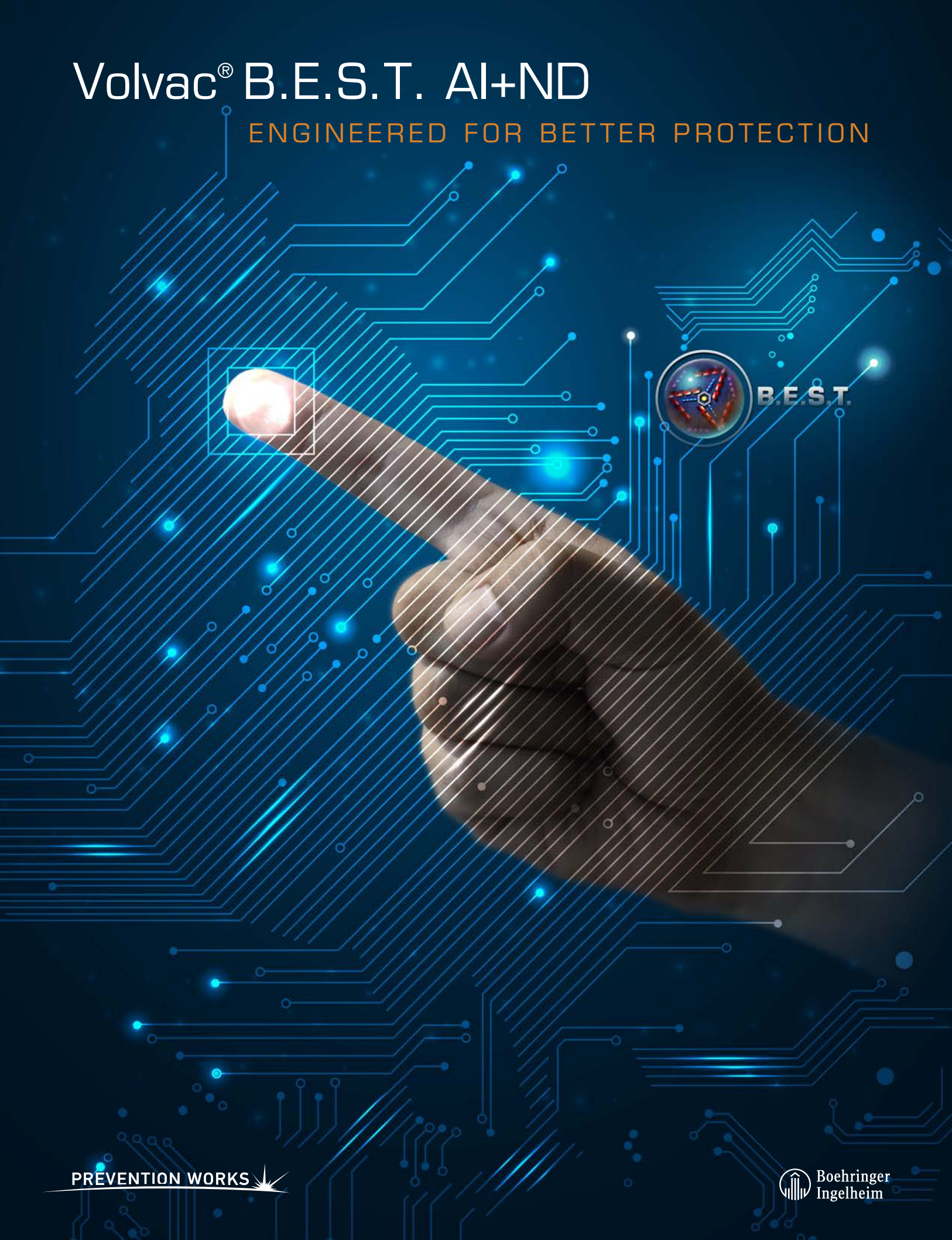
Volvac® B.E.S.T. AI+ND

ENGINEERED FOR BETTER PROTECTION



PREVENTION WORKS

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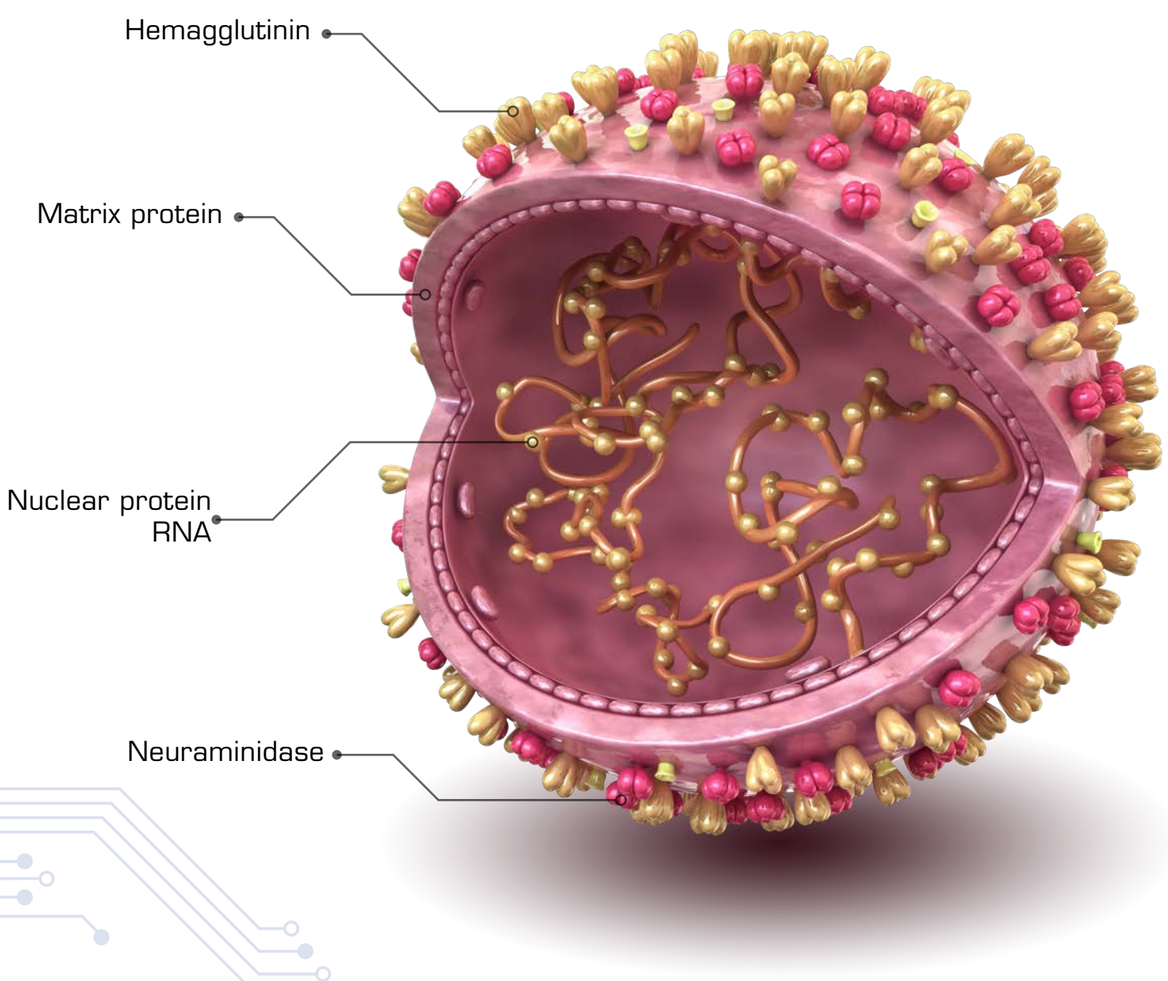
Avian Influenza and Newcastle disease:
SEVERE THREATS FOR THE POULTRY INDUSTRY

- Avian influenza (AI) and Newcastle disease (ND) are acute viral infections of poultry of all ages
- In severe and uncontrolled situations AI and ND are devastating for the poultry industry
- Highly pathogenic (HP) strains of particularly the H5 type of AI and genotype VII of ND have become predominant in many parts of the world (Asia, Middle East and Latin America) during the last years.



Avian influenza:
A MOVING TARGET

Avian influenza viruses evolve rapidly in the field and escape the protection provided by vaccination. This is also the reason why vaccinated birds can still be susceptible to infections.

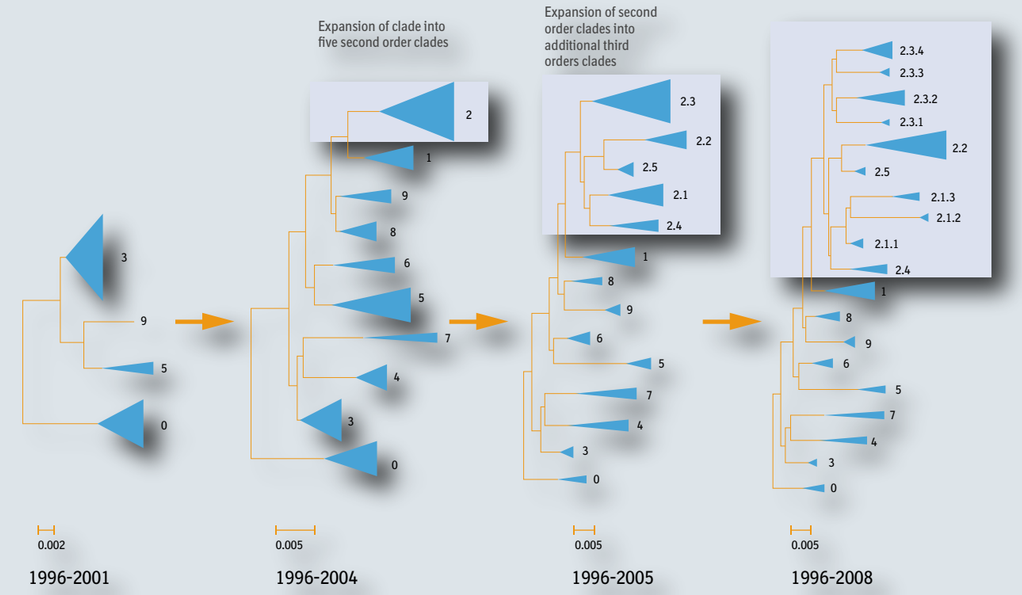
Avian influenza viruses can change by two mechanisms “drift” or “shift”.

Antigenic “drift” refers to small, gradual changes that occur through point mutations in the genes that contain the genetic material to produce the main surface proteins, hemagglutinin and neuraminidase. As a consequence the new strains may not be recognized by antibodies against earlier influenza strains.

Similar groups of viruses are put into groups (so called “clades”, see figure 1). Specific clades may change in a timeframe:

- Antigenic “shift” refers to an abrupt, major change which results in a novel avian influenza virus subtype that was not circulating among the population.
- Antigenic “drift” occurs more commonly, whereas antigenic “shift” only occasionally.

Figure 1: Clade Evolution of the HA of H5N1 over the years



http://www.who.int/influenza/gisrs_laboratory/201101_h5n1evoconceptualdiagram.pdf?ua=1

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The role of vaccination in the control of HIGH PATHOGENIC AVIAN INFLUENZA

CURRENT OPTIONS FOR VACCINATION ARE:

- **Inactivated or killed vaccines:** Conventional inactivated AI vaccines contain killed whole virus usually in an oil emulsion. Protection is based on the production of antibodies to neutralize the AI virus. This kind of vaccines may become less efficacious after a certain time because AI viruses mutate readily in the field and in this way they might escape the coverage given by such products.

- **Recombinant vaccines:** Genes encoding for important surface protein(s) of the AI virus are inserted into a host virus. The efficacy of live recombinant vaccines will depend on the replication of the host virus in the birds and the quality of expression of the proteins by the vector.

Table 1: Summary of the characteristics of the vaccines used against AI

	Recombinant Pox/AI	Recombinant HVT/AI	Recombinant ND/AI	Inactivated whole AI virus	Baculo expressed vaccine
Age at vaccination	1 day	1 day	12 days	14 days	10 days
Effect of MDA	+++	+	NA at age	NA at age	NA at age
Importance of the disease for which the vector is used	+	+++	+++	NA	NA
Onset of immunity	+/-2 weeks	+/-3-4 weeks	+/-1 week	+/-2 weeks	+/-2 weeks
Replication of the virus	Epithelial cells	Systemic in lymphocytes	Mucosa of the respiratory tract	NA	NA
Type of antigen	Complete live recombinant virus	Complete live recombinant virus	Complete live recombinant virus	Complete inactivated virus	HA protein of AI/H5
Vaccine administration	Wingweb punction	Injection (SC/IM) or in-ovo	Coarse spray or eye drop	Injection (SC/IM)	Injection (SC)
Control of mortality	+/++	++/+++	++/+++*	++/+++	+++
Control of virus shedding	+/++	++	++*	++/+++	+++
Duration of immunity	Medium	Long	Short	Long	Long
Broad protection	NR	NR	NR	No	Yes
Use in DIVA concept	+	+	+	+/-	+
Combination with ND	-	-	+	+/-	+

NA – Not applicable
NR – Not reported
*MDA dependent
SC - Subcutaneous
IM - Intramuscular

An innovative tool for the control of AVIAN INFLUENZA AND NEWCASTLE DISEASE IN POULTRY

Volvac® B.E.S.T. AI + ND represents an innovative option for the control of HPAI and ND.

- The Hemagglutinin (HA) protein is one of the surface proteins of the AI virus. It is important for the attachment and fusion of the virus with the cell wall, initiating the infection process. It is also the target of neutralizing antibodies. Once blocked by neutralizing antibodies, the virus will not be able to attach to the cell wall and infection will not take place.

- B.E.S.T. stands for **Baculovirus Expression System Technology**. The **Baculovirus Expression System Technology** is used for the large scale production of biologically active and functional recombinant proteins.

- The AI component in **Volvac® B.E.S.T. AI + ND** was specifically engineered at the R&D facilities of Boehringer Ingelheim by inserting the hemagglutinin (HA) viral sequence of the AI (H5 type) virus into the Baculovirus genome. The insert was generated using recombinant vaccine technology in order to highly resemble the HA protein of currently circulating H5 viruses, thus resulting in a product that provides the needed broad protection in the field.

- The similarity of the aminoacids sequences to the target challenge strains of the AI component in **Volvac® BEST AI + ND** is one of the main reasons why the vaccine shows such a broad spectrum of protection.

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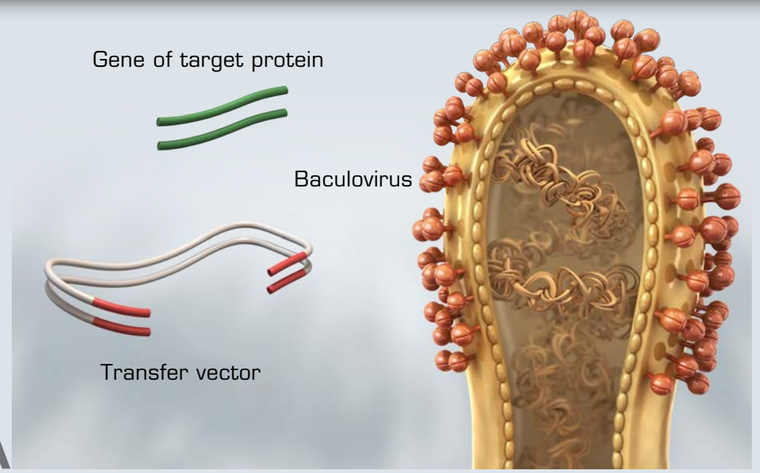
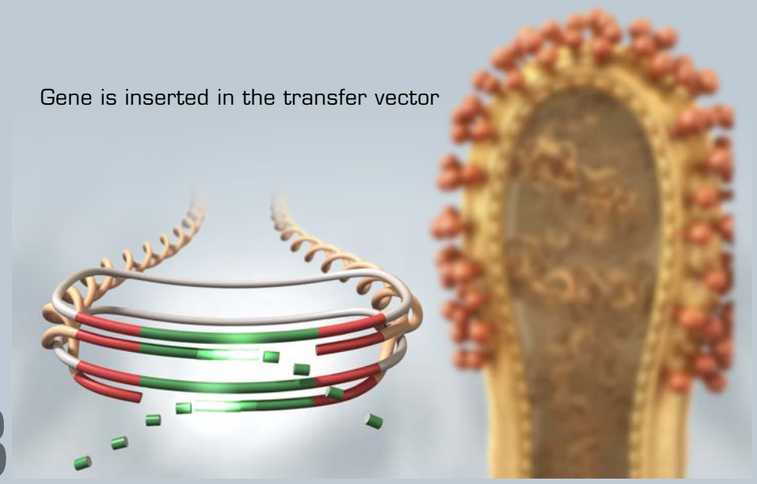
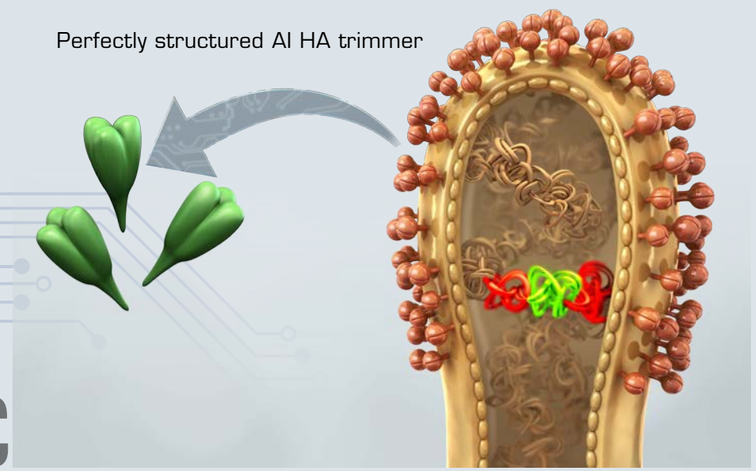




The specifically engineered gene sequence for the hemagglutinin (A) of AI H5 is included in the baculovirus genome by means of a transfer vector (B).

The inserted virus sequence results in the expression of a perfectly structured HA trimmer with high antigenic characteristics (C).



Unequalled Efficacy and Performance

THE PERFORMANCE OF VOLVAC® B.E.S.T. AI + ND HAS BEEN PROVEN IN VACCINATION-CHALLENGE EXPERIMENTS IN CHICKENS. THESE EXPERIMENTS WERE CARRIED OUT AT DIFFERENT REFERENCE LABORATORIES THROUGHOUT EUROPE (GERMANY, ITALY AND SPAIN).

- Specific Pathogen Free (SPF) chickens were vaccinated subcutaneously at 10 days of age
- Challenged 21 days after vaccination with isolates from different countries, years and clades
- In most cases the protection rate was 100% after challenge with HPAI (H5) virus
- Unvaccinated control birds died within 48 hours after challenge

Table 2: Summary of vaccination-challenge experiments with vaccine based on “Baculovirus Expression System Technology” HA (H5) protein

Group	Country of Origin of challenge virus	Species of Origin of challenge virus	Year of isolation	Virus Clade	Protection against mortality and clinical signs
1	Mexico (H5N2)	Chicken	2004	0	100%
2	Vietnam	Duck	2005	2.3.2	100%
3	Spain	Chicken	2006	2.2	100%
4	Egypt	Chicken	2008	2.2.1.1**	90%
5	Egypt	Chicken	2010	2.2.1*	100%
6	Egypt	Chicken	2010	2.2.1.1**	80%
7	Egypt	Chicken	2012	2.2.1*	100%

[* Also known as Clade A] [** Also known as Clade B]

Vaccination resulted in at least 80% protection against Egyptian Clade A (2.2.1), Clade B (2.2.1.1), Asian Clade 2.3.2, Eurasian Clade 2.2 and a Mexican H5N2 virus 21 days after vaccination.

In comparison, studies carried out using competitor products of different characteristics can be seen in the summary in table 3.

Table 3: Summary of experiments after vaccination with competitor products and challenge using Egyptian chicken H5N2 isolates of avian influenza

Group	Type of vaccine	Species of Origin of challenge virus	Year of isolation	Virus Clade	Protection against mortality and clinical signs
1	Full virus	Chicken	2012	A	40%
2	Full virus	Chicken	2012	A	80%
3	Full virus	Chicken	2011	B	60%
4	Recombinant AI full virus	Chicken	2012	A	10%
5	Recombinant AI full virus	Chicken	2011	B	65%
6	Recombinant AI full virus	Chicken	2011	B	40%
7	Recombinant HVT/AI	Chicken	2011	B	80%

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PREVENTION WORKS

Newcastle disease:
ANOTHER IMPORTANT CHALLENGE FOR THE POULTRY INDUSTRY

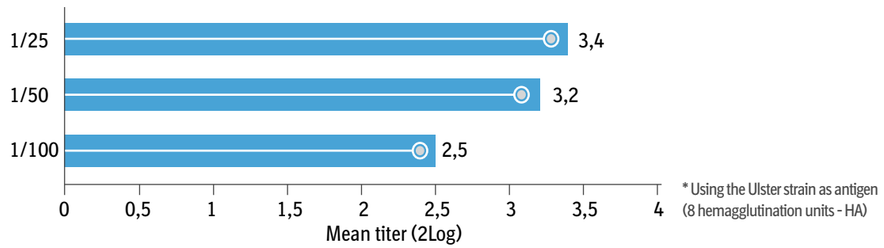
Volvac® B.E.S.T. AI + ND includes conventional inactivated ND virus for a convenient emulsion for the vaccination of chickens against AI (H5) and ND at the same time.

The efficacy of the ND component of an inactivated vaccine can be evaluated by means of the so called Protective Dose 50 test (PD_{50} test) as established in the European Pharmacopeia and OIE methodology.

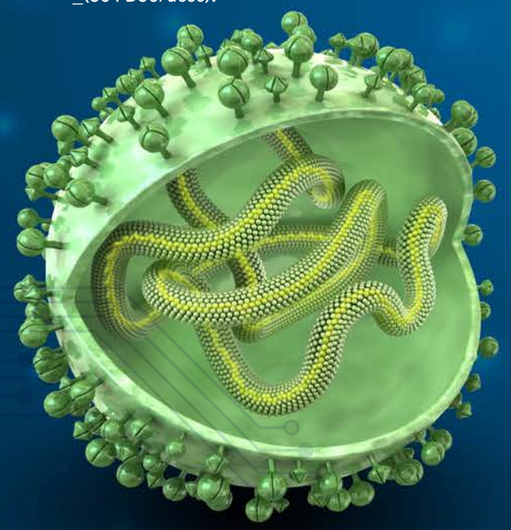
Table 4: Protection after vaccination with **Volvac® B.E.S.T. AI+ND** evaluated by means of the Protective Dose 50 test (PD_{50} test)

SPF birds 3 weeks of age	Number of birds	Dose/bird	% protection p.c. 3 weeks p.v. with NDV Herts 33
Group 1	20	1/25	100%
Group 2	20	1/50	100%
Group 3	20	1/100	90%
Group 4	10	-	0%

Chart 1: Antibody levels (2Log, HI Test*) after vaccination with Volvac® B.E.S.T. AI + ND vaccine



A high PD_{50} value is an indication for better efficacy of the ND inactivated vaccine. **Volvac® B.E.S.T. AI + ND** resulted in a PD_{50} value/dose (0.5ml) of >131, far much higher than the minimum requirements established for this type of products ≥(50 PD50/dose).



NEWCASTLE DISEASE VIRUS

THE B.E.S.T. WAY TO BUILD THE FUTURE TOGETHER

- Specifically engineered using recombinant vaccine technology to provide better protection and unequaled performance in the control of avian influenza and Newcastle disease
- Provides broad and better protection against HPAI
- Reduces the shedding and contributes to the control of avian influenza and Newcastle disease
- Maximize flock performance and provides better results with a cost-effective control against avian influenza
- Includes a highly effective component for Newcastle disease control

Volvac® B.E.S.T. AI + ND

B.E.S.T. PROTECTION AGAINST AVIAN INFLUENZA AND NEWCASTLE DISEASE

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Broad Protection

Safe Vaccine

Strong Inmune Response

Volvac® B.E.S.T. AI + ND

DIVA Concept

Higher Profit

Without Adverse Reactions

Volvac® B.E.S.T. AI + ND

Brings vaccination against avian influenza and Newcastle disease to a superior level

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