

# Evaluation of anti-neuraminidase antibodies in protection against influenza A H5N1 virus infections

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# Generation of NA-specific hyperimmune sera in rabbits

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## Objective

To determine whether N1 NA-specific antibodies can provide some protective immunity against avian H5N1 virus.

## Approach

- Rabbits were immunized with purified Baculovirus-expressed recombinant NA (rNA) proteins (4 X 100 µg + Titermax adjuvant)
  - A/Beijing/262/95 (Bj/262) human H1N1 virus
  - A/Hong Kong/483/97 (HK/483) HPAI H5N1 virus
  - NA of HK/483 virus shares ~80% amino acid identity with NA of human Bj/262 H1N1 virus.
- Rabbit was immunized with multiple doses of rHA as positive control
  - A/Vietnam/1203/04 (VN/1203) HPAI H5N1 virus

# Evaluation of NA-specific hyperimmune sera in reactivity against H5N1 virus *in vitro* and *in vivo*

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*In vitro*, cross-reactive antibody responses against H5N1 virus were evaluated by multiple assays

- Neuraminidase Inhibition assay (NI) – Fetuin substrate
- Plaque size reduction assay (PSR)
- Replication inhibition assay (RI) – MDCK, 96 well plate format
- NA-specific IgG ELISA
- Single radial hemolysis (SRH)

*In vivo*, cross-protection against HPAI H5N1 virus were evaluated in BALB/c mice.



# Anti-NA antibody titers to homologous human H1N1 or avian H5N1 virus

Rabbit antiserum against	Reciprocal antibody titers			
	NI	PSR	RI	ELISA IgG
Bj/262 rNA	320	5120	160	102400
HK/483 rNA	320	1280	80	102400
Normal serum (NS)	≤ 10	≤ 50	≤ 10	≤ 100

## Cross-reactive anti-NA antibody responses between human H1N1 and avian H5N1 viruses

Rabbit antiserum against	Reciprocal antibody titers					
	NI		RI		ELISA IgG	
	Bj/262	HK/483	Bj/262	HK/483	Bj/262	HK/483
<b>Bj/262 rNA</b>	<b>320</b>	<b><u>&lt; 10</u></b>	<b>160</b>	<b><u>20</u></b>	<b>102400</b>	<b><u>25600</u></b>
<b>HK/483 rNA</b>	<b><u>&lt; 10</u></b>	<b>320</b>	<b><u>10</u></b>	<b>80</b>	<b><u>12800</u></b>	<b>102400</b>

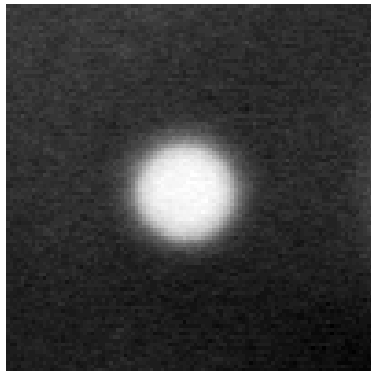
# Measurement of anti-NA antibodies by single radial hemolysis with HK/483 coated red blood cells

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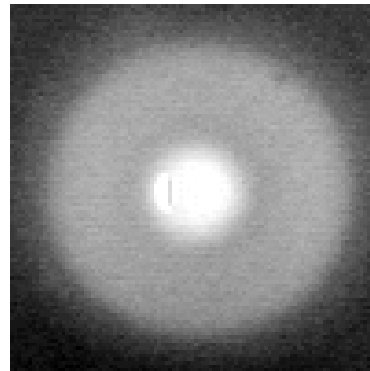
## Antisera

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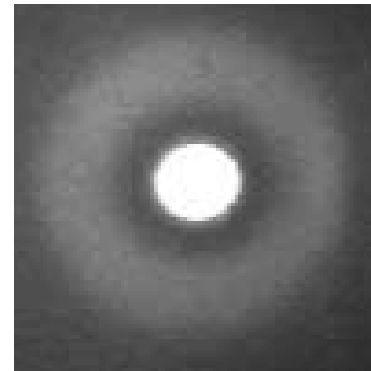
NS



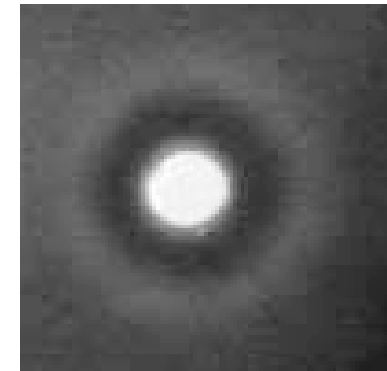
Positive control  
HK/483 virus



Rabbit  
HK/483 rNA



























Rabbit  
Bj/262 rNA)



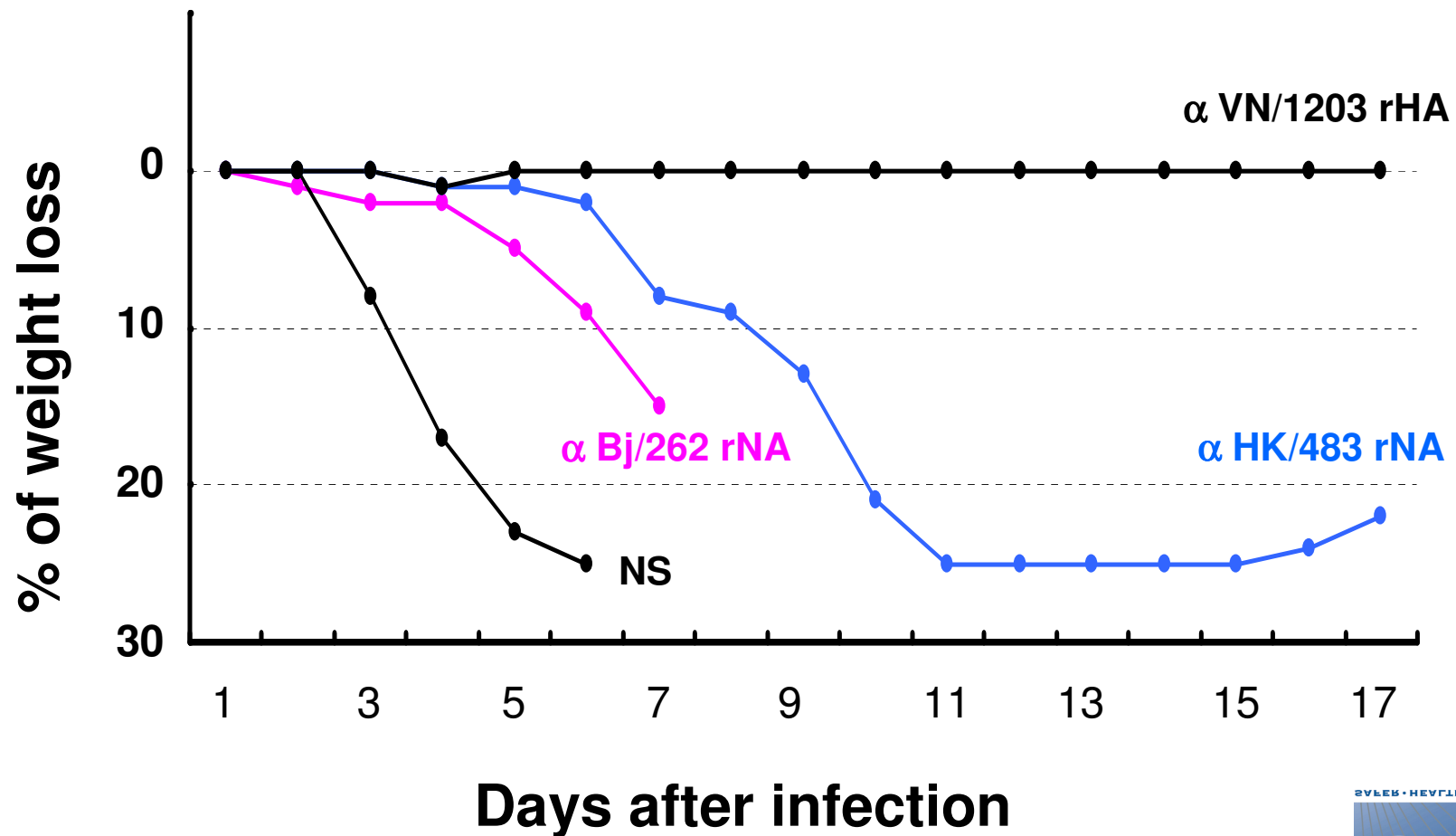
# In vivo protective effects of anti-NA antibodies against lethal HK/483 H5N1 virus infections

## Challenge with HK/483 virus

Rabbit serum Dose (ml)*	50 LD <sub>50</sub>			5 LD <sub>50</sub>		
	VN/1203 rHA	HK/483 rNA	Bj/262 rNA	VN/1203 rHA	HK/483 rNA	Bj/262 rNA
0.5						
0.1						
0.02						
0						

\* Mice received a single intraperitoneal injection with indicated doses of hyperimmune serum 24 hours before challenge.

# Weight loss in mice challenged with 5 LD<sub>50</sub> of HK/483 H5N1 virus



**In vivo protective efficacy against 3 LD<sub>50</sub> HK/483 virus using passive immunization with three doses of anti-NA serum in mice.**

Rabbit antiserum	Dose (ml)	Virus titers (day 6) (Log <sub>10</sub> EID <sub>50</sub> /ml)		% Maximum weight loss	No. survived /total no.(%)	Mean day to death
		Lung	Brain			
VN/1203 rHA	0.2	1.0 ± 0.3*	1.0 ± 0.4*	2*	7 / 7 (100)	>21*
HK/483 rNA	1, 0.5, 0.5	3.8 ± 1.4*	2.5 ± 0.7*	12*	6 / 7 (86)	11*
Bj/262 rNA	1, 0.5, 0.5	6.4 ± 1.8	4.8 ± 0.2	15*	0 / 7 (0)	8
NS	1, 0.5, 0.5	7.3 ± 1.5	4.6 ± 0.5	22	0 / 7 (0)	8

Passive immunization: - 24 hours (1 ml); + 2 days (0.5 ml); + 5 days (0.5 ml)

\**P* < 0.05 compared to NS mice.



# Summary

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- Using multiple assays, Bj/262 NA-specific antibodies showed limited cross-reactivity to H5N1 virus *in vitro*.
- Protection from severe disease and death due to HPAI H5N1 infection can be achieved by passive immunization with large doses of the homologous anti-NA antibodies in mice.
- Passive immunization with seasonal Bj/262 H1N1 anti-NA antibodies
  - ✓ only slightly reduced disease in early stages of H5N1 infection.
  - ✗ ineffective at reducing viral replications in lungs and brains.
  - ✗ failed to provide cross-protection from death.

These results indicate that influenza A H1N1 Bj/262 NA-specific antibodies afforded very limited cross-protective immunity against HPAI HK/483 H5N1 virus.

# Next steps for further NA research

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- **Develop new and standardized assays to evaluate anti-NA antibodies to seasonal and avian strains with pandemic potential in humans.**
- **Continue to use animal models (mice + ferrets) to better understand the extent of cross-protection afforded by anti-NA antibodies and evaluate NA-specific vaccine strategies.**

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