**Summary of GMO product environmental risk assessment**

**Nobilis® rHVT-ND (Innovax®-ND)**

1. **Introduction**

Nobilis® rHVT-ND is an active recombinant vaccine containing Herpesvirus Turkey (HVT) strain PB1 which inserted by F gene from Newcastle Disease Virus (NDV) strain Clone 30. Therefore, the rHVT-ND virus is able to provide protection against two different types of diseases, Marek’s Disease and Newcastle Disease. In addition, the benefits of the vaccine is to have a longer duration of immunity compared to similar conventional vaccines. In particular, vaccination with Nobilis® rHVT-ND does not introduce the whole NDV into the chickens, therefore the emergence of post-vaccination reactions that generally occur in conventional active ND vaccines can be eliminated.

Nobilis® rHVT-ND is manufactured by Intervet Inc. (Merck Animal Health) USA, which registered in other countries as Innovax®-ND or Innofusion®-ND or Marek’s Disease – Newcastle Disease Vaccine, Serotype 3, live Marek’s Disease Vector. Nobilis® rHVT-ND will be marketed by PT Intervet Indonesia for prevention and control of Marek’s Disease (MD) and Newcastle Disease (ND) in chickens in Indonesia.

Nobilis® rHVT-ND has been registered and obtained Free Sales Certificate (FSC) in various countries, such as USA (2010); Canada (2010); Yordania (2011); Thailand (2011); Kuwait (2012); Colombia (2013); Guatemala (2013); Mexico (2013); Morocco (2013); Panama (2013); Peru (2013); Philippines (2013); Rusia (2013); Trinidad and Tobago (2013); Ukraine (2013). Brazil (2014), and El Savador (2014).

According to Government Regulation No. 21 Year 2005 concerning Biosafety of Genetically Modified Organisms, and Regulation of the Minister of Environment No. 25 Year 2012 concerning Guideline for Preparation of Environmental Risk Assessment of Genetically Modified Organisms, then the Biosafety Technical Team for Genetically Modified Organisms has conducted Environmental Safety Assessment of Nobilis® rHVT-ND vaccine. This assessment was based on genetic information and environmental safety information which consists of modified genetic properties, genetic stability of the GMO, potential spread of the GMO, natural host of the parental microorganisms of the GMO, and possibility to pose a risk to the environment, as described in below paragraphs.

1. **Information of Genetically Modified Organisms (GMO)**

**II.1. General description of the GMO**

The GMO used in the construction of Nobilis® rHVT-ND is Herpesvirus of Turkey (HVT) strain PB1 as the parental microorganism which carries the inserted F gene of the Newcastle Disease Virus (NDV) strain Clone 30 as the donor gene.

**II.2. Characteristic information of the GMO**

The parental microorganism of the GMO is HVT serotype 3 strain PB1 (NCBI accession number AF291866) which is a non-pathogen Marek’s disease virus strain and has been widely used as the vaccine strain to prevent Marek’s disease (Calnek *et al.,* 1997).

The gene donor of Nobilis® rHVT-ND vaccine is the F gene from NDV strain Clone 30, which is included in the Lentogenic strain. The F gene has been used as a gene donor in the production of GMO for animal vaccines. The function of protein F is as a mediator of viral envelope fusion with the plasma membrane (Ganar *et al.,* 2014 and Kapczynski *et al.,* 2013). In detail, sequence of the F gene of the NDV strain Clone 30 can be seen at GenBank [www.ncbi.nih.gov/genbank/](http://www.ncbi.nih.gov/genbank/) with access number Y18898.1.

The flanking region is located in *Bgl*II restriction site located in nucleotide 138,193 in the open reading frame (ORF-086) HVT PB1, which is non-essential region of the HVT. The number copies inserted is one copy. This insertion does not have a detrimental impact and does not affect the virulence of parental virus, it even provides added value in protection against Newcastle Disease (Sondermeijer, 1993).

The stability of inserted NDV F gene and its promoters in HVT PB1 when passage *in vitro* is shown in Report 04R/0238. The F gene sequence and HVT flank regions are stable after 11 passages in chicken embryo fibroblast (CEF) culture cell, demonstrated by HVT/ND-F having 100% sequence identity similar to the Master Seed Virus. The NDV-F genes are stable in the HVT genome and do not convert HVT/NDV-F to virulent when they are passage in vivo (Report 7. Genetic and phenotypic stability of HVT/NDV-F following back passage through the natural host).

**II.3. Methods of the Genetic Construction**

Recombinant virus originated from classical recombination linear recombinant homologous vector (containing the NDV F gene with the promoter sequence which is flanked by HVT sequence) and the complete HVT genome.

The complete steps of cloning are found in flow chart “Cloning and Generation of HVT/NDV-F” and Sondermeijer *et al.,*1993. In brief, the HVT/NDV-F genetic construction method is as follows:

1. Unique short segment (Us) of HVT DNA created by partial digestion of the HVT genome with *Sau*3A enzyme is then ligated with pGME3Z vector to become pMD07. Plasmid pMD07 is a recombinant plasmid containing non-essential HVT genome insertion region.
2. Construction of recombinant vector is made by inserting long terminal repeat (LTR) promoter region of the Rous Sarcoma Virus (RSV) in the pRSVcat plasmid. In the downstream part of the RSV LTR promoter, which is fragment of *Nde*I-*Hind*III digestion in the pRSVcat plasmid of 0.6 kb, unique region for several restriction sites is possible to insert one or several genes into recombinant vector. The recombination of the pVEC01 plasmid containing LTR which is flanked by the HVT genome sequence from pMD07 plasmid of 0.3 kb and 0.4 kb subsequently was the pVEC04 plasmid.
3. cDNA from ND Clone 30 virus is include in λgt10 library. Replica filter from the λgt10 library was grown in the E. coli strain NM514 and then screened/selected to obtain F gene from NDV. The fragment of F gene was subcloned into pGEM4Z plasmid and manipulated by sites cutting using *Bal-31* enzyme to eliminate some sequences in the upstream and downstream parts of the F gene, hereinafter referred to as pNDV01 plasmid. The NDV F gene in pNDV01 was cloned at *Bam*HI site from pVEC04 plasmid in the downstream part of LTR promoter to make the pNDV04 plasmid.
4. The pNDV04 plasmid was made linear and co-transfection was carried out with HVT-PB1 into the CEF culture. Recombinant HVT virus containing F gene of the NDV were detected by indirect immunofluorecent using anti-ND antibodies.

**II.4. Characteristic of Genetic Modification**

Nobilis® rHVT-ND is recombination of Herpesvirus of Turkey (HVT) strain PB-1 that is inserted with F gene of Newcastle Disease Virus (NDV) strain Clone 30.

Genetic modification in the form of insertion of the F gene of the NDV to HVT genome does not affect the virulence of its parents (HVT), on the contrary it provides added value in the form of F protein expression of NDV which gives protection against NDV infection.

The HVT-ND recombinant was stable when it was passaged 11 times in vitro on CEF culture cells and genotypically and phenotypically stable in the in vivo study after 5 times back-passage in its natural host.

**II.5. The possibility of genes inserted in GMO for animal vaccines transferred to other organisms**

The F gene inserted in HVT genome is stable in accordance with description in point II.2. To find out the possibility of transferring the donor gene (F gene) to another organism, a BLAST analysis on the nucleotide of the F gene of the NDV strain Clone 3 with access number Y18898.1 was carried out.

BLAST analysis result shows that the F gene does not have homology with the sequences of other organisms’ genes, therefore transferring genes to other organisms is not possible.

**II.6. Conclusions**

Based on the results of the study of genetic information it can be concluded that:

1. Expressions of the interest genes (F genes from ND virus) inserted in the HVT genome did not change the virulence properties of HVT and could provide added value in the form of multiple protection against Marek's disease and ND.
2. The HVT-ND recombinant virus contains one copy of F gene that are genotypically and phenotypeically stable.
3. The F gene that is inserted in the GMO (HVT-NDV-F) cannot spread or move to other organisms.
4. **Environmental Safety Information**

**III.1. Spread Ability of Microorganism**

Microorganisms for the GMO vaccine Nobilis® rHVT-ND have the same properties as the HVT virus whose parental are not pathogenic and are not excreted in chicken excreta so that they do not pose a risk to the environment (Sondermeijer, 1993).

**III.2. Information on Host Coverage Range of GMO virus for Vaccine**

Parental microorganism of the Nobilis® rHVT-ND vaccine is an PB1 strain HVT virus. The natural host of the HVT virus is turkey (*Calnek et al., 1997*).

**III.3. Molecular Study Information**

Genetic modification of HVT strain PB1 with insertion of F gene from ND virus strain Clone 30. The insertion location is in the open reading frame (ORF-086)HVT PB1, which is a non-essential part of HVT.

The purpose of this gene modification is to make a GMO vaccine containing recombinant HVT virus that expresses F protein of the ND virus, therefore the use of GMO vaccine Nobilis® rHVT-ND can induce immunity and provide dual protection against Marek’s disease and ND.

The HVT-ND-F recombinant virus contains one copy of the F genes that are genotypically and phenotypically stable, and there is no possibility of the spread or transfer of F gene which are inserted into GMO (HVT-NDV-F) to other organisms so it is safe in the environment.

Marker that can be used to identify GMO is sequence of the F gene from ND virus. The technique to identify this sequence is Polymerase Chain Reaction (PCR) technique using primers as follows: Forward primer F2: TGAACATGTCGCATCCCTGC; and Reverse primer R7: ATTCTTCATGCAATTGTCGG.

**III.4. Possibility of Negative Impact of GMO Vaccine Microorganisms on The Environment**

The results of the BLAST nucleotide analysis of the F gene shows that the homology of the genome sequences of other organisms is not found, so the possibility of gene transfer to other organisms is not possible.

GMO vaccine Nobilis® rHVT-ND does not form spores because the parental of this vaccine are included in the group of viruses. The study results showed that there was no spread of the virus through contact between vaccinated chickens and unvaccinated chickens (*Report 5. Test for Spreading of the Recombinant Herpesviruses of Turkey HVT/NDV-F to Various Avian Species after Intramuscular Inoculation*). This can indicate that the possibility of spreading recombinant vaccine virus from chicken farms are very unlikely to occur.

Vaccine viruses can spread to environment and infect their natural hosts, turkeys, but like the nature of their parental this virus is not pathogenic.

**Conclusion**

1. GMO vaccine Nobilis® rHVT-ND is safe for the environment because it does not risk spreading microorganisms to the environment.
2. Based on the stability study of genotype and phenotype, GMO microorganisms in the GMO vaccine Nobilis® rHVT-ND were declared stable.
3. Biosafety Technical Team for Genetically Modified Organisms of Environmental Safety considers that the proposed GMO vaccine Nobilis® rHVT-ND is safe for the environment.
4. If new data and information are found that are not in accordance with the environmental safety data obtained to date, then the environmental security status of the GMO vaccine Nobilis® rHVT-ND needs to be reviewed.
5. If it has been declared safe in the environment, then the product is proven to pose a risk to human and animal health, the applicant must take control and control measures and destroy the GMO vaccine Nobilis® rHVT-ND in the Indonesian territory.
6. GMO vaccine Nobilis® rHVT-ND may not be used as a chicken vaccine before obtaining an Environmental Safety Certificate from the Ministry of Environment and Forestry.

**Bibliography**

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5. Report No. 04R/0238. Genetic Stability of the HVT-NDV F recombinant by sequence analysis of the insertion site and flanking regions. Pg. 1–8.
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* Flow Chart Cloning and Generation of Innovax-ND. Internal Documents Global R&D MSD Animal Health.
* Schematic representation of pNDV04. Internal Documents Global R&D MSD Animal Health.
* Sequence of NDV04. Internal Documents Global R&D MSD Animal Health [Report No. 04R/0238].

1. Blast Result of the NDV-F gene.
2. Newcastle disease virus cDNA to complete genomic RNA, clone 30. [GenBank: Y18898.1]