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# Approaches for the Detection of Neuraminidase (NA)-Specific Antibodies in Sera from Humans and Animals

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**Approaches investigated:**

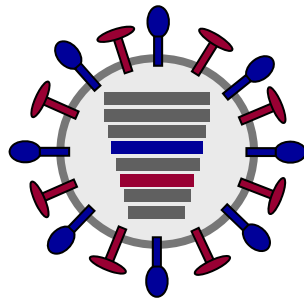
- Use of specifically designed recombinant virus
- Chemical assay (WHO manual)
- Use of vaccine monovalents as target protein in Western blot

# MNT with Recombinant Influenza Viruses

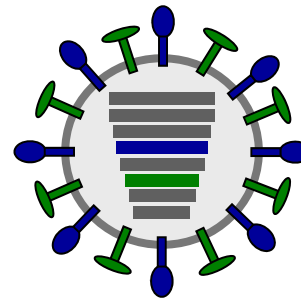


Recombinant Influenza viruses generated by reverse genetics to decipher antibody specificities in sera from vaccinated (pandH1N1) ferrets by MNT

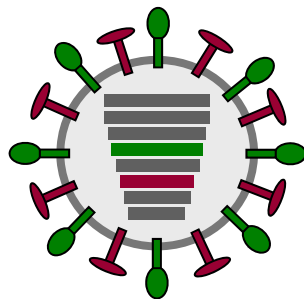
⇒ Schematic representation of recombinant viruses with distinct surface antigen configurations and resulting intended AB reactivities for application in MNT



**H1N1** (as in vaccine)  
→ detect AB to H1-HA  
and N1-NA



**H1N2**  
→ detect AB to H1-HA



**H9N1**  
→ detect AB to N1-NA



**H9N2**  
→ no detection of AB  
(negative control)

⇒ Acceptor viruses for reverse genetics: A/FPV/Ro/34 (H7N1) (att. Mutant) and A/PR8/34 (H1N1)

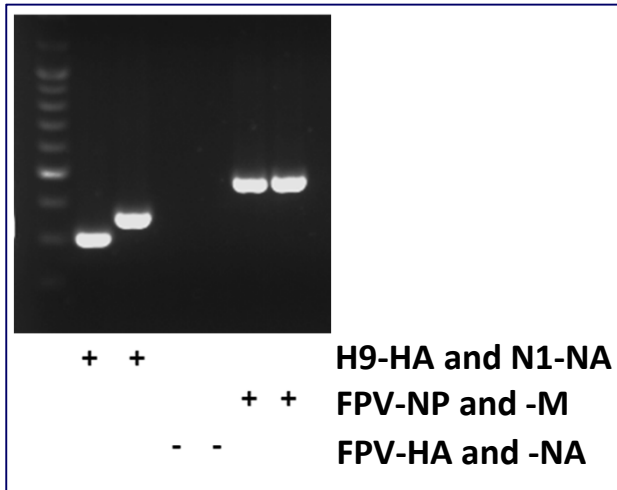
# Characterisation of Recombinant Influenza Viruses



Example: H9N1-FPV

## Genotypic: rec. H9N1

RT-PCR amplification of segments  
by use of specific primers

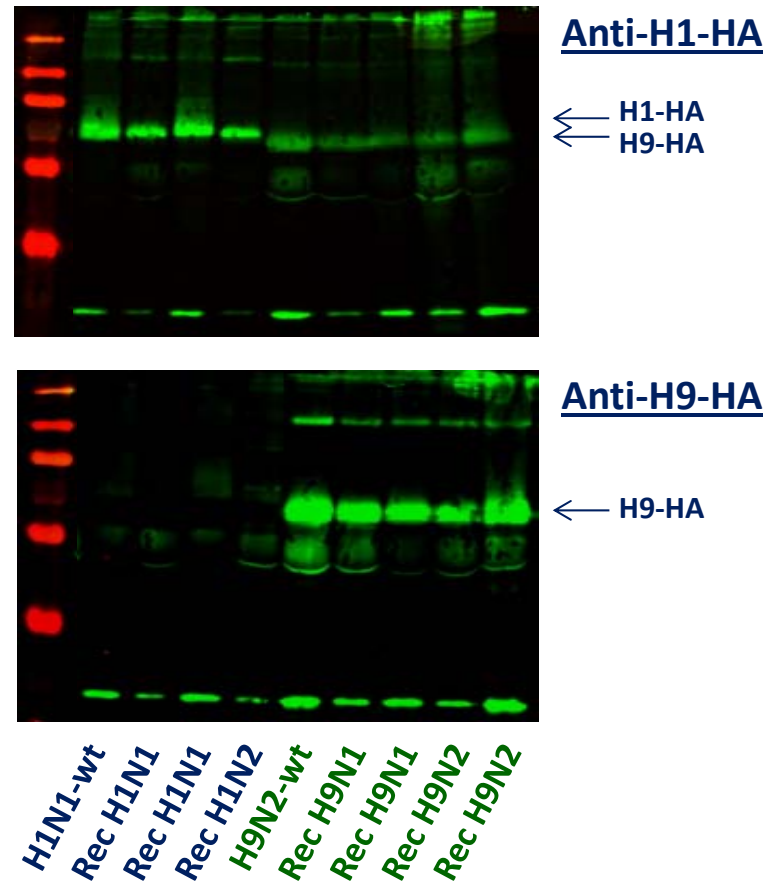


## Confirmation of genotype

- Surface AG-genes H9N1
- Internal genes of FPV (acceptor)

## Phenotypic: Hemagglutinin-Identity

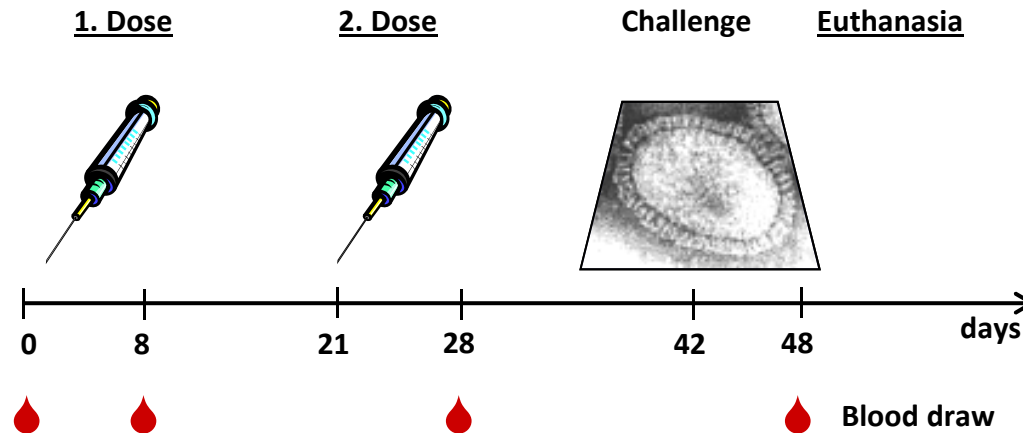
Western blot using HA-subtype  
specific antibodies



# Ferret Vaccination Study



## Overall design of study:



## Specific features:

- Two doses of pandH1N1-specific vaccine
- Blood draw for serological analysis (pre-vacc, and 1 week after 2<sup>nd</sup> dose)

- Challenge with recombinant influenza viruses
  - H1N1/FPV
  - H1N2/FPV
  - H9N1/FPV

# Serological Data from Ferret Study – Day 0



## HI- and MNT titer of all animals at day 0 (pre-vacc):

MNT by classical WHO protocol (1 day)

Animals for vaccination

Control Animals

Ferret Nr.	H1N2_FPV		H9N1_FPV		H1N1v_FPV		pdmH1N1v		Test virus
	HAI	MNT	HAI	MNT	HAI	MNT	HAI	MNT	
165 Vaccine	20	5	40	5	20	5	5	5	
166 Vaccine	20	5	40	5	40	5	5	5	
167 Vaccine	28	-	14	5	10	5	5	5	
168 Vaccine	20	5	40	5	14	5	5	5	
169 Vaccine	5	5	40	5	20	5	5	5	
170 Vaccine	5	5	5	5	5	5	5	5	
171 Vaccine	20	5	40	5	20	5	5	5	
172 Vaccine	12	-	14	5	-	5	5	5	
173 Vaccine	-	5	-	5	40	5	-	5	
177 Vaccine	7	5	10	5	20	5	5	5	
178 Vaccine	10	5	20	5	20	5	5	5	
179 Vaccine	-	-	-	-	-	-	-	-	
180 Vaccine	14	5	24	5	20	5	5	5	
182 Vaccine	5	5	14	5	20	5	5	5	
183 Vaccine	7	5	28	5	57	5	5	5	
140 (-)	7	5	40	10	5	5	5	5	
162 (-)	5	5	6	5	5	5	5	5	
163 (-)	5	5	5	5	5	5	5	5	
164 (-)	5	5	24	5	10	5	5	5	
174 (-)	5	5	24	5	5	5	5	5	
175 (-)	5	5	5	5	5	5	5	5	
176 (-)	5	5	20	7	5	5	5	5	
181 (-)	10	5	24	5	28	5	5	5	
197 (-)	7	5	28	5	5	5	5	14	

# Serological Data from Ferret Study – Day 28



## HI- and MNT titer of vaccinated animals 1 week after 2<sup>nd</sup> dose:

MNT by classical WHO protocol (1 day)

Ferret Nr.	H1N2_FPV		H9N1_FPV		H1N1v_FPV		pdmH1N1v		Test virus
	HAI	MNT	HAI	MNT	HAI	MNT	HAI	MNT	
165 Vaccine	2560	5120	24	5	2560	5120	10240	≥20480	
166 Vaccine	1280	1280	20	10	1280	1280	3620	5120	
167 Vaccine	640	640	20	5	640	640	2560	3620	
168 Vaccine	1280	1280	40	5	1280	1280	7241	3620	
169 Vaccine	226	320	20	5	160	320	1280	1280	
170 Vaccine	3620	5120	5	5	2560	5120	10240	14482	
171 Vaccine	1280	1280	20	5	453	640	2560	2560	
172 Vaccine	2560	2560	20	5	1280	1280	10240	5120	
173 Vaccine	1280	-	5	5	1280	1280	3620	7241	
177 Vaccine	1280	640	5	5	640	640	1280	2560	
178 Vaccine	640	1280	40	5	640	905	5120	5120	
179 Vaccine	1810	2560	5	5	1810	2560	≥20480	14482	
180 Vaccine	320	640	5	5	320	320	1280	2560	
182 Vaccine	640	640	5	5	640	1280	5120	2560	
183 Vaccine	-	-	-	-	-	-	-	-	

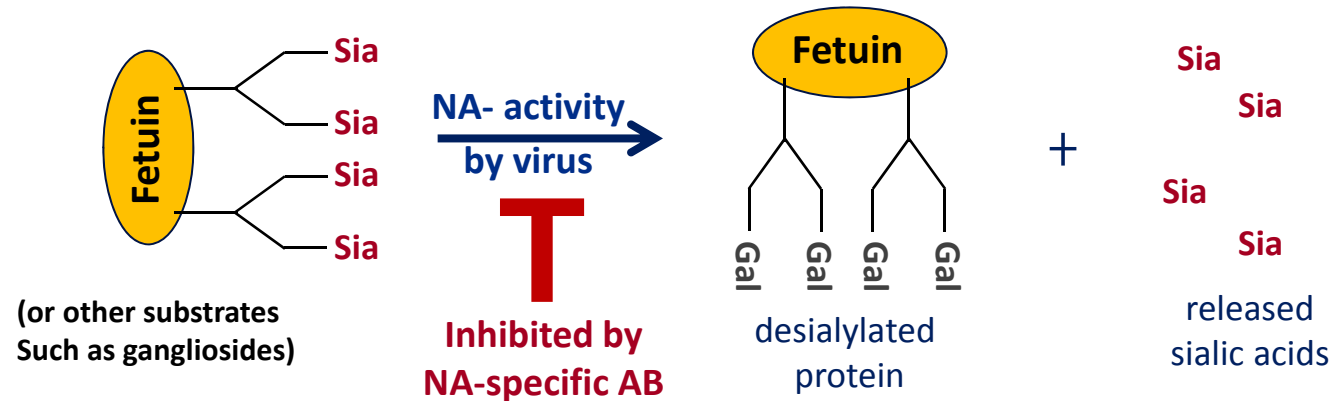


Powerful immune response detectable against H1-HA in HI and MNT

No response at all detectable against N1-NA ( → MNT with H9N1)

⇒ Reactivity against H1N1 most probably solely due to H1-HA component

# Alternative Assays for NA-specific Antibodies



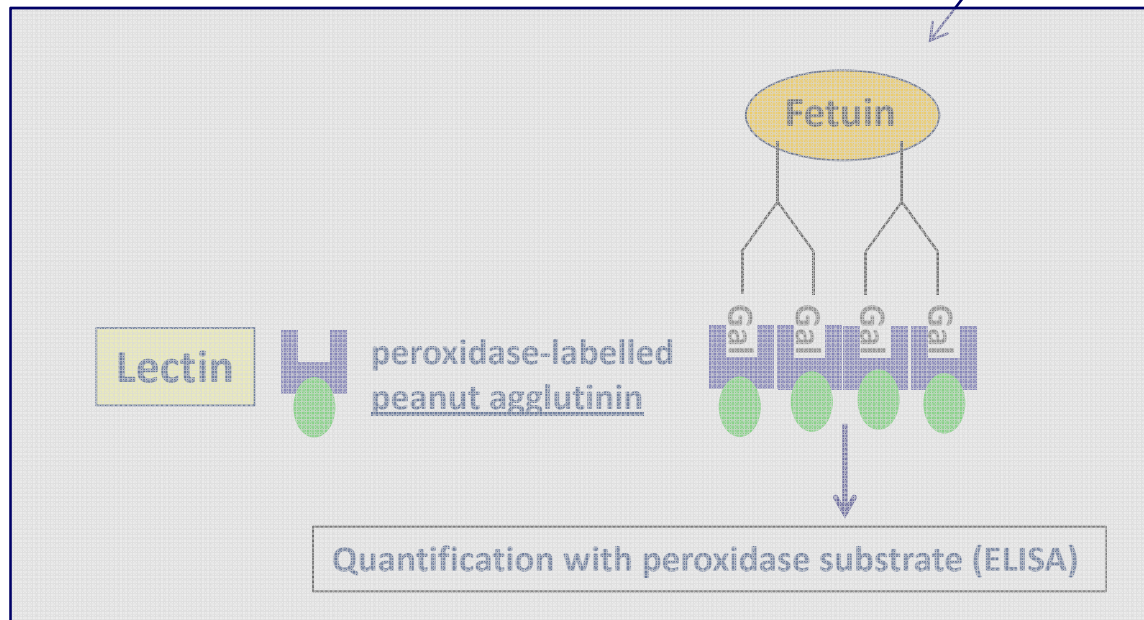
(or other substrates  
Such as gangliosides)

## Chemical conversion (WHO manual)

conversion to formol-pyruvate by periodate oxidation  
formation of **chromophore** by TBA  
extraction of chromophore

**spectrophotometry**

(also: Sandbulte et al., 2009)

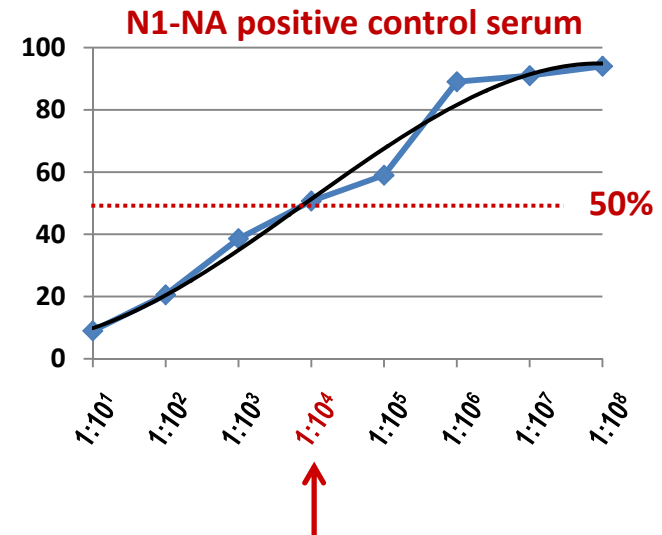
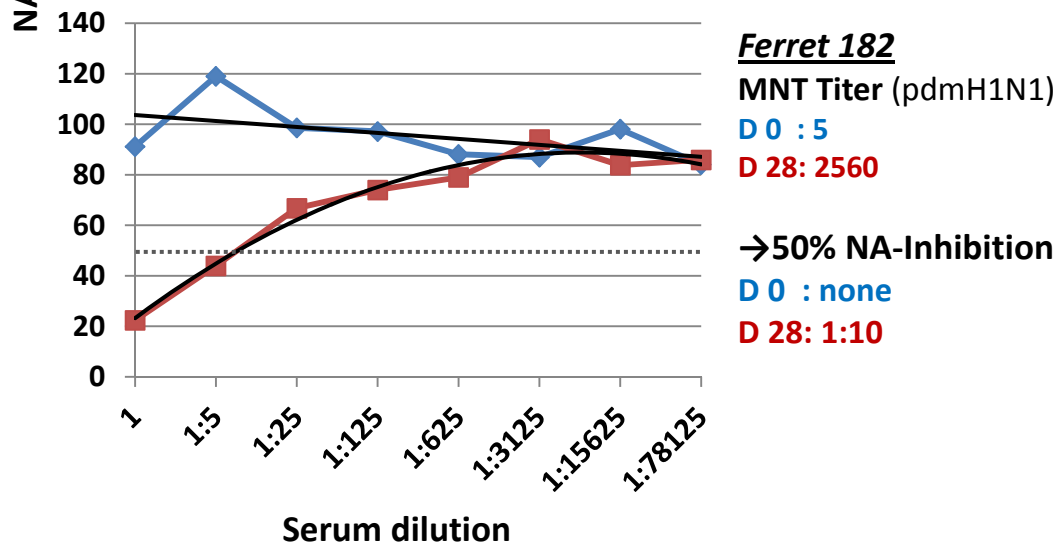
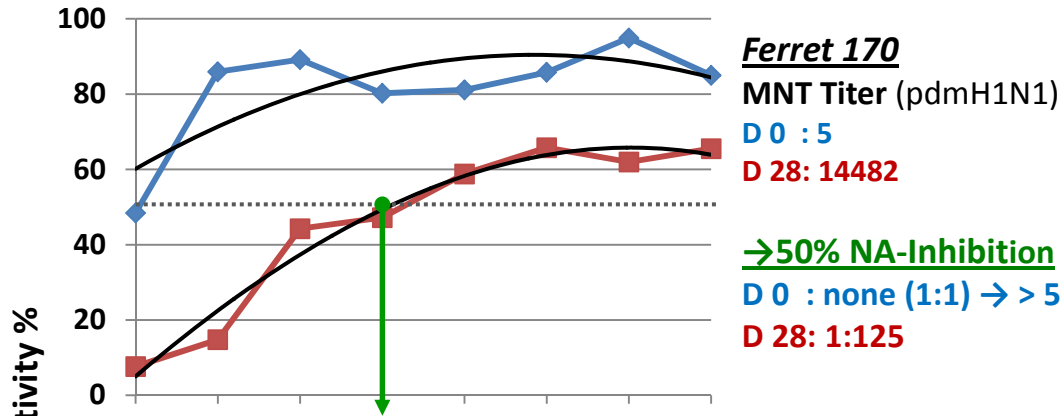




# NA-AB Titer in Ferrets by “WHO”-Assay



## Examples of original data



# Comparison of measured anti-NA titers



**Summary of data:  
MNT vs anti-NA**

Ferret Sera		
	MNT	anti-NA
D0	5	none
D28	14480	125
D0	5	none
D28	2560	10
D0	5	none
D28	20480	80000

Human Sera		
	MNT	anti-NA
D0	5	none
D42	10240	5625
D0	5	none
D42	2480	5000
D0	5	none
D42	20480	2500
D0	5	125
D42	3620	5000
D0	5	5625
D42	1280	>80000

## Conclusions from ferret study (H1N1):

Obviously, NA-specific antibodies are not easily captured in standard MNT – even when using specifically designed influenza viruses

But: anti-NA AB clearly detectable in chemical assay

However: No correlation between MNT results and anti-NA titers (but not too surprising)

## ⇒ Potential improvements (ongoing investigations):

- **Longer incubation period after infection** (to capture more NA-AB-sensitive replication cycles)
  - standard: 22-25 hrs (about 3 influenza replication cycles)
  - extend to 3 – 5 days
- **Lower initial starting infectious dose** to allow for more NA-AB-sensitive replication cycles

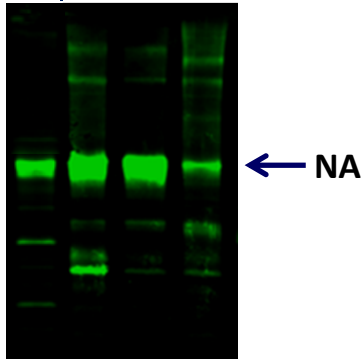
# Detection of NA-AB Titer by Western Blot



## Pandemic H1N1

### NA-content in concentrated

recH9N1 | X Y Z : Vaccines



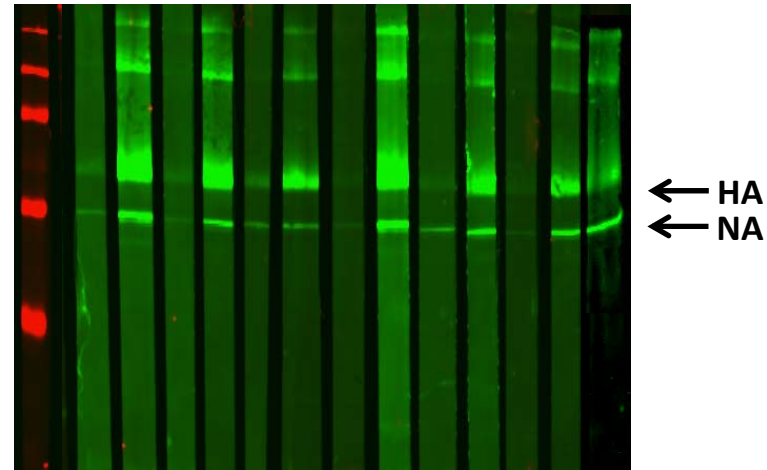
Although currently not a regulatory requirement all tested vaccines contain detectable amounts of NA

### Western blot for antibody detection:

Vaccine X antigen run in SDS-PAGE (as NA source)

HA- and NA-bands detected with sera from vaccinated children

Subject No.: 205 201 203 201 204 5-2 Positive control serum



B A B A B A B A B A B A

Before (B), after (A)  
vaccination

⇒ **NA-antibodies clearly detectable – and correlate well with fetuin-assay results**

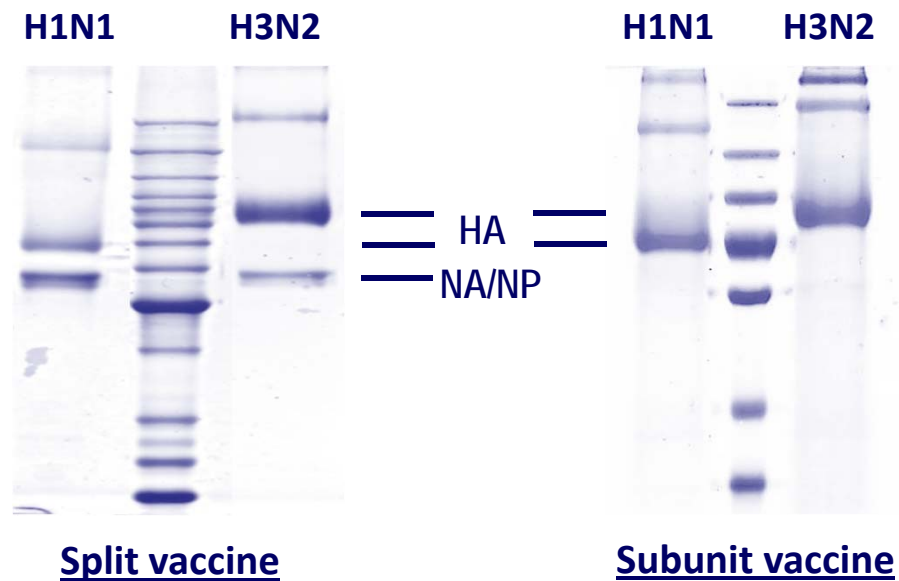
⇒ **Vaccines induce anti-NA antibodies**

**Quantification:** eg Odyssey technology (currently evaluated)  
use positive control serum for standard curve

# Detection of NA-AB Titer by Western Blot – Antigen source

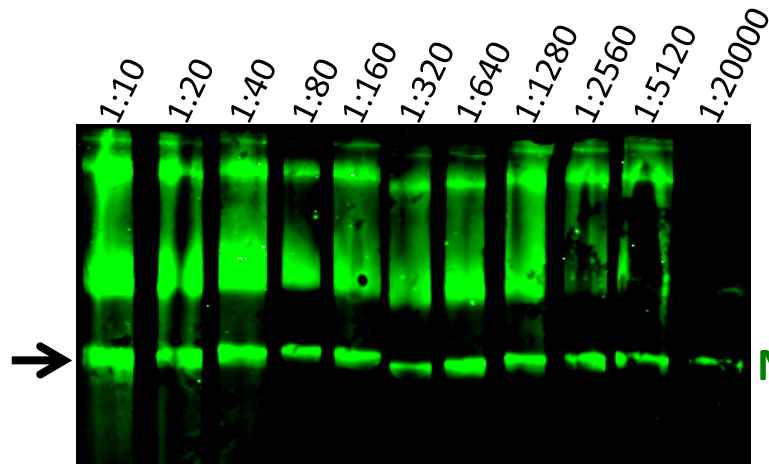


Protein profile of seasonal vaccine monovalent bulks:



↓  
Use split vaccine as protein substrate for the detection of anti-NA antibodies in WB

# Detection of NA-AB Titer by Western Blot

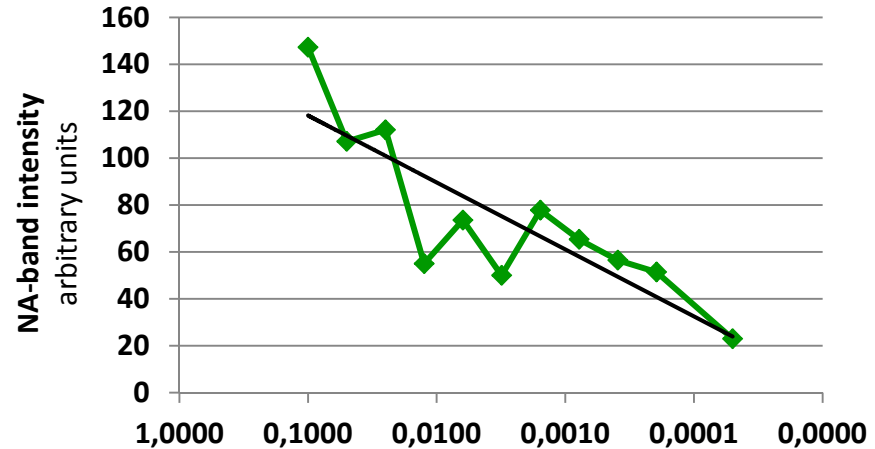


## Western blot strips (1-11):

H3N2 monovalent split vaccine bulk analysed with different dilutions of anti-N2-NA serum (NIBSC) (as primary antibody)

## Blot NA-band intensities against serum dilution

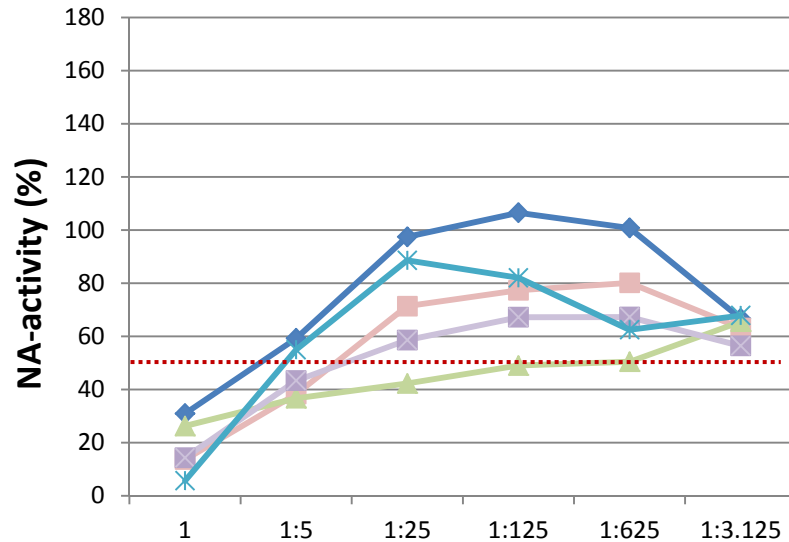
Could serve as an approach for quantification of NA-specific AB against standard serum (more calibration data needed)



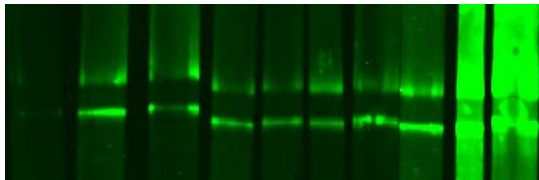
# Comparison of NA-AB detection by chemical assay and WB



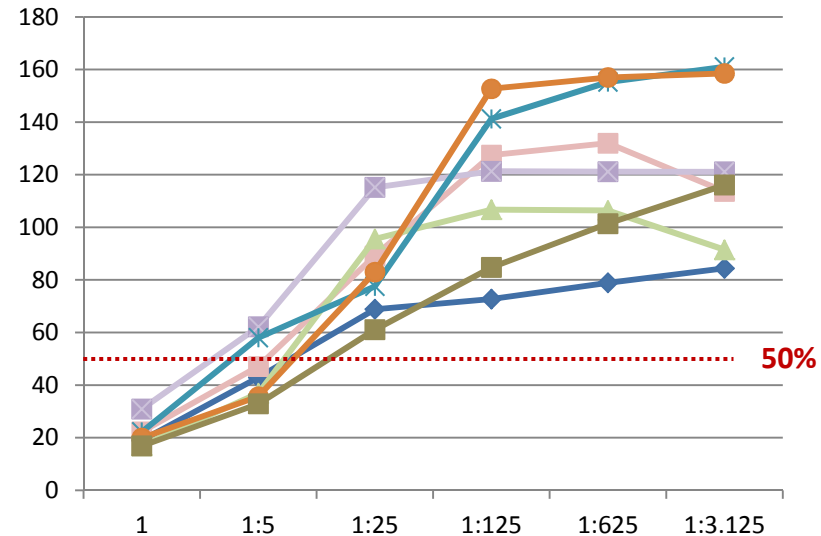
H1N1-virus tests



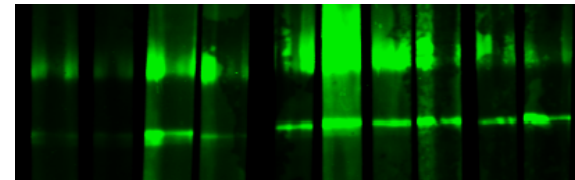
CH CJ HW JH BN SH EK SB HJT CS



H3N2-virus tests



CH CJ HW JH BN SH EK SB HJT CS



**At present, no clear correlation – more data for standardisation/optimisation needed**

→ Eg Optimisation of serum concentrations (1st and 2nd antibody) / antigen amount...  
multiple repeats to get more robust/reliable data

**=> Nevertheless considered a valuable approach for detection of NA-specific antibodies**



**That's all  
Thank you**