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Antigenic characterisation of haemagglutinin proteins derived from different avian influenza virus subtypes.

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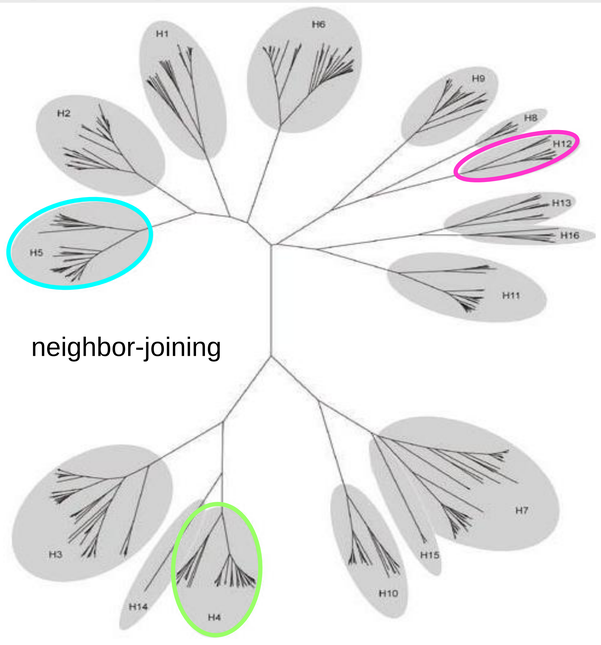
Introduction:

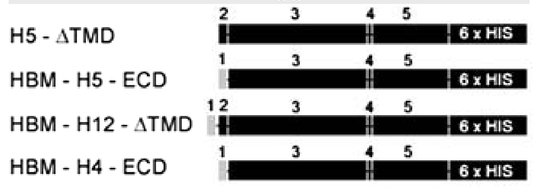
The spontaneous emergence of HP H5N1 and H7N7 and the high mutation rate of influenza viruses point out that a correct prediction of new HPAIV is impossible. A comprehensive surveillance and antigenic knowledge of all circulating reassortants between the16 heamagglutinin and 9 neuraminidase subtypes will lead to a more efficient preparedness for new highly pathogenic strains, irrespective of the subtype they will be. In this study, we present a first set of data about the repertoire of subtype-specific and intersubtype-conserved epitopes of haemagglutinin proteins derived from different avian influenza virus subtypes (A/tufted duck/Switzerland/V504/06(H5N1) (HP), A/duck/Cz/56(H4N6) (LP), A/mallard/Switzerland/WV4060166/2006(H12N2) (LP) relevant for further examination in differential diagnostics and multivalent vaccination approaches.

Genetic relationship of HA full-length coding region among all 16 subtypes of influenza virus.



neighbor-joining

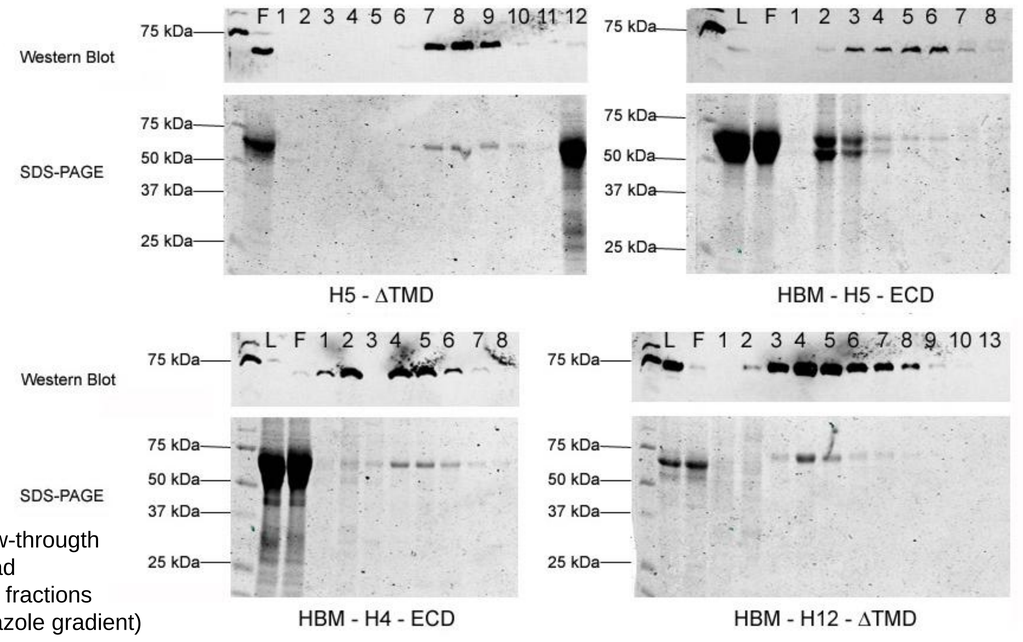
Methods: Make-up of recombinant haemagglutinin.



1: HBM secretion signal
2: AIV secretion signal
3: HA1 ectodomain
4: fusion peptide
5: HA2 ectodomain

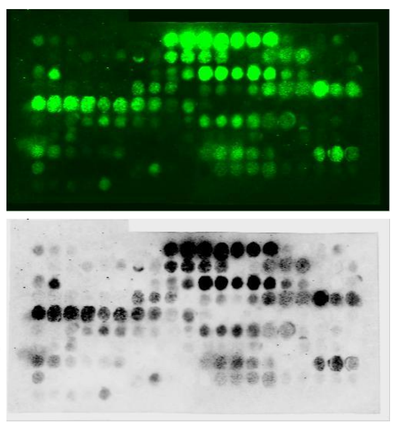
1: cloning of truncated HA ORF in pFASTBAC & C-terminal fusion to 6xHis tag
2: expression of rec. HA in High Five cells
3: purification w/ Ni-NTA FPLC (imidazole gradient)
4: immunisation of rabbits, boosts 200-500 μg purified rec. HA 4 and 8 weeks after immunisation
5: PepSpot analysis using
- jpt Peptide Technologies GmbH
- Odyssey Infrared Imaging System (LI-COR® Biosciences)
- polyclonal sera of rabbits (2. bleeding)

Results: Purification of baculovirus-expressed recombinant haemagglutinins. The purified proteins were used to immunise rabbits.



F: flow-through
L: load
1-13: fractions (imidazole gradient)

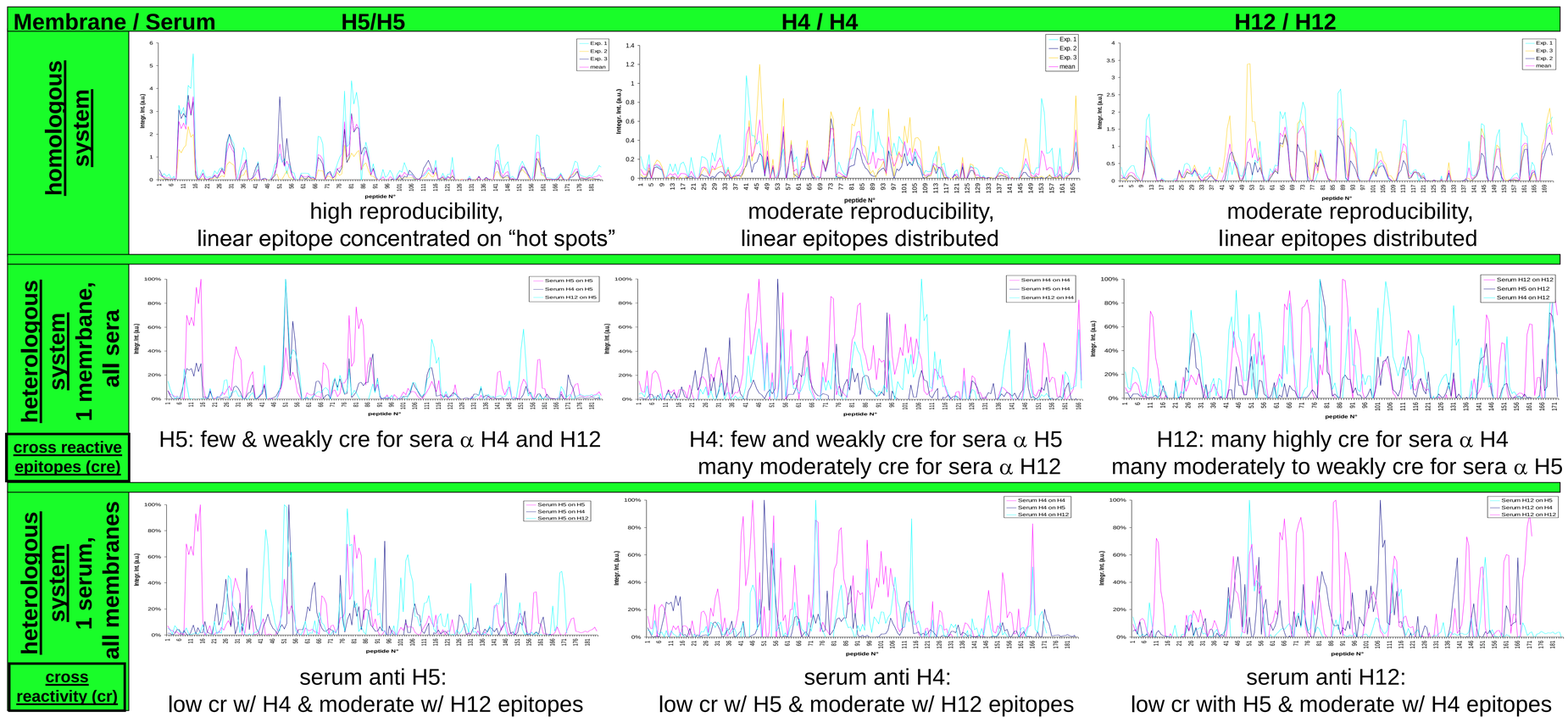
Results: PepSpot analysis membrane H5.



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
A	0.55	0.25	0.11	0.02	0.02	-0.02	0.00	0.04	3.07	2.69	2.92	2.75	3.72	2.83	3.45	0.23	0.00
B	-0.01	0.08	0.07	0.19	0.07	0.28	0.50	0.26	1.61	2.05	1.61	1.37	0.04	-0.05	0.38	0.50	0.90
C	0.59	1.58	0.00	0.05	0.07	-0.01	-0.04	0.18	0.58	1.17	5.86	4.92	2.63	3.75	2.65	0.54	0.17
D	0.09	0.04	0.15	0.05	0.10	0.03	0.97	0.90	0.71	0.01	0.37	0.47	0.09	0.49	0.56	0.36	0.58
E	2.96	2.10	2.30	2.27	1.12	1.45	1.51	1.06	0.24	0.93	0.14	0.00	-0.01	-0.04	-0.10	-0.15	0.01
F	0.21	0.01	0.22	0.33	0.86	0.61	0.26	0.11	0.06	0.22	1.00	1.12	1.17	0.75	0.30	0.53	0.10
G	0.17	0.06	0.38	0.01	-0.04	0.00	0.00	0.00	-0.01	0.10	0.03	0.01	0.02	0.21	-0.01	0.00	0.15
H	0.82	0.65	0.20	0.07	0.15	0.21	-0.02	0.03	0.01	0.01	0.33	0.59	0.74	0.46	0.24	-0.02	-0.01
I	0.56	0.00	0.00	0.00	0.00	-0.01	0.05	0.63	0.04	0.00	-0.02	0.12	0.14	0.23	0.38	0.06	0.08
J	0.10	0.08	0.13	0.05	0.51	0.02	0.01	0.00	0.02	0.01	0.03	0.01	0.00	0.01	0.03	0.01	0.02

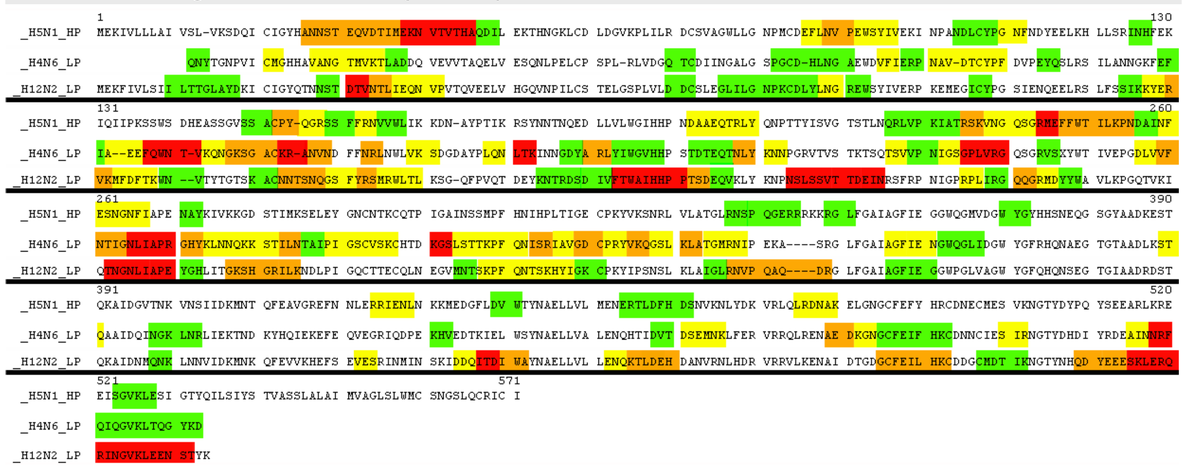
quantification values after background substraction (integrated intensity)

Results: Epitope reactivity for each membrane shown as integrated intensity (arbitrary units) or normalised in %, (highest value set as 100%).



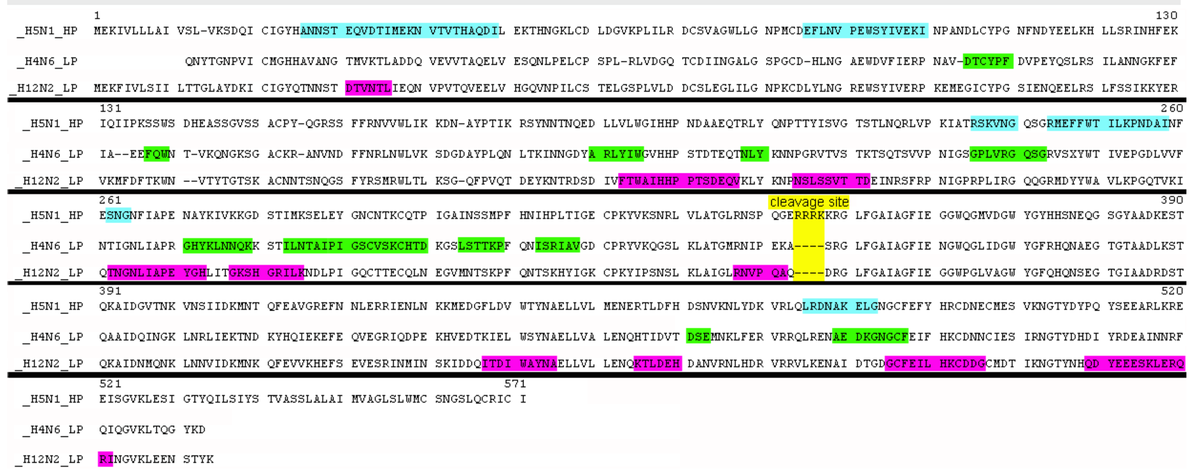
Results: Semi-quantitative analysis of the reactivity of linear epitopes on PepSpot membranes with their corresponding (homologous) rabbit antisera.

integrated intensities (dot blot) **100 >70%** **70 > 40%** **40 > 20%** **20 > 10%**



- linear epitopes are detectable in H5, H4 and H12, in both the HA1 and HA2 ectodomain
- H4 shows most epitopes with homologous serum
- the strongest reactive linear epitopes are subtype-specific (compared with the alignment to the right) and their localisation is not inter subtype-conserved

Results: Identification of HA subtype-specific epitopes, based on the substraction of the heterologous from the homologous sera signals. H5 H4 H12



- ☞ linear epitopes are detectable in H5, H4 and H12, in both the HA1 and HA2 ectodomain
- ☞ H4 shows most epitopes with homologous serum
- ☞ the strongest reactive linear epitopes are subtype-specific (compared with the alignment to the right) and their localisation is not inter subtype-conserved

- ☞ subtype-specific linear epitopes are present in H5, H4 and H12 in the HA1 and HA2 ectodomain
- ☞ subtype-specific linear epitopes in H5, H4, H12 react moderately to strongly in homologous system, (compared with the alignment to the left)

Discussion:

- ☞ as shown by peptide scanning, linear epitopes are present in HA1 and HA2 ectodomains of H5, H4 and H12, most of them in HA1
- ☞ subtypes differ in the number of specific linear epitopes; localisation of subtype-specific epitopes is not conserved among the subtypes tested
- ☞ sera against tested subtypes vary in cross-reactivity (reactivity to and number of detected epitopes)
- ☞ the conclusion of Green et al. (1) “Twenty peptides ... of the HA1 molecule of the influenza virus were synthesized... 18 peptides did not correspond to the known antigenic determinants of the hemagglutinin molecule” seams to be not valid as a general argument against linear peptides tested with sera against folded recombinant HA
- ☞ our data support a recent report indicating the existence of reactive linear epitopes (2)
- ☞ our results show, that linear epitopes are detected by polyclonal sera derived from rabbits immunised with native recombinant AIV haemagglutinin
- ☞ impact of inter subtype-conserved epitopes on vaccine development and of subtype-specific epitopes on differential diagnostics has to be evaluated

References:

1. Green, N., Alexander, H., Olson, A., Alexander, S., Shinnick, T.M., Sutcliffe, J.G., Lerner, R.A. (1982). Immunogenic structure of the influenza virus hemagglutinin. Cell 28: 477-487.
2. Shen, S., Mahadevappa, G., Oh, H.-L. J., Wee, B.Y., Choi, Y.-W., Hwang, L.-A., Lim, S.G., Hong, W., Lal, S.K., Tan, Y.-J. (2008). Comparing the antibody responses against recombinant hemagglutinin proteins of avian influenza A (H5N1) virus expressed in insect cells and bacteria. J Med Virol 80:1972-1983.