final volume per microtitre well	100 µl	
	Calculated starting serum dilution	1:10 excluding virus and RBC volume	
	# of sample replicates	Duplicate	
Control of virus titre	Back titration of virus to check 4HAU		
HI Assay end-point estimation	'HI endpoint' definition	Highest dilution of serum that prevents complete haemagglutination	
	'HI titre' definition	Reciprocal of HI end-point	
	Acceptable replicate titres	Within two-fold or require repeating	