MSD ANIMAL HEALTH
TECHNICAL SERVICES BULLETIN
#1022 MSD

HVT Dual Construct Vaccine:
Safe and efficacious protection (control / aid in control) of MD, ND, and ILT

INTRODUCTION

The herpes virus of turkeys (HVT) has been shown to persist within vaccinated flocks giving rise to sustained immune responses; making it the ideal backbone for an HVT dual construct vaccine. Genes inserted into the HVT genome encode specific immunogenic proteins which are expressed within infected host cells and are subsequently recognized by the immune system of the bird and stimulate antibody and cell mediated immunity. The immune responses elicited by HVT vaccination provide life-long immunity once a minimum threshold of immunogenic protein production is reached.

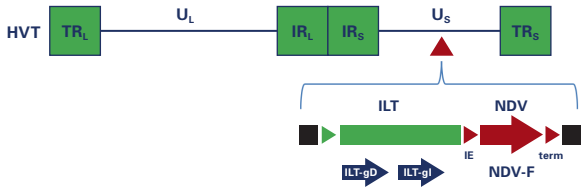
Innovax®-ND-ILT incorporates genes encoding the gD and gI proteins of infectious laryngotracheitis virus (ILTV) and the F protein of Newcastle disease virus (NDV) for simultaneous protection against ND and ILT in addition to protection against Marek's disease (MD). ND and ILT can occur simultaneously in a flock, but it will not be possible to use a combination of HVT-ND and HVT-ILT vaccines to provide concurrent protection against both pathogens due to the observed interference among HVT construct vaccine and a choice must be made as to which construct vaccine to use. Innovax®-ND-ILT solves this problem and protects flocks against both ND and ILT at the same time.

CONSTRUCTION OF INNOVAX®-ND-ILT

Scientists begin with an HVT vaccine that is broken into DNA fragments which are cloned and conserved on plasmids.

These cloned, overlapping DNA segments on plasmid backbones are inoculated into chicken embryo fibroblast (CEF) cells and allowed to reassemble at the overlap sites, and then they are linearized and the plasmid carrier is digested with appropriate enzymes. A "cassette" of DNA that includes genes for the production of gD, gI and F protein is inserted at a specific site called "US 2",

Figure 1. Schematic of the construction of Innovax®-ND-ILT



TRL – terminal repeat long region
UL – unique long region
IRL – internal repeat long region
IRS – internal repeat short region
US – unique short region
TRS – terminal repeat short region

connected by promoter amino acid sequences on one end and terminator amino acid sequences on the other end (Figure 1).

The entire genome is then tested with Southern Blot Hybridization using a series of restriction enzymes and DNA probes to ensure that all of the segments have assembled exactly as intended, and without any plasmid DNA incorporated.

SAFETY

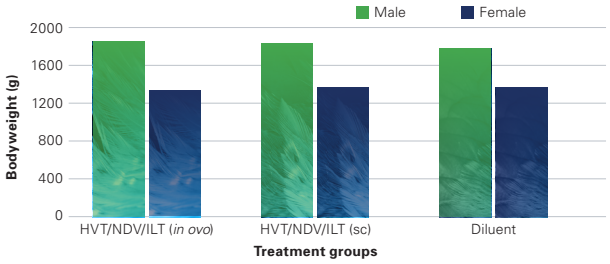
- Phenotype and genotype are stable

	IFA positive plaques/total (% Expression)		
Stock	ILT gD	ILT gI	NDV F
Passage 0	121/121 (100%)	147/147 (100%)	137/137 (100%)
P+5	90/90 (100%)	107/107 (100%)	92/92 (100%)

  - Vaccine back-passaged 5 X in chickens
  - Genetic stability determined by PCR and sequencing
  - Phenotypic stability determined by monoclonal or polyclonal antibodies in Immunofluoresence antibody (IFA) assay
- Master seed virus (MSV) was inoculated *in ovo* or subcutaneously at >10x the field dose
- Vaccine did not affect hatchability of 18 day old embryos
  - 87% hatchability with MSV, vs. 83% with diluent

- No reversion to virulence after 5 back-passages
- Innovax®-ND-ILT has the same tissue tropism and host range (chicken, turkey, quail) to the parent strain
- No significant difference in average body weights of birds receiving MSV or placebo
- Innovax®-ND-ILT does NOT shed to contact controls: it cannot seed a house or spread to neighboring farms. But missed birds stay missed: vaccination accuracy is needed

Average bodyweight

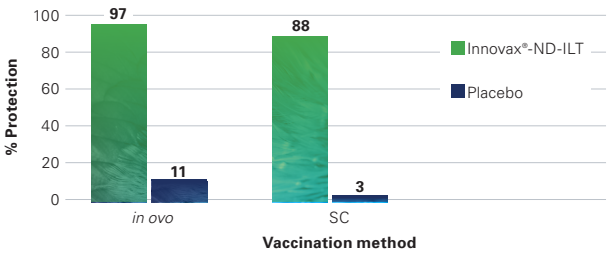


EFFICACY

Marek's Disease Efficacy

- SPF eggs vaccinated *in ovo* at 18 days' embryonation or SPF chicks vaccinated subcutaneously (SC) at day 1 with < 3000 pfu (plaque forming units) of vaccine
- Birds challenged at 5 days of age by intraperitoneal injection (as per 9 CFR) with virulent GA 5 Marek's disease strain
- Birds were evaluated over 49 days for clinical signs and gross lesions of Marek's disease

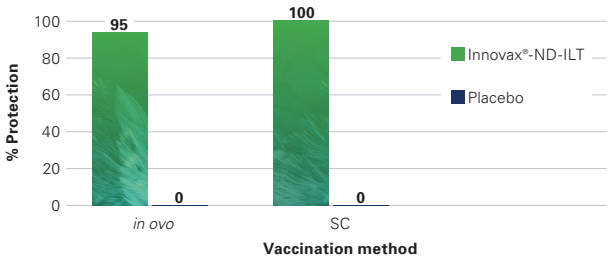
Marek's disease efficacy



Newcastle Disease Efficacy

- SPF eggs vaccinated *in ovo* at 18 days' embryonation or SPF chicks vaccinated subcutaneously (SC) at day 1 with < 3000 pfu of vaccine
- Birds challenged at four weeks by intramuscular injection (as per 9 CFR) with virulent Texas GB challenge strain
- Birds were evaluated over 14 days for clinical signs of Newcastle disease or death

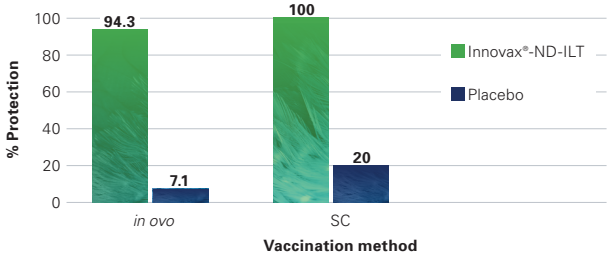
Newcastle disease efficacy



Infectious Laryngotracheitis Efficacy

- SPF eggs vaccinated *in ovo* at 18 days' embryonation or SPF chicks vaccinated subcutaneously (SC) at day 1 with < 3000 pfu of vaccine
- Birds challenged intratracheally at four weeks (as per 9 CFR) with virulent challenge ILT Lot 96-3 provided by the USDA
- Birds were evaluated over 10 days for clinical signs of infectious laryngotracheitis or death

ILT efficacy



CONCLUSION

Innovax®-ND-ILT is a HVT dual-construct vaccine that offers protection against Marek's disease, Newcastle disease and infectious laryngotracheitis. The vaccine has demonstrated a strong stability, safety and efficacy profile and provides long lasting disease prevention without the spread of live ND or ILT vaccine virus. Producers no longer need to make a choice between using a HVT-ND or a HVT-ILT vaccine at the hatchery.

Detailed information about the studies summarized in this technical bulletin can be found in Gergen, L *et. al.* 2019, Avian Pathology Vol 48, No. 1, pages 45 - 56. *https://doi.org/10.1080/03079457.2018.1546376*

Innovax®-ND-ILT contains cell-associated live recombinant turkey herpesvirus (strain HVT/NDV/ILT), expressing the fusion protein of Newcastle disease virus and the glycoproteins gD and gI of infectious laryngotracheitis virus: $10^{3.3}$ – $10^{4.3}$ PFU. **POM-V**. Further information is available from the SPC, datasheet or package leaflet. MSD Animal Health UK Ltd. Registered office Walton Manor, Walton, Milton Keynes MK7 7AJ, UK. Registered in England & Wales no. 946942. Advice should be sought from the medicine prescriber.

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