Criteria for Seropositivity: Standardization for Serologic Confirmation of Avian Influenza A (H5N1) Virus Infection

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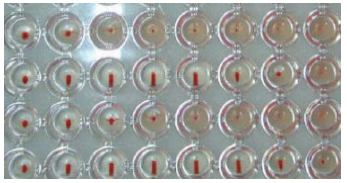
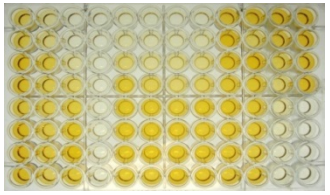
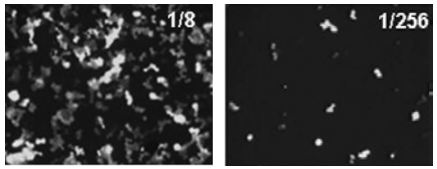
Serologic Assays for Detection of H5N1 Virus Antibodies

- **Hemagglutination-Inhibition (HI)**
  - **Horse or turkey/chicken RBC**

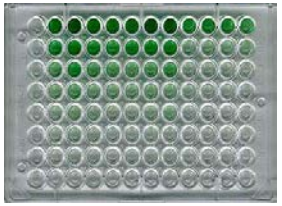


- **Neutralization assays**
  - **Microneutralization (MN) or Virus Neutralization (VN)**
  - **Pseudotype viral particle neutralization (PN)**

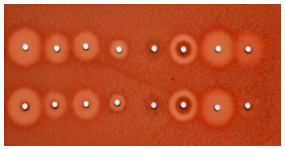




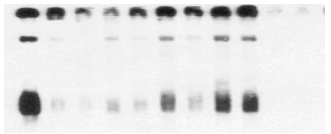
- **ELISA using rHA**



- **Single radial hemolysis (SRH) Assay**

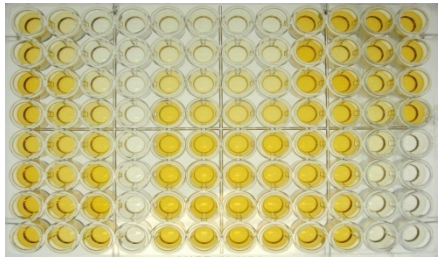


- **Western blot**

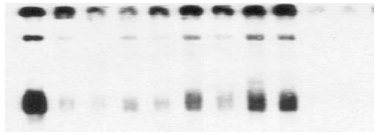


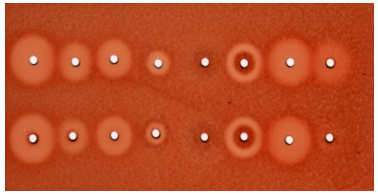
Criteria for Seropositive Results for Serologic Tests used in 1997 H5N1 Investigations

- **Microneutralization assay**
  - **Titer of ≥1:80* in 2 independent assay**
  - **Seroconversion (≥4-fold rise) between acute/convalescent paired sera**



- **Confirmatory assay to enhance specificity**
  - **Western Blot (CDC) with H5 rHA**
  - **SRH (Hong Kong Dept. of Health Lab)**





Using MN starting dilution = 1:20 convention

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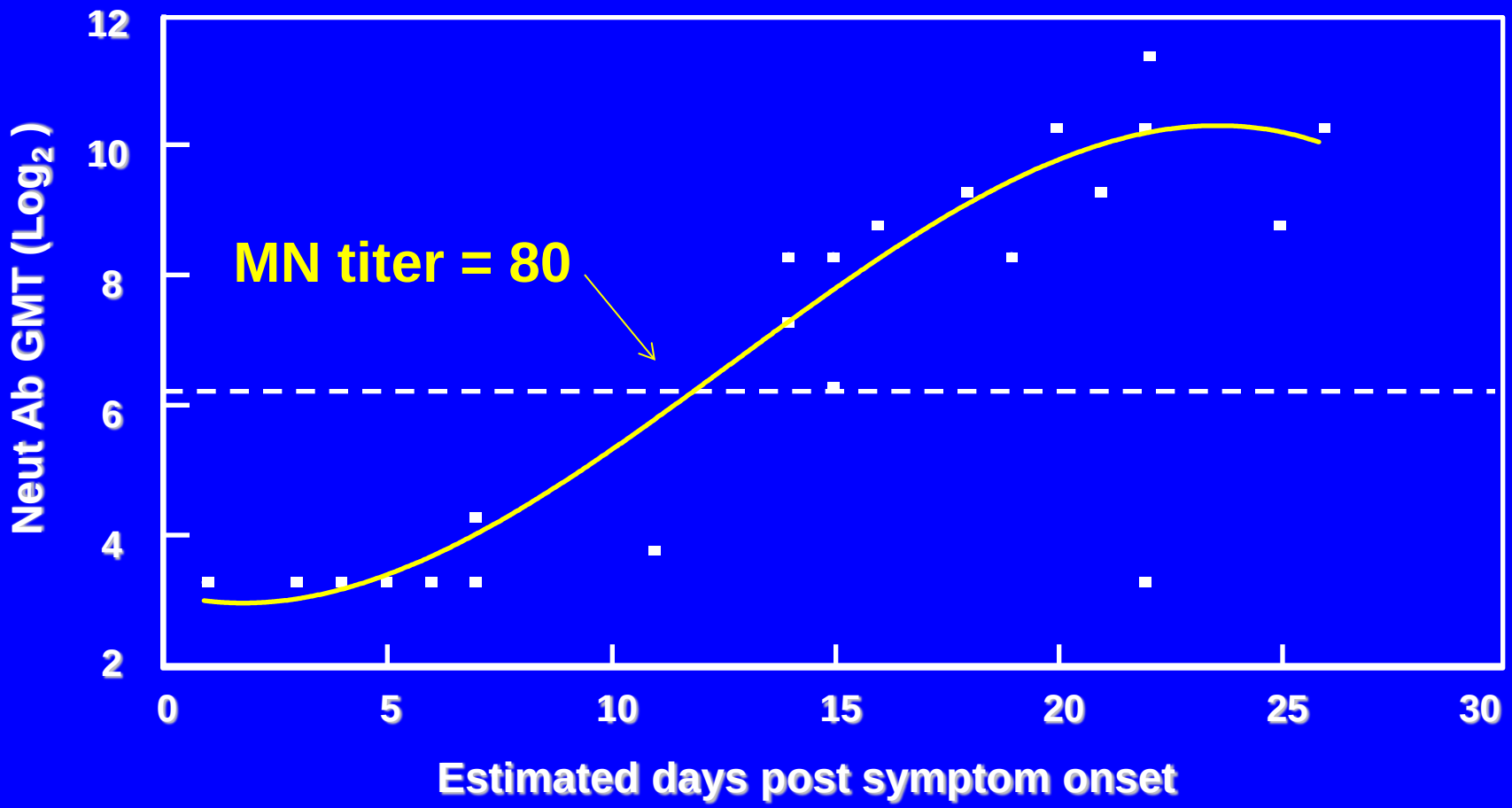
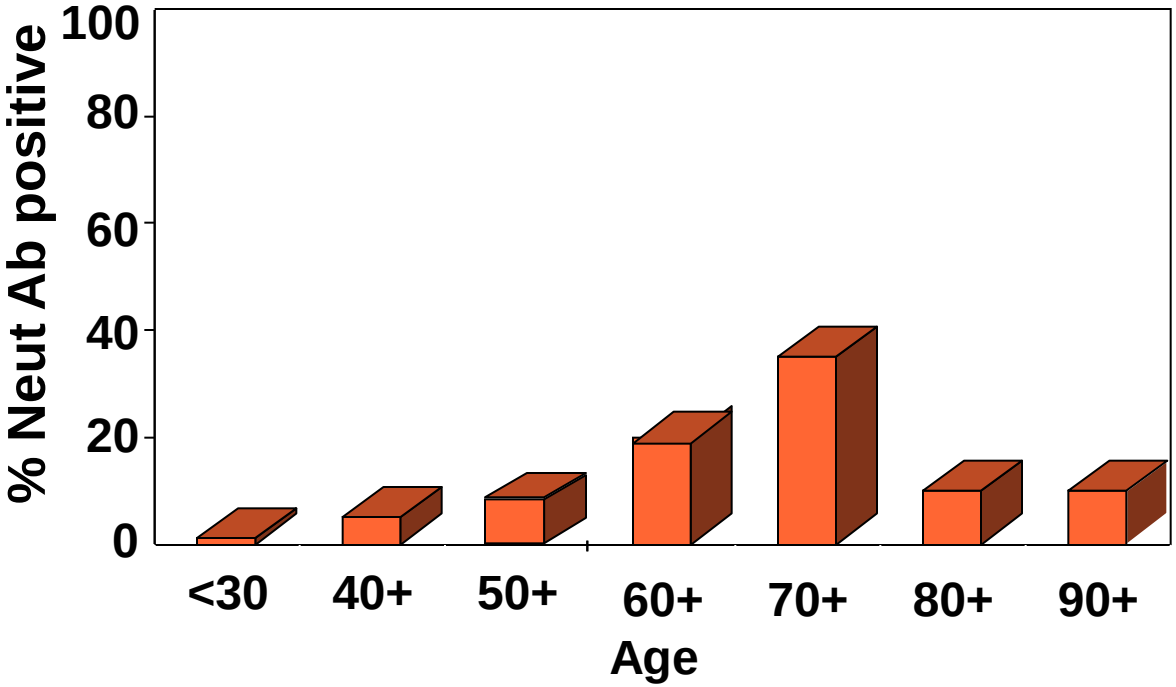
Kinetics of Antibody Response in H5N1 Virus-infected Humans Determined by Microneutralization (MN) Assay



Age and Sensitivity, Specificity of H5 Serologic Tests

- Children aged <15 yrs MN+WB tests
  - Sensitivity: 88% (n=8); Specificity: 100% (n=24)
- Adults aged 18-59 yrs MN+WB tests
  - Sensitivity: 80% (n=8); Specificity: 96% (n=85)
- Frequency of Seropositives for H5 MN antibody in non-exposed baseline samples increased with age



WHO Criteria for Serodiagnosis of Human H5N1 Virus Infection (2006)

- **Person meeting clinical definition of H5N1 case AND:**
  - **Serological confirmation with appropriately timed paired sera:**
    - ***≥4-fold rise in H5N1 neutralization antibody titer***
    - ***Convalescent neutralizing antibody titer ≥1:80 (1:20 starting dilution)***
  - **Serological criteria for single serum collected ≥14 days after symptom onset**
    - ***H5N1 neutralization antibody titer ≥1:80***
    - ***Positive result using a different assay***
      - ***Horse RBC HI titer of ≥1:160 or greater or positive H5-specific western blot result***

***http://www.who.int/cdr/disease/avian_influenza/guidelines/case_definition2006**

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H5N1 Seropositive Criteria used in Selected Studies in Persons with Poultry Exposure

Population	Country/ Year	Criteria	Confirm Assay?	Reported seroprevalence
Villagers	China, 2004	cRBC HI≥20	MN	3%
Villagers	Turkey, 2006	ELISA + cRBC HI≥20	MN ≥10	0%
Villagers	Thailand, 2008	MN≥10	No	5.6%
Villagers	Cambodia, 2006	MN≥80	WB	1%
Villagers	Cambodia, 2007	PN≥20 (to screen)	MN ≥80 hRBC HI ≥160	2.6%
Poultry workers	China, 2006	tRBC HI =320	MN = 640	0.9%
Poultry workers	China, 2007-08	HI- no criteria	MN - no criteria	0.8%
Poultry workers	China, 2010	hRBC HI≥160	No	2.6%

Decline in Serum Antibody Response to H5N1 Virus over Time in Asymptomatic Persons*

(Vong et al., JID 2009)

Subject	MN titer at estimated time post exposure		Fold drop in titer
	1-2 months	10-11 months	
A	80	20	4
B	320	40	8
C	1280	320	4
D	640	20	32
E	640	160	4
F	640	80	8
G	160	20	8

** Using serological data from 11 severely ill H5N1 (cl 1.1) patients, a fractional polynomial regression model predicted rising titers of ≥80 2 weeks post onset, peak titer achievement at 5-6 weeks and a titer >80 beyond 2 years (Buchy et al., 2010)*

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Factors to Consider for Development of H5N1 Virus Antibody Seropositivity Criteria - I

- **Timing of sera collection in relationship to H5N1 virus exposure or illness onset**
  - **>14 days post symptom onset and >> 14 days post exposure**
  - **Before waning of antibody response (6-9 months?)**
- **Use of relevant clade/virus antigen in assays**
- **Sensitivity of assay**
  - **Use criteria with high sensitivity to detect antibody in RT-PCR confirmed cases (mild to severe)**
    - **Cutoff of ≥1:10 ≥1:20 may be too low**
- **Specificity of assay**
  - **Use criteria that results in low/no detection of positives in age-matched unexposed persons**
    - **Adsorption of cross-reactive antibodies to seasonal influenza viruses (infection or vaccination)**

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Factors to Consider for Development of H5N1 Virus Antibody Seropositivity Criteria - II

- Do we need different criteria for different situations?
  - Persons with symptomatic illness and serological confirmation of H5N1 virus infection versus
    - Patients with more severe disease generally had higher antibody titers than those with clinically mild illness, regardless of age
  - Seroprevalence studies to identify asymptomatic or clinically mild illness
- Is a confirmatory assay still needed or recommended?
- If so:
  - hRBC HI ≥160 may be too stringent?
  - hRBC HI may be insensitive for some H5N1 clades
  - Western blot lacks specificity

- Should the H5N1 seropositive criteria be applied to other avian influenza A virus subtypes (H7, H9)?

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