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	HI,MN,WB	HI,MN,WB	HI,MN	HI,MN	HI,MN

- Main factors we evaluated

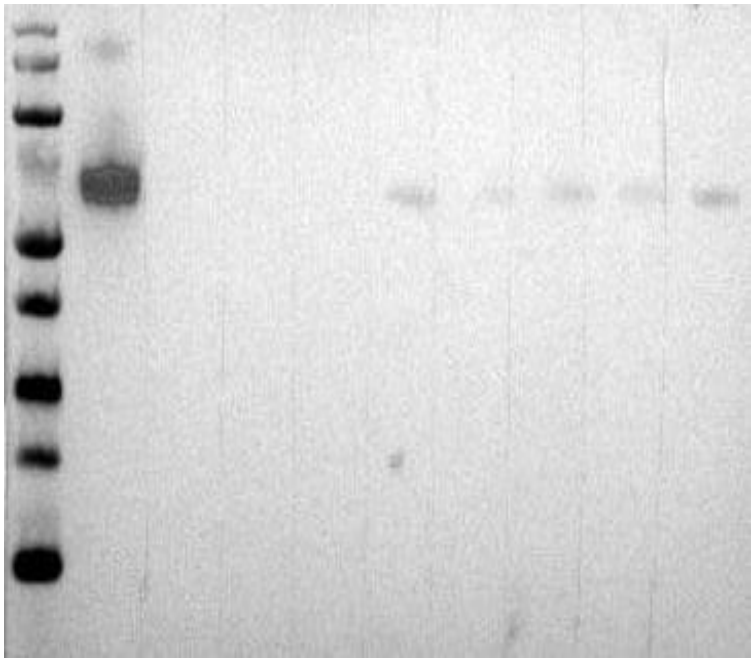
WB(Gold standard)
Concentration(HA protein) and dilution(sera) modification

HI
Evaluation on different type of RBCs
RBC adsorption

MN
Reducing virus concentration(100TCID50 VS 50TCID50)
Cells numbers
BSA concentration

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

Plasma causes trouble

Plasma(heparin)

No.of samples	Day after onsets	HRBC HI Titer	mMN titer	Day after onsets	HRBC HI Titer	mMN titer	Seroconvertion	
							HI	mMN
1	6	320	5	28	160	5	3(30%)	7(70%)
2	12	160	5	14	20	5		
3	5	320	5	19	160	20		
4	8	160	5	17	160	10		
5	10	320	5	31	640	80		
6	6	160	5	13	160	40		
7	9	160	5	18	320	20		
8	7	10	5	13	320	640		
9	8	20	5	22	320	20		
10	9	40	5	28	1280	80		

Sera

No.of samples	Day after onsets	HRBC HI Titer	mMN titer	Day after onsets	HRBC HI Titer	MN titer	Seroconvertion	
							HI	mMN
1	5	10	5	25	640	160	6（75%）	6(75%)
2	0	5	5	24	40	20		
3	5	10	5	35	320	80		
4	7	5	5	46	320	80		
5	5	10	5	27	160	20		
6	6	10	5	26	320	80		
7	0	5	5	63	5	5		
8	5	5	5	28	5	5		

Hemagglutination-Inhibition (HAI) assay

- Comparison between HRBC and TRBC

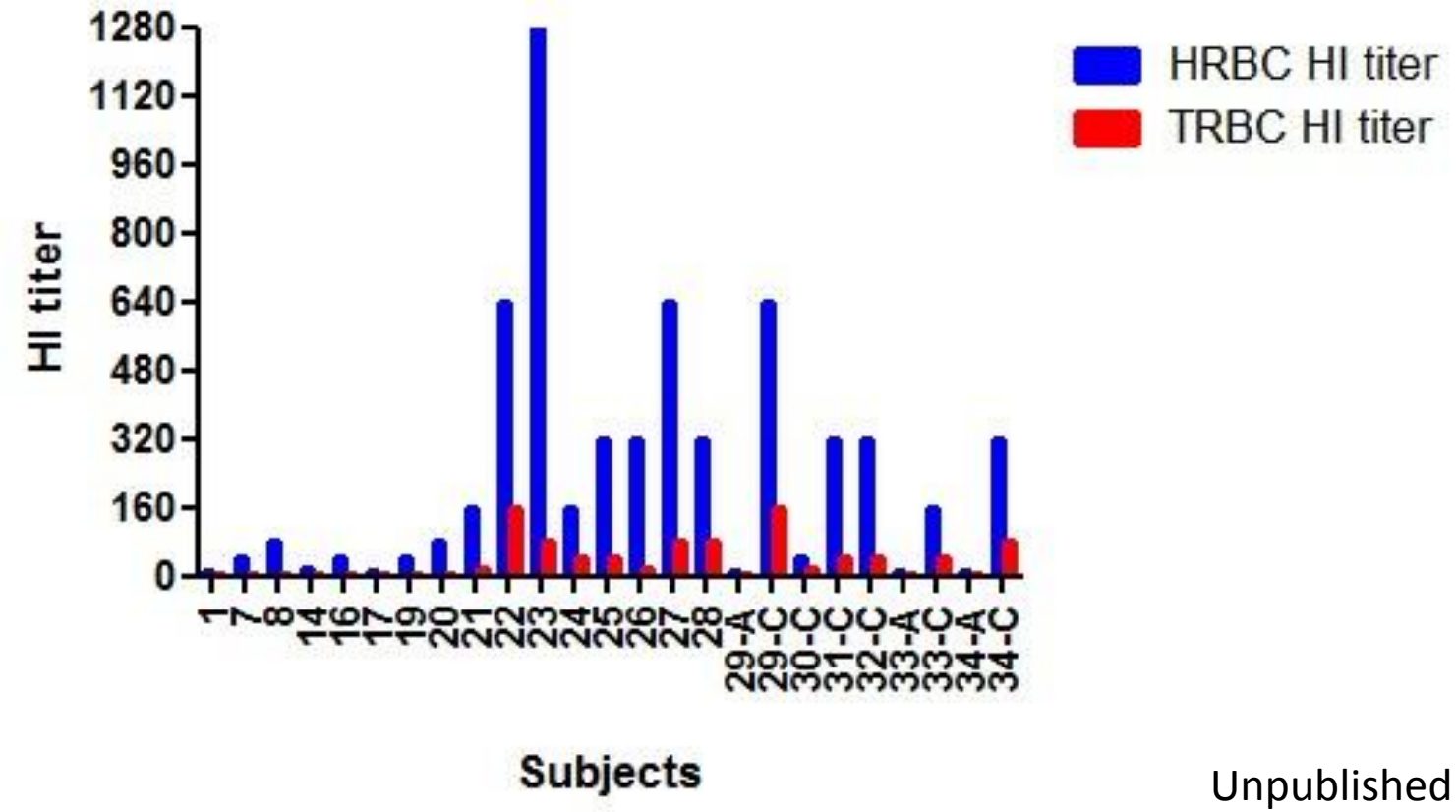
  Virus concentration: 4HAU

  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⩾20		74(61-87)	100
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⩾20		95(89-101)	93(86-100)
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 "confirmatory method"**

Unpublished data

Cut-off value for HI and MN assays

Validation the cut-off value by using 15 convalescence H7N9 human sera 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

Microneutralization (MN) assay

MN		mMN
100TCID50	→	50TCID50
1% of BSA	→	0.5% of BSA
1.5 ×10^5/ml	→	1×10^5/ml

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	47(47.5 years-old)		
⩾10		70(57-83)	100
⩾20		65(51-79)	100
⩾40		45(31-59)	100
mMN	47(47.5 years-old)		
⩾10		100	100
⩾20		85(75-95)	100
⩾40		55(41-69)	100

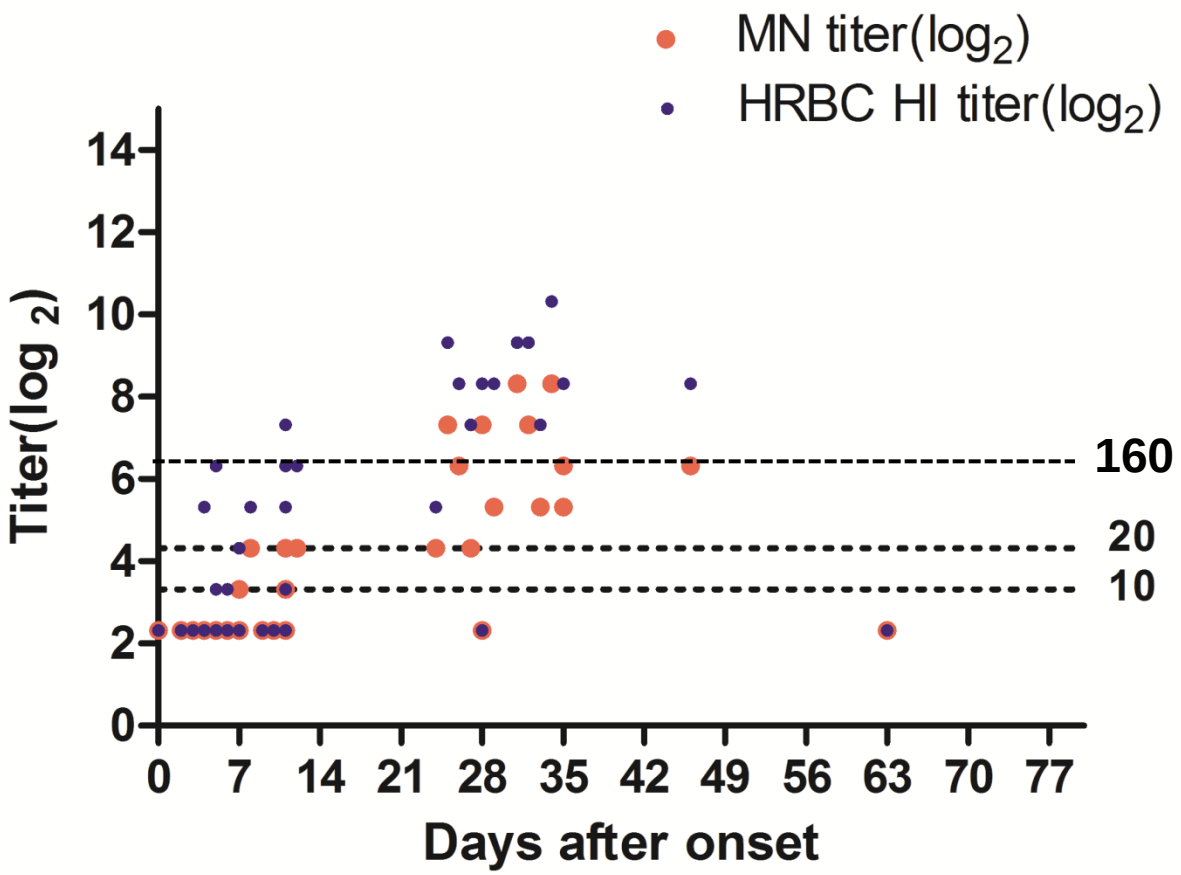
***: Using WB as "confirmatory method"**

Cut-off value for MN assays

Validation the cut-off value by using 15 convalescence H7N9 human sera and 258 non-patient sera

Group	N	mMN≥10 % (95% CI)	mMN≥20 % (95% CI)	mMN≥40 % (95% CI)	mMN≥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

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.

- A reversal sera treatment order is recommended
- Plasma is not proper for case diagnosis and serology survey

• Modified MN increase the sensitivity and has a better consisitence with WB.

• Cases diagnosis:

- 4-fold rise titer in H7N9 HI or mMN
- Convalescent HI titer: ≥160 or mMN titer ≥10
- Sera with a horse HI titer of 20-80 should be confirmed by mMN or WB.

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