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RESEARCH

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Standardization of Quality Control Protocol for Evaluation of Recombinant HVT-H5 Vaccine

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ABSTRACT

Background: There is no international quality control protocol for evaluation of recombinant H5 vaccines had been established yet so, these vaccines are evaluated by applying the conventional QC tests such as identity, safety, purity, potency and efficacy that were not satisfactory in vaccine evaluation.
Objective: The present study was conducted to standardize a protocol for quality control and evaluation of the recombinant HVT-H5 vaccine.
Methods: The protocol was designed for evaluation of H5 identity besides regular vaccine safety, purity and potency. The identity of H5 insert was evaluated using real-time PCR while the vaccine potency was evaluated using HVT virus titration, reduction of viral shedding during challenge test.
Results: Five vaccine batches were selected randomly and coded as A, B, C, D and E; these were examined and proved to be identical by quantitative real- time PCR in which the H5 gene titer was 7.364, 7.499, 7.767, 8.049, 8.000 logs 10 copies / ml respectively. The result of in- house ELISA was not significant for H5 gene detection and titration using polyclonal H5 serum. The HVT titer in CEF was 3045, 3200, 3400, 3750 & 4000 PFU/dose for batch A, B, C, D, and E respectively. The selected batches safety was satisfactory by 10 fold field doses injection in SPF chicks. The challenge test results revealed 80% protection for A, B and C batches while D and E batches gave 90% protection. The shedding of challenge virus was significantly low with mean of 2 logs 10 in the vaccinated group compared with control group.
Conclusion: the developed evaluation protocol could depend mainly on q PCR for identity and titration of H5 insert gene in addition to vaccine safety and efficacy.

Keywords: Evaluation; identity; potency; real-time PC; Recombinant HVT-H5 Vaccine; Safety.

BACKGROUND

Avian influenza (AI) virus type A is a member of family Orthomyxoviridae affecting much variety of birds, mammals, and man. (**Saif, Y.M. *et al.,* 2003)**.The majority of AI vaccines are inactivated oil emulsion vaccines which protect poultry against clinical signs, death and decrease virus transmission among poultry and to humans. (**Swayne, D.E. 2003).**

Although of the massive use of inactivated AI vaccines in Egypt, the outbreak continues to occur due to many reasons such as: the frequent viral structure changes via antigenic shift and antigenic drift, so the vaccine should be continuously updated according to circulating field strains. **(Tosh, P.K. and Poland, G.A. 2008).** Inactivated vaccines do not prevent infection and birds may shed variable quantities of virus depending on the homology between the hemagglutinin of vaccine and field strains. **(Becker, A.L. 2004).** AI vaccination requires effective biosecurity and monitoring system to be able to prevent AI virus introduction and reducing its spread, this biosecurity measures is not well implemented in Egypt. **(OIE /FAO /**

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IZSVe scientific conference, 2007) and inactivated vaccines require booster doses for long term protection and limit virus transmission. (**Peyre, M. *et al* 2009).**

On the other hand, other vaccine types were developed with enhanced efficacy, and cross-protection such as gene-based vaccines (rFP-H5, rND-H5 and rHVT-H5). **(Rao, S.S. *et al.,* 2008).** Both recombinant fowl pox and Newcastle disease virus (rNDV) vaccines are ineffective as single dose primary vaccine in presence of maternal immunity or immunity against vector. In this case, they are effective if used as a priming vaccine. **(OIE, 2015)**. Recently a new live cell-associated recombinant turkey herpesvirus avian influenza (A/swan/ Hungary/4999/2006 (H5N1)) clade 2.2 virus (rHVT-H5) vaccine was developed, that is effective in presence of maternal immunity for both the vector (HVT) and the insert (H5) and is applicable at the hatchery (**Rauw, F. *et al.,* 2012a**), in which both humeral and cellular immunity induced. **(Kapczynski, D.R. *et al.,* 2015).**

No international quality control protocol for evaluation of recombinant H5 vaccines had been established yet, however, these vaccines are evaluated by applying the conventional QC tests such as identity, safety, purity, potency and efficacy. But **Kaczynski, D.R. *et al.,* 2015** tried conventional QC for evaluation of rHVT-H5 vaccine and he found that the HI antibodies average titers were between 2^5 and 2^6 using the homologous HA antigen. However, when using heterologous HA antigens, the HI antibodies average titers was between 2^1 and 2^3. Nonetheless, the vaccine provides good protection against those heterologous viruses in challenge test, which may indicate that other immune factors (CMI) contribute to this protection against AIV than antibodies. So, there is a need for the establishment of a standard protocol for evaluation of recombinant H5 vaccines as follow: identity testing and titration testing for H5 gene insert by qPCR and ELISA, vaccine safety & purity, vaccine efficacy and its relatedness with vaccine identity and antigen amount.

MATERIALS AND METHODS

Vaccines

- Five randomly selected batches coded as A, B, C, D and E of VECTORMUNE® HVT AIV (Recombinant Avian influenza Marek's disease vaccine) contains a genetically engineered Marek's disease virus of serotype 3 (turkey herpesvirus or HVT) expressing HA gene of a highly pathogenic avian influenza (HPAI) H5N1 clade 2.2 A/Swan/Hungary/499/ 2006.
- Reassortant inactivated avian influenza virus vaccine, inactivated (H5N1 subtype, Re-5 strain), used for the preparation of monospecific H5 serum in rabbit to be used in ELISA according to **Hussain, I. *et al.,* 2004.**
- Recombinant HVT-ND vaccine, select one batch randomly for H5-ELISA.
- Marek's disease vaccine (Rismevac) ®: batch number A304B with a titer of 3200 PFU/dose, used in H5-ELISA test.

H5 avian influenza virus strain:
A/chicken/Egypt/12378 N3-CLEVB/2006/H5N1 strain was used as positive control for real - time PCR (standard sample).

Viral antigen:
Reassortant avian influenza virus (H5N1 subtype, Re-5 strain) antigen was used as positive control in H5- ELISA according to **Burleson, F.G., *et al.* 1992.**

Challenge virus:
The challenge virus used in the present study was A/chicken/Egypt/15755/2015 (H5N1) which represents currently circulating HPAI H5N1 clade 2.2.1.2 **(Arafa, A.S. *et al.,* 2015)** viruses in Egypt. The challenge was administered by intranasal inoculation at a dose of 10^6 EID_{50}/0.1ml **according to OIE, 2004.**

Cell Culture:
- Primary chicken embryo fibroblasts (CEF) were prepared from 10 days old SPF chicken embryo; that obtained from Kom Oshiem Farm, Fayoum, Egypt and used for titration of HVT virus vaccine by plaque assay according to **OIE, 2004** and used also for Cell-ELISA according to **Dutta, S.K. *et al.,* 1983 & Burleson, F.G., *et al.,* 1992**.
- MDCK cell line used for detection of H5 in Cell-ELISA according to **Dutta, S.K. *et al.,* 1983**.

SPF Embryonated Chicken Eggs (ECE):
9-11 day old SPF ECE obtained from Kom Oshiem Farm, Fayoum, Egypt used for detection of extraneous viruses.

Chickens:
One day old SPF chicks obtained from SPF poultry farm at Koum Osheim El-Fayoum, Egypt, were used for vaccine safety and efficacy.

Nucleic acid extraction kits:
- DNeasy Blood and tissue Qiagen extraction kit (Cat No. /ID: 69506).
- QIA amp Viral RNA Mini Kit (Qiagen extraction kit) Cat No. / ID52906.

PCR condition
Real-time PCR was conducted on the rHVT-H5 vaccine using primer pair and probe designated according to **Capua, I. and Alexander, D. J. (2009)** and superscript ^{R} III Platinum ^{R} one-step q real - time-PCR system Invitrogen kit by life technologies (lot no. 1447688). The fluorescence data were read by Bio-Rad thermocycler. The quantification of H5 gene insert was accomplished using standard curve generated from tenfold serial dilutions of the standard H5 sample in the range of 10^1 to 10^9 copies / ml.

Cell-ELISA:
Used for detection of H5 recombinant protein according to Burleson, F.G., *et al.,* (1992) & Dutta *et al.,* (1983).

H5 ELISA:
Used for H5 antigen detection according to Burleson, F.G., *et al.,* (1992).

Vaccine safety:
According to OIE, 2008.

Extraneous virus detection (vaccine purity):
According to OIE 2008.

Potency test:
Titration of live HVT vector in CEF (Plaque assay)
According to Burleson, F.G., *et al.,* 1992, Bussey, K. A., 2010 and Tse, L. V. *et al.,* 2013.

Experimental design for vaccine efficacy:
The vaccine is diluted by the manufacturer's diluent at room temperature according to the indicated dosage before administration. Fifteen, one day old SPF broiler chicks were used for each vaccine batch in this experiment. They were divided into 3 groups, each group kept in separate isolation unit with adequate water and balanced ration:

Group (I): Comprised 20 SPF one day old chicks are vaccinated with 0.2 ml of VECTORMUNE® HVT AIV vaccine S/C and experimentally challenged with A/chicken/Egypt/15755/2015 (H5N1) 4 weeks post vaccination.

Group (II & III): each consisted of 15 unvaccinated SPF chickens.
Group II challenged with A/Chicken/Egypt/15755/2015 (H5N1) and serving as positive control, while Group III was neither vaccinated nor challenged and served as negative control.
Four weeks post vaccination, ten birds was selected randomly from a group I & II to be challenged. After the challenge, birds were monitored daily for clinical signs and deaths.

Virus shedding

Quantification of the AIV challenge strain in the oropharyngeal swabs was determined by quantitative real-time reverse transcription–polymerase chain reaction (qRT-PCR) targeting the influenza A matrix (M) gene, as previously described by **Rauw, F *et al.,* 2011**. The results were expressed as the number of M gene copies per milliliter of swab samples.
Oropharyngeal swabs were taken in 3rd, 5th, 7th & 10th days post challenge and stored at -80 C before processing.

Measured parameters for vaccine efficacy

The mean virus load shed per group was calculated only for live positive shedders per group per day.
The level of protection (survival rate) conferred by the rHVT-H5 vaccine was computed as the proportion of surviving birds after challenge.

RESULTS

Quantitative real-time PCR
The results obtained by q real-time PCR was shown in fig. (1&2) and mentioned in a table (1).

Table (1): Results of quantitative real-time PCR

Target	Content	H5 gene titer (log 10 copies/ml)
H5	A	7.364
H5	B	7.499
H5	C	7.767
H5	D	8.049
H5	E	8.000
H5	Neg. Ctrl (NTC)	0
H5	Neg. Ctrl	0

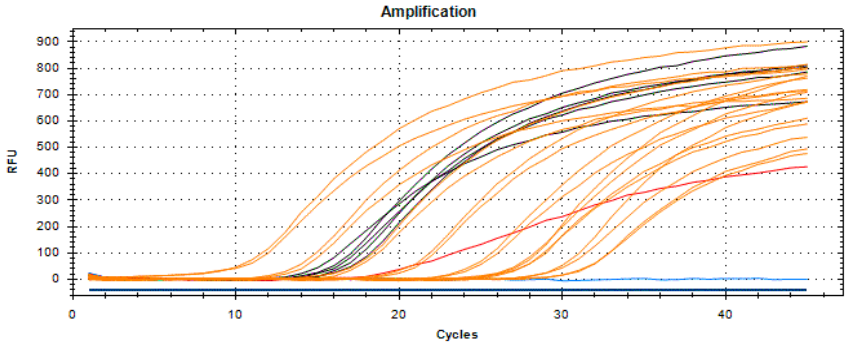

Fig. (1) Show amplification plot of quantitative real - time PCR, blue negative, orange standard, black unknown.

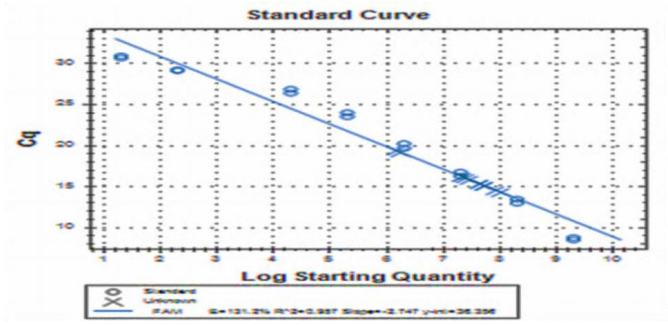

Fig. (2): Show the standard curve for quantifying H5 gene insert in vaccine samples, Efficiency % 133.32, Slope -2.718,Y intercept 36.165,R^2 (CORRELATION COEFFICIENT)0.958.

ELISA

The result of Cell-ELISA is not significant due to high false positive result in negative cell control in Cell-ELISA as shown in the table (2 & 3).

The result of H5-ELISA is also not significant due to high false positive result with rHVT-ND & rispen antigens as shown in the table (4).

Table (2): 24 hour incubated CEF monolayer inoculated with the tenfold serially diluted vaccine. The optical density (O.D) result was as follow:

	Serum dilutions			
	1/10	**1/20**	**1/40**	**1/80**
Control +ve	1.602	0.601	0.967	0.501
Control –ve	1.44	0.923	0.763	0.556
10^{-4}	1.4	0.52	1.58	0.46
10^{-5}	1.5	0.47	1.5	0.49
10^{-6}	1	0.46	0.92	0.48
10^{-7}	0.85	0.36	0.89	0.36
10^{-8}	0.68	0.29	0.73	0.31
10^{-9}	0.56	0.26	0.640.46	0.32

Table (3): 24 hour incubated MDCK monolayer inoculated with tenfold serially diluted vaccine, the optical density (O.D) result was as follow:

	Serum dilution			
	1/10	**1/100**	**1/200**	**1/400**
Control +ve	1.86	1.055	0.881	0.615
Control –ve	0.76	0.75	0.35	0.54
10^{-4}	1.19	0.83	1.05	0.37
10^{-5}	0.69	0.57	1.15	0.34
10^{-6}	1.07	0.49	0.97	0.33
10^{-7}	0.96	0.41	1.15	0.34
10^{-8}	1.05	0.38	1.13	0.36
10^{-9}	1.01	0.35	1.2	0.21

Table (4): Result of H5 ELISA by optical density (O.D):

Vaccine batch	**Vaccine dil.**	**Mean of 1/10 serum dil.**	**Mean of 1/100 serum dil.**	**Mean of 1/10 anti-IBD serum dil.**	**Mean of 1/10 anti-reo serum dil.**
Batch 30	1/25	2.2	0.76		
	1/50	2.5	0.62		
Batch 32	1/25	2.3	0.66		
	1/50	2.3	0.69		
Batch 34	1/25	3.3	0.92	0.056	0.058
	1/50	3.2	0.97	0.053	0.059
Batch 42	1/25	2.7	0.72		
	1/50	2.5	0.74		
Resmivac®	1/25	2.8	0.57		
	1/50	2.78	0.64		
Re-5 H5 antigen	1/25	4.3	3.45		
	1/50	3.79	2.98		
r HVT-ND	1/25	1.75	0.41		
	1/50	1.77	0.51		

Vaccine safety

There are no clinical signs, deaths, pathological lesion & systematic reactions on chicks was observed so the vaccine is safe.

Extraneous virus detection (vaccine purity)

The result of intr allantoic inoculation of 9 days old SPF ECE for other HA viruses detection was negative so, the vaccine is pure.

Potency test

HVT virus titration in CEF

The oldest vaccine batch (A) show the lowest HVT virus titer (3045 PFU/ml) but, the most recent one (E) show the highest virus titer (4000 PFU/ml) as shown in the table (5).

Table (5): Results of HVT titration in CEF cells

Vaccine batch	HVT virus titer (PFU/ml)
A	3045
B	3200
C	3400
D	3750
E	4000

Vaccine efficacy in SPF chicks:

In general, only 2/10 (80%) for batch A, B and C, 1/10 (90%) for batch D and E of the birds in group I and 10/10 (100%) in Group II were died as described in the table (6).The virus shed varied significantly ($P < 0.05$) between the two challenged groups, The mean number of virus copies shed by Group II was significantly higher ($P < 0.05$) than that of Group I. The amount of virus shed by Groups I was lower (by 2 or 3 logs) than in Group II as described in the table (6).

After infection with influenza A (H5N1), oropharyngeal swabs may be superior to cloacal swabs for diagnosing AI infection under field conditions. Duration of virus excretion before death was very short. So, we take tracheal swabs only. **(Swayne, D.E. *et al.,* 2001).**

Table (6): Shedding titer variation between the negative non - vaccinated challenged and vaccinated challenged groups:

vaccine batch	protection %	reduction in mean daily virus shedding (log10 PCR copies/ml)
A	80	2.1
B	80	2.4
C	80	2.51
D	90	3.35
E	90	3.35
Control	0	0

DISCUSSION

Although up till now the majority of commercially available AI vaccines are inactivated vaccines, a new trend for development the recombinant vaccines was arisen to overcome the problem of inactivated vaccine **(Kapczynski, D.R. *et al.,* 2010)**.

In Egypt, however, the use of classical inactivated vaccines is hampered by the interference with MDA; the frequent antigenic drift of the AIV that requires continuous updating of vaccines to keep up the corresponding efficacy; the poor quality of vaccine application at farm level and the limited duration of the induced immunity and, therefore, the necessity for booster vaccinations. **(FAO, 2013)**. So, the recombinant H5 vaccines are gaining use for their ability to overcome maternal antibody interference & induce protection against heterologous isolates (multiple lineages of HPAI). (**Kapczynski, D.R. *et al.,* 2015).**

The current study was planned to evaluate the live rHVT-H5 vaccine by identity, purity, safety and potency. The real-time PCR was successfully used to detect and titrate H5 gene insert in the randomly selected batches of rHVT-H5 vaccine in which there is no signal of amplification was observed in negative control (no template control NTC) to ensure that the observed amplification signal from samples is related to H5 gene insert only. The specificity of quantitative real- time PCR was examined by the absence of amplification signal for the negative control of available avian pathogen isolates other than H5.

The real-time PCR result shows that all vaccine batches used in this study contain H5 gene insert and they are identical that confirmed also by high protection % in challenge test against H5N1 virus.

The study shows that the vaccine is very sensitive to any change in temperature as shown in real-time PCR results, the most recent batch has H5 titer higher than the old batch So, long period of storage and handling errors can decrease vaccine potency and reduce effectiveness and protection as shown in challenge test result, the E batch with 10^8 copies /ml give protection 90% while batch C with $10^{7.7}$copies /ml give protection 80%. These agreed with **Centers for Disease Control and Prevention (CDC, 2011).**

In Cell-ELISA results, the negative cell control give high false positivity which can be explained by the endogenous peroxidase activity that physiologically present in many cell types such as RBCs, granulocytes, monocytes, neurons, muscle cells, liver & kidney cells that can react with the chromogen and substrate producing high non-specific background identical to specific immunoperoxidase and lead to false positivity in negative cell control this agreed with **Helle Grann Wendelboe MS and Kirsten Bisgaard BSc, 2009 and Elias, J.M. 2003** who said that this activity must be completely blocked to prevent false positivity especially in case of bloody/inflamed tissues. So, we try to block the endogenous peroxidase activity by the treatment of cell culture with diluted H_2O_2 without significant changes in the reactions.

In H5 ELISA result, show high O.D with rispen & r HVT-ND antigens. These results may be due to impurities that present in the used H5 monospecific serum which react non-specifically with negative cell control in cell-ELISA, Respin & r HVT-ND antigens in H5-ELISA lead to a false positive result. This results agreed with **Chantle S.M., and Clayton A.L. 1988, Crowther J.R. 2001, Law B. and Malone, M.D. 1996** who found out that the common source of these impurities was proteins aggregates and bacterial contamination.

The non- specific reactions in negative controls also agreed with **Coudert, F. and Ahluwalia, R. 1984, and Cheng, Y.Q *et al.,* 1984** who noticed this in ELISA and Cell-ELISA for detection of antibodies against MDV respectively and referred these non-specific reactions to unpurified antigen and/or serum. The serum must be treated before used to eliminate these nonspecific reactions in negative controls. This findings suggested that non-specific binding might be overcome by using monoclonal monospecific H5 antibody to exclude any protein aggregates and contaminants that might cause non-specific binding **(Chantle S.M., and Clayton A.L. 1988, Crowther J.R. 2001, Kemeny D.M. and Chantle S. 1988).**

The rHVT+H5 vaccine titer for A, B, C, D & E batches was 3045, 3200, 3400, 3750 & 4000 PFU/dose respectively in CEF cells and these vaccine batches were potent in challenge test that agreed with **the parameters of Code of Federal Regulation USA ''Part 133.331-9 CFR ch. 1, 1-1-97 Ed.''** which recorded the minimum titration level of Marek's disease must be not less than 2000 PFU/ dose for potent vaccines.

The challenge test showed that at 4 weeks post vaccination, the r HVT-H5 vaccine conferred 80% protection in batches A, B and C and 90% protection in batches D and E against the high-dose experimental challenge infection. This results agreed with **De Vriese, J. *et al.***

(2009), Kilany, W.H. *et al.* (2012) and Rauw, F. *et al.* (2012b) studies on rHVT-H5 vaccine, who reported 80 to 100% clinical protection after challenge with H5N1virus in broilers and SPF chicks (with or without maternal derived antibodies) vaccinated with rHVT-H5.

The amount of viral shed by group I was lower by 2 or 3 logs than in positive control group, this reduction in viral shedding was agreed with **Kilany, W. H. *et al.,* 2014** who found that the amount of virus shed by group I vaccinated with r HVT-H5 vaccine with 73.3% protection was lower by 2 logs than positive control group.

The reduction in viral shedding result reflect the antigenic and / or genetic identity between vaccinal H5 gene insert and challenged virus so, there is a link between identity and efficacy of the vaccine that agreed with **Jadhao, S.J. *et al.,* 2009, Middleton, D. *et al.,* 2007, Romer-Oberdorfer, A. *et al.,* 2008, Veits, J. *et al.,* 2006, Taylor, J. *et al.,* 1988, Veits, J. *et al.,* 2008, Van der Goot, J.A. *et al.,* 2008 and Kilany, W.H. *et al.,* 2011** studies which suggest that, for both inactivated and vectored HA vaccines, a more closely matched HA is more effective and may suppress virus shedding to a greater extent.

From the qPCR and challenge test results, we can notice that the vaccine batch with higher antigen content (high H5 gene titer) generally provide better protection and greater decrease in virus shedding. The vaccine batch A has H5 titer $10^{7.3}$ copies /ml with protection % 80 but the vaccine batch E has higher H5 titer 10^{8} copies / ml with protection % 90. These agreed with **Swayne, D.E. *et al.,* 1999, Webster, R.G. *et al.,* 2006, Wood, J.M. *et al.,* 1985, Uchida, Y. *et al.,* 2014, Maas, R. *et al.,* 2009, Swayne, D.E. 2001, Di Trani, L. *et al.,* 2003 and Sasaki, T. *et al.,* 2009** who reported that sufficient antigen content is critical for potent vaccine and the quantification of antigen content may be a method to determine potency.

In conclusion, the developed evaluation protocol will depend mainly on qPCR for identity and titration in addition to safety and efficacy.

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REFERENCES

Arafa, A.S., Naguib, M.M., Luttermann, C., Selim, A.A, Kilany, W.H., Hagag, N., Samy, A., Abdelhalim, A., Hassan, M.K., Abdelwhab,E.M., Makonnen, Y., Dauphin, G., Lubroth, J., Mettenleiter, T.C., Beer, M., Grund, C., and Harder, T.C., (2015) . Emergence of a novel cluster of influenza A (H5N1) virus clade 2.2.1.2 with putative human health impact in Egypt, 2014/15.Eurosurveillance 20, 2-8.

Becker, A. L. (2004). USDA funds avian flu vaccine bank for poultry. http://www.cidrapbusiness.net/cidrap/content/influenza/avianflu/news/nov0504vaccine.html Accessed July 2008.

Burleson, F.G., Chambers, T.M. and Wied brauk D.L. (1992). Virology-A Laboratory Manual. Academic Press, Inc., San Diego, 1992-250 PP.ISBN 978-0-12-144730-4.

Bussey, K. A., Bousse, T. L., Desmet, E. A., Kim, B. and Takimoto, T. (2010). PB2 residue 271 plays a key role in enhanced polymerase activity of influenza A viruses in mammalian host cells. J Virol 84(9): 4395-4406.

Capua, I. and Alexander D.J. (eds.) (2009). CD-Rom, Avian Influenza, and Newcastle Disease, © Springer-Verlag Italia 2009.

Centers for Disease Control and Prevention (CDC, 2011). General recommendations on immunization: recommendations of the Advisory Committee on Immunization Practices. MMWR 2011; 60(no. RR-2): 23-27. http://www.cdc.gov/vaccines/ pubs/ACIP-list.htm.

Chantle, S.M. and Clayton, A.L. (1988). The use of ELISA for rapid viral diagnosis: Viral antigen detection in clinical specimens. Kemeny D.M., Challacombe SJ. (Ed.) ELISA and other solid phase immunoassays-Theoretical and practical aspects. John Wiley & Son. UK. 13. 279-301.

Cheng, Y.Q., Lee, L.F., Smith, E.J., and Witter, R.L. (1984). An Enzyme-Linked Immunosorbent Assay for the Detection of Antibodies to Marek's Disease Virus. AVIAN DISEASES vol. 28 no. 4. pp. 900-911. Published by: American Association of Avian Pathologists, Stable URL: http://www.jstor.org/stable/1590266.

Code of Federal Regulation USA (2008). Part 133.331-9 CFR ch.1; 1-1-97 ED.

Coudert, F. and Ahluwalia, R. (1984). Antigen requirements & specificity of a microplate enzyme-linked immunosorbent assay (ELISA) for Marek's disease viral antibodies in chicken, mouse & rabbit sera. Annales de Recherches Veterinaries, INRA Editions, 1984, 15 (4), pp.529-534. <Hal-00901537> https://hal.archives-ouvertes.fr/hal-00901537.

Crowther, J.R. (2001). Systems in ELISA. Methods in molecular biology, Vol 149-The ELISA guidebook Humana Press. Totowa, New Jersey. 2. 9-44.

Crowther, J.R. (2001). Stages in ELISA. Methods in molecular biology, Vol 149-The ELISA guidebook. Humana Press. Totowa, New Jersey. 3. 45-82.

De Vriese, J., Gardin, Y., Palya, V., Dorsey, K.M., Lambrecht, B., Van Borm, S. and Van den Berg, T. (2009). Efficacy of r HVT-AIV vector vaccine in broilers with passive immunity to HVT and AIV, against challenge with H5N1 HPAIV. In D. L. Suarez (ed) Proceedings of the VII International Symposium on Avian Influenza (p. 43). Athens, GA.

Di Trani, L., Cordioli, P., Falcone, E., et al. (2003). Standardization of an inactivated H17N1 avian influenza vaccine and efficacy against A/Chicken/Italy/13474/99 high-pathogenicity virus infection. Avian Dis 2003; 47(3 Suppl.): 1042-6.

Dutta, S.K., Talbot, N.C., and Myrup, A.C. (1983). Detection of equine herpesvirus-1 antigen and the specific antibody by enzyme-linked immunosorbent assay. Am. J. Vet. Res., v.44, p.1930-1934, 1983.

Elias, J.M. (2003). Immunohistochemical methods. In: Elias JM, ed. Immunohistopathology. A Practical Approach to Diagnosis. 2nd ed. Chicago, IL: ASCP Press; 2003:1–110.

Food and Agriculture Organization (FAO) (2013). Report on FAO Technical Meeting on Avian Influenza Hatchery Vaccination (p. 15). December 3, 2013, Beijing, China.

Helle Grann Wendelboe MS and Kirsten Bisgaard BSc, (2009). IHC Staining methods, fifth edition, background, pp 115-121, Chapter 17 2009.

Hussain, I., Rasool, M.H., and Mahmood, M.S., (2004). Production of hyperimmune serum against infectious bursal disease virus in rabbits. Pakistan Veterinary Journal 24, 179-183.

Jadhao, S.J., Lee, C.W., Sylte, M., and Suarez, D.L. (2009). Comparative efficacy of North American and antigenically matched reverse genetics -derived H5N9 DIVA marker vaccines against highly pathogenic Asian H5N1 avian influenza viruses in chickens. Vaccine 2009 Oct 19; 27(44):6247-60.

Kapczynski DR, Esaki M, Jackwood MW, and Dorsey KM. (2010). Vaccination of SPF chickens with a recombinant HVT expressing the HA from H5N1 highly pathogenic avian

influenza protects against lethal challenge. 59th Western Poultry Disease Conference, Vancouver, Canada, 18–21 April 2010; P. 124.

Kapczynski, D.R., Esaki, M., Dorsey, K.M., Jianga, H., Jackwood, M., Moraes, M., and Gardind, Y. (2015). Vaccine protection of chickens against antigenically diverse H5 highly pathogenic avian influenza isolates with a live HVT vector vaccine expressing the influenza hemagglutinin gene derived from a clade 2.2 avian influenza virus Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).Vaccine 2015 Feb 25; 33 (9)1197-1205 Journal Homepage: www.elsevier.com/locate/vaccine.

Kemeny, D.M., and Chantle, S. (1988). An introduction of ELISA. Kemeny D.M., Challacombe S.J. (Ed.) ELISA and another solid phase immunoassays-Theoretical and practical aspects. John Wiley & Son. UK. 1. 1-29.

Kilany, W.H., Abdelwhab, E.M., Arafa, A.S., et al. (2011). Protective efficacy of H5 inactivated vaccines in meat turkey poults after challenge with Egyptian variant highly pathogenic avian influenza H5N1 virus. Vet Microbiol 2011 May 12; 150:28-34.

Kilany, W., Dauphin, G., Selim, A., Tripodi, A., Samy, M., Sobhy, H., Von Dobschuetz, S., Safwat, M.,Saad, M., Erfan, A., Hassan, M., Lubroth, J and Jobre, Y., (2014). Protection conferred by recombinant turkey herpesvirus avian influenza (r HVT-H5) vaccine in the rearing period in two commercial layer chicken breeds in Egypt, Avian Pathology , 43:6,514-523, DOI:10.1080/03079457.2014.966302.

Kilany, W.H., Hassan, M.K., Soad, A., Alaa, A.F., Gardin, Y., Palya, V. and Aly, M.M. (2012, April 1–4). Evaluating the efficacy of r'HVT-H5 vaccine in commercial broiler chickens carrying maternally derived antibody (MDA) to AI under field conditions in Egypt. The VIIIth International Symposium on Avian Influenza (p. 55). London, UK.

Law B. and Malone M.D., (1996). Enzyme-linked immunosorbent assay (ELISA) development and optimization. Law B. (Ed.) Immunoassay - A Practical Guide. 1996. Taylor & Francis Ltd, London, UK. 7. 127-149.

Maas, R., Tacken, M., Van, Z.D., and Oei, H. (2009). Dose- response effects of avian influenza (H7N7) vaccination of chickens: serology, clinical protection and reduction of virus excretion. Vaccine 2009 Jun 2; 27(27):3592-7.

Middleton, D., Bingham, J., Selleck, P., et al. (2007). Efficacy of inactivated vaccines against H5N1 avian influenza infection in ducks. Virology 2007 Mar 1; 359(1):66-71.

OIE, 2004. Manual of standards for diagnostic tests and vaccines, 5 Ed.

OIE /FAO / IZSVe: scientific conference, Co-organized & supported by European Union vaccination: a tool for the control of A.I, Verona (Italy, 20-22 march 2007).

OIE, Terrestrial Manual 2008 Chapter 1.1.8. - Principles of veterinary vaccine production. Website http://web.oie.int/eng/normes/MMANUAL/2008/pdf/1.1.08_VACCINE_PRODUCTION.pdf.

OIE, 2015 on website http://www.oie.int/fileadmin/Home/fr/Health_standards/tahm/2.03.04_AI.pdf.

Peyre, M., Samaha, H., Makonnen, Y.J ., Saad, A. , Abd-Elnabi, A. , Galal, S., Ettel, T. , Dauphin, G. , Lubroth, J. , Roger, F. , and Domenech, J. (2009). Avian influenza vaccination in Egypt: Limitations of the current, strategy. J. Mol. Genet. Med. (2009), 3(2), 198-204.

Rao, S. S., Styles, D., W. Kong, W., Andrews, C., Gorres, J.P., and Nabel, G.J. (2008). A gene – based avian influenza vaccine in poultry. Poultry Science, 2009, 88:860–866 doi: 10.3382/ps.2008-00360.

Rauw, F., Palya, V., Gardin, Y., Tatar-Kis, T., Dorsey, M.K., Lambrecht, B. and Van den Berg, T. (2012a). Efficacy of r HVT-AI vector vaccine in broilers with passive immunity against challenge with two antigenically divergent Egyptian clade 2.2.1 HPAI H5N1 Strains. Avian Diseases, 56, 913–922.

Rauw, F., Palya, V., Tatar-KIS, t., Dorsy, M.K., Van den Berg, T., Lambrecht, B. and Gardin, Y. (2012b, April 1-4). Lack of interference between two recombinant HVT vaccines expressing the H5 protein of influenza or the F protein of Newcastle disease administered simultaneously to day-old chicks and efficacy against challenges performed at 4 or 8 weeks of age. In I. Brown, D.E. Swayne & T. Kuiken (Eds.), Proceedings of the VIIIth International Symposium on Avian Influenza (P.94). London, UK.

Rauw F, Palya V, Van Borm S, Welby S, Tatar-Kis T, Gardin Y et al. (2011). Further evidence of antigenic drift and protective efficacy afforded by a recombinant HVT-H5 vaccine against challenge with two antigenically divergent Egyptian clade 2.2. 1 HPAI H5N1 strains. Vaccine 2011; 29(14):2590–600. Doi: 10.1016/j.vaccine.2011.01.048 PMID: 21292007

Romer-Oberdorfer, A., Veits, J., Helferich, D., and Mettenleiter, T.C. (2008). Level of protection of chickens against highly pathogenic H5 avian influenza virus with Newcastle disease virus- based live attenuated vector vaccine depends on homology of H5 sequence between vaccine and challenge virus. Vaccine 2008 May 2; 26(19):2307-13.

Saif, Y.M., Barnes, H.J., Fadly, A.M., Glisson, J.R., McDoudald, L.R. and Swayne, D.E., (2003). "Influenza", in (Diseases of poultry),A book,11th Ed.,Chap.5,PP.135-160,Iowa state press, A Blackwell publishing company, Iowa state university press: Ames, IA.

Sasaki, T., Isoda, N., Soda, K., et al. (2009). Evaluation of the potency, optimal antigen level and lasting immunity of inactivated avian influenza vaccine prepared from H5N1 virus. Jpn J Vet Res 2009 Feb; 56(4):189-98.

Swayne, D.E. (2003). Introduction and Welcome. Avian Diseases: 47. (3), 783-784.

Swayne, D.E., Beck, J.R., Garcia, M., and Stone, H.D. (1999). Influence of virus strain and antigen mass on the efficacy of H5 avian influenza inactivated vaccines. Avian Pathol. 1999; 28(3):245-255.

Swayne, D.E., Beck, J.R., Perdue, M.L., and Beard, C.W. (2001). The efficacy of vaccines in chickens against highly pathogenic Hong Kong H5N1 avian influenza. Avian Dis 2001 Apr; 45(2):355-65.

Taylor, J., Weinberg, R., Kawaoka, Y., Webster, R.G., and Paoletti, E. (1988). Protective immunity against avian influenza induced by a fowlpox virus recombinant. Vaccine 1988 Dec; 6(6):504-8.

Tosh, P. K., and Poland, G.A. (2008). Emerging vaccines for influenza. Expert Opin. Emerg. Drugs 13:21–40.

Tse, L. V., Marcano, V. C., Huang, W., Pocwierz, M. S. and Whittaker, G. R. (2013). Plasmin-mediated activation of pandemic H1N1 influenza virus hemagglutinin is independent of the viral neuraminidase. J. Virol. 87(9): 5161-5169.

Uchida, Y., Takemae, N., and Saito, T. (2014). Application of reverse genetics for producing attenuated vaccine strains against highly pathogenic avian influenza viruses. J. Vet. Med. Sci. 2014 Aug; 76(8):1111-7.

Van der Goot, J.A., Van, B.M., Stegeman, A., Van de Water, S.G., de Jong, M.C., Koch, G. (2008). Transmission of highly pathogenic avian influenza H5N1 virus in Pekin ducks is significantly reduced by a genetically distant H5N2 vaccine. Virology 2008 Dec 5; 382(1):91-7.

Veits, J., Romer-Oberdorfer, A., Helferich, D., et al.(2008). Protective efficacy of several vaccines against highly pathogenic H5N1 avian influenza virus under experimental conditions. Vaccine 2008 Mar 20; 26(13):1688-96.

Veits, J., Wiesner, D., Fuchs, W., et al.(2006). Newcastle disease virus expressing H5 hemagglutinin gene protects chickens against Newcastle disease and avian influenza. Proc Natl Acad. Sci. U S A 2006 May 23; 103(21):8197-8202.

Webster, R.G., Webby, R.J., Hoffmann, E., et al. (2006). The immunogenicity and efficacy against the H5N1 challenge of reverse genetics-derived H5N3 influenza vaccine in ducks and chickens. Virology 2006 Aug 1; 351(2):303-11.

Wood, J.M., Kawaoka, Y., Newberry, L.A., Bordwell, E., and Webster, R.G. (1985). Standardization of inactivated H5N2 influenza vaccine and efficacy against lethal A/Chicken/Pennsylvania/1370/83 infection. Avian Dis 1985 Jul; 29(3):867-72.

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