Measurement of antibodies response to avian influenza A(H7N9) virus in confirmed human cases by multiple serology methods

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Serological method evaluation on new emerging avian influenza A(H7N9)virus

- **Sera panels**

Description	H7N9 patient group			Non H7N9 patient group		
	Single serum sample of acute-phase	**Single serum sample of convalescent-phase**	**Paired serum samples**	**General population**	**Seasonal flu antibody positive**	**Poultry workers with H9N2 or H5N1 positive antibody**
No.of serum samples	21	7	19	94	100	64
Average ages(range)	57(3.8-87)	53(30-75)	35(4-69)	21(18-39)	24(1-79)	40.5(8.9-70)
Testing methods	HI,MN,WB, NI(partial)	HI,MN,WB, NI(partial)	HI,MN,WB, NI(partial)	HI,MN	HI,MN	HI,MN

- Main factors we evaluated

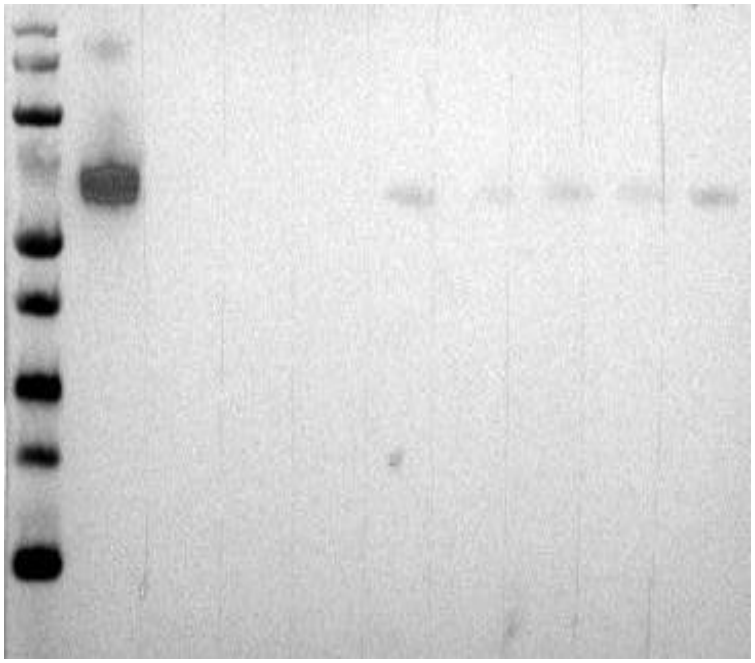
WB(Gold standard)
Concentration(HA protein) and dilution(sera) modification

HI
Evaluation on different type of RBCs
RBC adsorption

MN
Reducing virus concentration(100TCID50 VS 50TCID50)

Western-blot assay

Marker 1 2 3 4 5 6 7 8 9



1 anti H7N9 rabbit sera
2 anti-H3 human sera
3 anti-H5 human sera
4 anti-H9 human sera
5 Case1 MN=20
6 case2 MN=20
7 case3 MN=10
8 case4 MN=40
9 case5 MN=40

HA protein (Sino Biological, Inc.): 250ng
Dilution of human sera:1:1000

Hemagglutination-Inhibition (HAI) assay

Study populations	No. of serum samples	RDE treated-RBC adsorption	RBC adsorption-RDE treated
		No. of serum samples with HI titer of ≥20 **HRBC**	No. of serum samples with HI titer of ≥20 **HRBC**
General populations with natural infection of seasonal H1,H3,Bv,By	100	0	3
Poultry workers with H5N1 antibodies and H5N1 cases	15	2	2
Poultry workers with H9N2 antibodies	49	0	3
Total	164	2	8

Serum samples	RDE treated-RBC adsorption	RBC adsorption-RDE treated	Testing Virus
	HI titer	HI titer	
AH1 FS(NIC)	640	80/160	LOT#20130701

Hemagglutination-Inhibition (HAI) assay

- Comparison between HRBC and TRBC

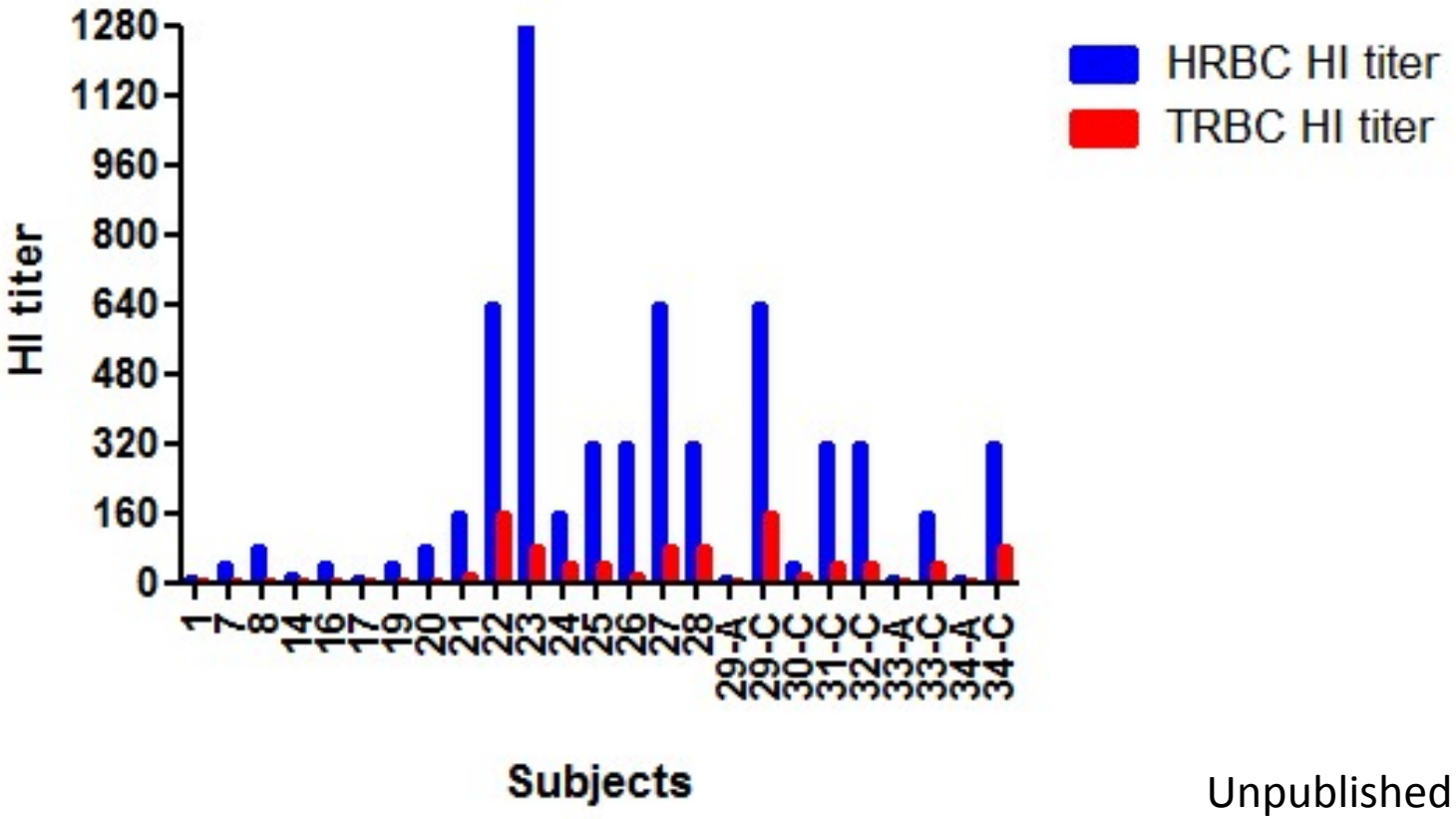
  Virus concentration: 4HAU

  RBC standardization

  HRBC: 1.5E+08/ml with 0.5%BSA(Roche)-adapted from USCDC

  TRBC: 4E+07/ml (WHO Manual 2011）

  HRBC is 2-16 fold higher than TRBC



Unpublished data

Sensitivity and specificity of TRBC HI and HRBC HI

Testing results	No.of study subjects(average age)	Sensitivity % (95%CI)*	Specificity % (95%CI)*
TRBC HI	43(50.2 years-old)		
HI≥40		58(43-73)	100
HI≥80		32(18-46)	100
HI≥160		11(2-20)	100
HRBC HI	47(47.5 years-old)		
HI≥40		90(81-99)	93(86-100)
HI≥80		75(63-87)	96(90-101)
HI≥160		65(51-79)	100

***: Using WB as "Gold standard"**

Unpublished data

Microneutralization (MN) assay

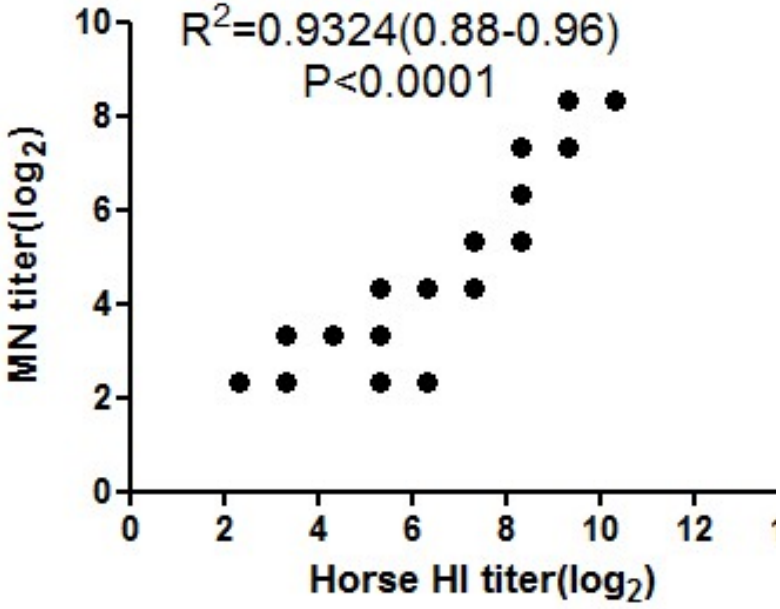
Sensitivity and specificity of MN with different concentration of virus

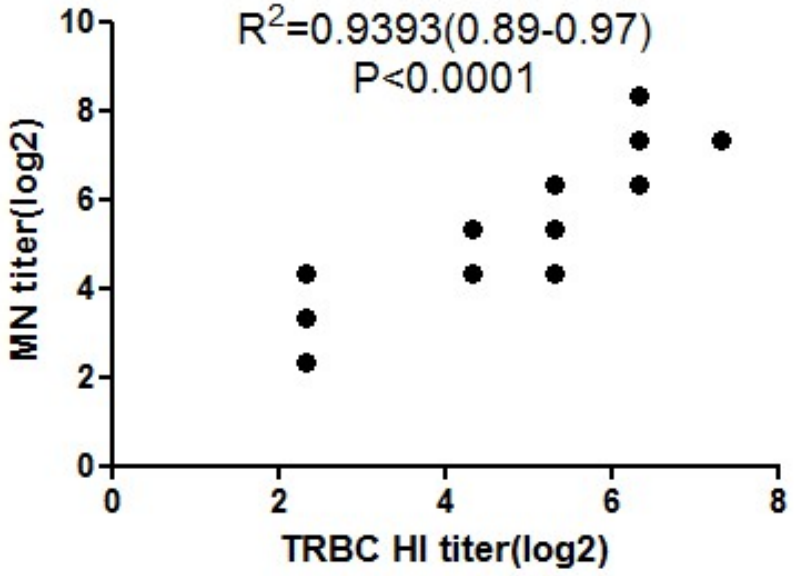
Testing results	No.of study subjects(average age)	Sensitivity % (95%CI)*	Specificity % (95%CI)*
MN(100TCID$_{50}$)	47(47.5 years-old)		
MN≥10		70(57-83)	100
MN≥20		65(51-79)	100
MN≥40		45(31-59)	100
MN(50TCID$_{50}$)	47(47.5 years-old)		
MN≥10		100	100
MN≥20		85(75-95)	100
MN≥40		55(41-69)	100

***: Using WB as "Gold standard"**

Unpublished data

Correlations between MN, HRBC HI and TRBC HI





Cut-off value for HI and MN assays

Validation the cut-off value by using 15 convalescence H7N9 human sera(14d after onsets) and 258 non-patient sera

Group	N	HI⩾20 % (95% CI)	HI⩾40 % (95% CI)	HI⩾80 % (95% CI)	HI⩾160 % (95% CI)
Sensitivity	15(ages<60yr)	87(70-104)	87(70-104)	80(60-100)	80(60-100)
Specificity	258(ages<60yr)	97(95-99)	98(96-100)	100	100
		MN⩾10 % (95% CI)	**MN⩾20 % (95% CI)**	**MN⩾40 % (95% CI)**	**MN⩾80 % (95% CI)**
Sensitivity	15(ages<60yr)	87(70-104)	87(70-104)	73(51-95)	53(28-78)
Specificity	258(ages<60yr)	100	100	100	100

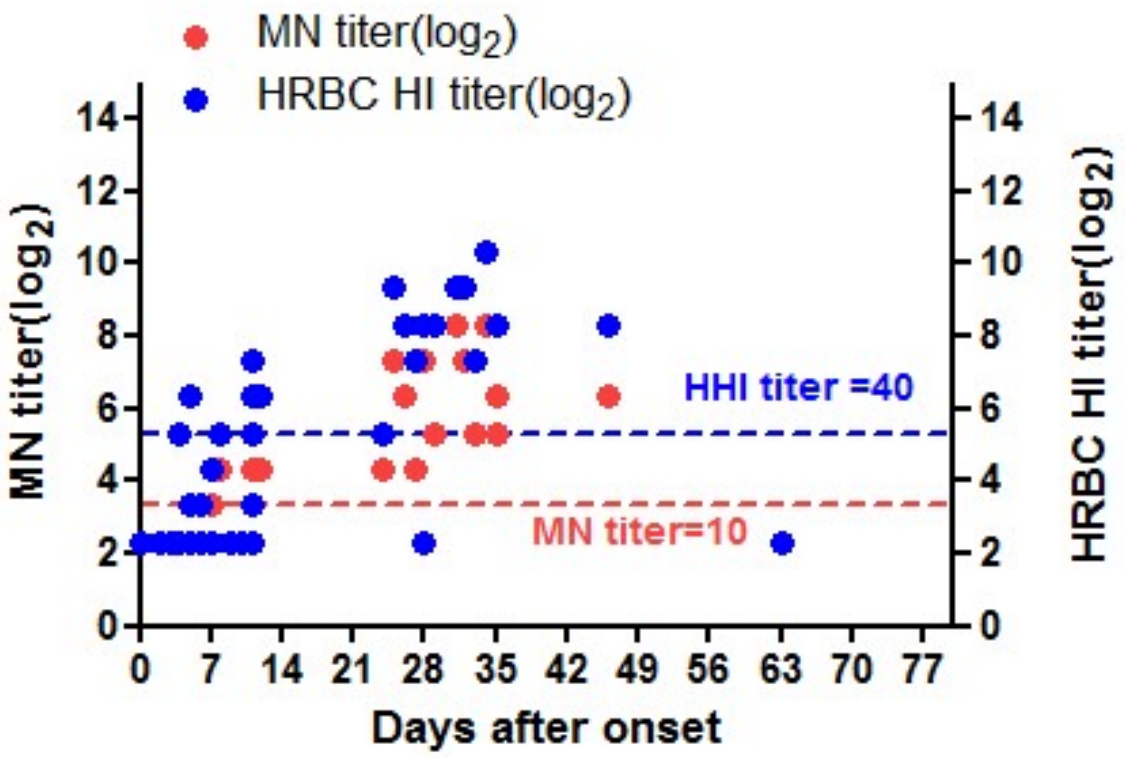
- HI

A horse HI titer of ≥ 40 shows a good sensitivity and specificity .
Sera with a horse HI titer of ≥80 show a lower sensitivity but a 100% specificity.

- MN

MN≥ 10 or 20 shows a good sensitivity and an excellent specificity.
MN ≥ 10 also shows a better sensitivity than MN ≥ 20 when using WB as a gold standard.

Spectrum of antibodies to H7N9 virus by along the days after onsets



Conclusion

• Although the H7N9 virus could bind to both avian-type (α2, 3-linked sialic acid) and human-type (α2, 6-linked sialic acid) receptors, horse RBCs increase the sensitivity of HI in detecting antibody response to H7N9 virus comparing to turkey RBCs.

• Reducing the concentration of virus increase the sensitivity of MN.

• Single sera with a horse HI titer of ≥80 or a MN titer of ≥10 can be confirmed to be sera positive for H7N9 virus as an excellent specificity were observed.

• A horse HI titer of ≥ 40 could be a cut-off value for serology survey but need to be confirmed by MN.

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