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Veterinary Medicines Division

## Committee for Veterinary Medicinal Products (CVMP)

# CVMP assessment report for Nobilis Multriva Gm+REOm (EMEA/V/C/006614/0000)

Vaccine common name: Avian infectious bursal disease and avian reovirus vaccine (inactivated)

**Assessment report as adopted by the CVMP with all information of a commercially confidential nature deleted.**

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# Introduction

The applicant Intervet International B.V. submitted on 25 November 2024 an application for a marketing authorisation to the European Medicines Agency (The Agency) for Nobilis Multriva Gm+REOm, through the centralised procedure under Article 42(4) of Regulation (EU) 2019/6 (optional scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 18 July 2024 as no other marketing authorisation has been granted for the veterinary medicinal product within the Union.

Nobilis Multriva Gm+REOm is presented as an emulsion for injection for chickens containing inactivated infectious bursal disease virus, strain GB02, inactivated infectious bursal disease virus, strain 89/03, inactivated avian reovirus, strain ARV-1 and inactivated avian reovirus, strain ARV-4 as active substances and light liquid paraffin as adjuvant. The target species is chickens.

This vaccine is intended for use as a booster vaccination following priming with either live or inactivated vaccines in the vaccination schedule. The vaccine is to be administered intramuscularly as a single dose of 0.3 ml in the breast or thigh region from 8 weeks of age onwards, but no later than 3 weeks before the onset of lay.

At the time of submission, the applicant applied for the following indications:

For the active immunisation of chickens for passive immunisation of the progeny of the vaccinated chickens to reduce mortality and clinical signs of disease caused by very virulent (CS89) and classical (STC) strains of infectious bursal disease virus (IBDV), and to reduce viraemia and clinical signs of disease caused by avian reovirus (ARV) genotypes 1 and 4.

Onset of immunity: - IBDV and ARV: 4 weeks post-vaccination.

- IBDV and ARV in progeny: 1 day of age

Duration of immunity: - IBDV and ARV: 80 weeks post-vaccination.

- IBDV and ARV in progeny: 3 weeks of age.

Cross protection has been established for IBDV antigenic variant strains (variant E and GLS).

Cross protection has been established for ARV genotypes 2, 3 and 5.

The vaccine is presented in packs containing 1 bottle of 300 ml (1000 doses) or 600 ml (2000 doses).

The rapporteur appointed is Jacqueline Poot and the co-rapporteur is Christine Miras.

The dossier has been submitted in line with the requirements for submissions under Article 8 of Regulation (EU) 2019/6 – full application.

On 9 April 2025, the CVMP adopted an opinion and CVMP assessment report.

On 2 June 2025, the European Commission adopted a Commission Decision granting the marketing authorisation for Nobilis Multriva Gm+REOm.

## *Vaccine Antigen Master file (VAMF)*

The following VAMF certificates were submitted with the marketing authorisation application:

- EMEA/V/VAMF/00005 - Infectious Bursal Disease virus, strain GB02, inactivated
- EMEA/V/VAMF/00006 - Infectious Bursal Disease virus, strain 89/03, inactivated

- EMEA/V/VAMF/00007 - Reovirus, strain ARV-1, inactivated
- EMEA/V/VAMF/00008 - Reovirus, strain ARV-4, inactivated

# Part 1 - Administrative particulars

## *Summary of the Pharmacovigilance System Master File*

The applicant has provided a summary of the pharmacovigilance system master file which fulfils the requirements of Article 23 of Commission Implementing Regulation (EU) 2021/1281. Based on the information provided the applicant has in place a pharmacovigilance system master file (PSMF) with the services of a qualified person responsible for pharmacovigilance and has the necessary means to fulfil the tasks and responsibilities required by Regulation (EU) 2019/6.

## *Manufacturing authorisations and inspection status*

**Active substance**

Four vaccine antigen master file certificates were submitted with this application. The GMP status for the manufacturers in these certificates is valid.

**Finished product**

The finished product is manufactured at Merck Sharp & Dohme Animal Health S.L. in Salamanca, Spain.

A manufacturing authorisation was issued on 24 November 2020 by the competent authority of Spain (AEMPS). A GMP certificate confirming compliance with the principles of GMP is provided. The certificate was issued on 16 February 2022, referencing an inspection on 16 December 2021, by the competent authority of Spain (AEMPS).

Intervet International B.V., Wim de Körverstraat 35 5831AN Boxmeer, the Netherlands, performs batch release. Proof of establishment in the EEA was provided.

A manufacturing authorisation was issued on 7 October 2022 by the Ministry of Agriculture, Nature and Food Quality of the Netherlands. A GMP certificate confirming compliance with the principles of GMP is provided. The certificate was issued on 22 January 2024, referencing an inspection on 28 – 31 August 2023, by the Ministry of Agriculture, Nature and Food Quality of the Netherlands.

## *Overall conclusions on administrative particulars*

The summary of the pharmacovigilance system master file is considered to be in line with legal requirements.

The GMP status of the active substance(s) and of the finished product manufacturing sites has been satisfactorily established and are in line with legal requirements.

# Part 2 - Quality

## *Quality documentation (physico-chemical, biological, and microbiological information)*

### Qualitative and quantitative composition

Nobilis Multriva Gm+REOm is an inactivated viral poultry vaccine presented in bottles containing 300 ml (1000 doses) and 600 ml (2000 doses).

The vaccine consists of the following antigens: inactivated infectious bursal disease virus strain GB02, inactivated infectious bursal disease virus strain 89/03, inactivated avian reovirus strain ARV-1, inactivated avian reovirus strain ARV-4 as active substances and light liquid paraffin as adjuvant. Polysorbate 80, sorbitan oleate and PBS are included as excipients.

### Container and closure system

The product is packed in a polyethylene terephthalate (PET) container (compliant with Ph. Eur. 3.2.2, USP 661, USP 87 and USP 88), closed with a halogenated butyl rubber stopper (Ph. Eur. 3.2.9) and aluminium caps. Container and stopper are sterilised by ionising radiation at a minimum dose of 25 kGy. Example certificates of analysis for the packaging materials are provided in the dossier.

The container sizes are consistent with the vaccination schedule and intended use. The containers and closures are in compliance with the pharmacopoeial requirements and their sterilisation is adequate.

### Product development

The Nobilis Multriva inactivated vaccine range can be regarded as the successor of the Nobilis inactivated vaccine range containing the inactivated avian metapneumovirus (AMPV), infectious bronchitis virus (IBV), Newcastle disease virus (NDV), infectious bursal disease virus (IBDV), egg drop syndrome virus (EDSV) and/or avian reovirus (ARV) antigens. The Nobilis Multriva vaccine dose is 0.3 ml vaccine dose, compared to the vaccine dose of 0.5 ml of the Nobilis inactivated vaccine range manufactured by the applicant. IBDV D78 strain was replaced by the combination of IBDV GB02 (vIBDV) and IBDV 89-03 (VarE). This combination should provide protection against all classic, very virulent and different variant IBDV strains. ARV 1733 and 2408 (both genetic class 1b) are replaced by a particular combination of two new strains (ARV-1 and ARV-4) originating from the genetic class 1b and 4 to provide protection against all described genotypes.

The adjuvant (oil phase of the emulsion) is the same as for the Nobilis inactivated range manufactured by the applicant.

The control tests were updated replacing animal testing where possible.

The antigens, adjuvant and excipients are qualitatively and quantitatively the same throughout the whole Nobilis Multriva range.

## *Description of the manufacturing method*

Active substances are manufactured as described in the respective vaccine antigen master files. For

the four antigens standard manufacturing methods are used. Virus strains are cultured on Vero cells and subsequently inactivated and concentrated where appropriate. The processes were appropriately validated, control tests are adequate to assure the quality and consistency of the antigens.

The final product is manufactured by adding the inactivated antigen suspensions to PBS. Oil soluble constituents (sorbitan oleate and polysorbate 80) are dissolved in liquid paraffin and the solution is sterilised. The oil solution and the aqueous suspension are subsequently mixed and emulsified. An example formulation calculation is provided.

Bottles are filled and closed with a stopper and secured with a cap. The final product is stored at 2-8°C.

The process is considered to be a standard manufacturing process. The major steps of the manufacturing process have been validated by three consecutive batches of the full-scale finished product (Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS). It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible and consistent manner. The in-process controls are adequate for this type of manufacturing process.

## *Production and control of starting materials*

## Starting materials listed in pharmacopoeias

Starting materials listed in pharmacopoeias and used for manufacturing of antigens are listed in the respective VAMFs. Certificates of analysis have been provided and all conform to the relevant specifications.

Starting materials listed in pharmacopoeias and used in the production of the finished product are:

| | |
|---|---|
| Disodium phosphate dihydrate | Ph. Eur. Monograph 0602 |
| Paraffin, light liquid | Ph. Eur. Monograph 0240 |
| Polysorbate 80 | Ph. Eur. Monograph 0428 |
| Sodium chloride | Ph. Eur. Monograph 0193 |
| Sodium dihydrogen phosphate dihydrate | Ph. Eur. Monograph 0194 |
| Sorbitan oleate | Ph. Eur. Monograph 1041 |

Example certificates of analysis are provided for these starting materials and all materials comply with Ph. Eur. requirements.

## Starting materials not listed in a pharmacopoeia

### *Starting materials of biological origin*

Starting materials of biological origin not listed in pharmacopoeias and used for manufacturing of antigens are listed in the respective antigen master files. The seed materials are sufficiently characterised and appropriate tests have been performed to assure their quality. Other materials of biological origin conform to the in-house specifications as illustrated by certificates of analysis. The materials used conform to the requirements. No other starting materials of biological origin are used in the manufacturing of the finished product.

***Starting materials of non-biological origin***

Starting materials of non-biological origin not listed in pharmacopoeias and used in the manufacturing of antigens are listed in the respective VAMFs. No other starting materials of non-biological origin are used in the manufacturing of the finished product.

***In-house preparation of media and solutions consisting of several components***

Adequate information regarding the qualitative and quantitative composition of culture media used in the manufacturing of the antigens, their treatment processes and their storage conditions is provided in the respective VAMFs. The risk of contamination with extraneous agents was evaluated and considered negligible for each of the four antigens. An evaluation of the risk of presence of extraneous agents in the finished product is provided. The conclusion is acceptable.

Information regarding the qualitative and quantitative composition of solutions used in the manufacturing of the final product, their treatment processes and their storage conditions is provided in the dossier. All components are either tested for or treated to ensure that there are no contaminants.

A TSE risk assessment on the finished product is provided in accordance with EMEA/410/01. The risk that the vaccine transmits TSE to chicken is estimated as practically zero.

## *Control tests during the manufacturing process*

The control tests performed during the manufacture of the antigens are described in the respective VAMFs. These in-process control tests have been appropriately validated and are deemed to be sufficient to control all the critical steps in the manufacturing of the antigens.

The only control test performed during the manufacturing of the finished product is filling volume. The relevant test method (weighing) is considered to fall under GMP. This in-process test is deemed to be sufficient to control the critical step in the manufacturing of the finished product.

## *Control tests on the finished product*

A description of the methods used for the control of the finished product (appearance, viscosity, accelerated stability [stability of the emulsion], identity and potency, type of emulsion, free formaldehyde, sterility) and the respective specifications were provided.

The proposed tests are considered adequate to control the quality of the finished product. The tests performed on the finished product were appropriately validated and limits have been set.

## *Batch-to-batch consistency*

The results of in-process and finished product testing on three consecutive batches of the full-range finished product (Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS) and one additional batch of Nobilis Multriva RT+IBm+ND+Gm+REOm (no EDS antigen) are presented in tabular format. All batches conformed with all requirements, consistent results were obtained.

These batches are considered appropriate and the results representative also for this vaccine.

## *Stability*

The stability of the bulk antigens is addressed in the respective VAMFs. From the data provided, the antigen's storage period has been adequately demonstrated.

The proposed shelf life of the product is 24 months at 2-8°C. Long-term stability data are provided for a number of batches. Batches were tested for appearance, viscosity, sterility (at the end of the storage period, to confirm integrity of closure) and potency for each of the antigens.

For all batches ($n$=17), appearance and viscosity conformed with the requirements throughout the 27-months storage period. Batches ($n$=3) remained sterile for up to 51 months. Potency for all of the antigens was tested at different times and was shown to remain stable and within specifications for at least 27 months of storage thereby supporting the proposed 24 months shelf life.

Stability data was provided on vials from two batches stored for 3 days at 37°C after broaching. Batches were fully tested and shown to be stable. The data support the claimed 10 hour in-use stability at 20-25°C.

## *Overall conclusions on quality*

Nobilis Multriva Gm+REOm is an emulsion for injection for chickens containing inactivated avian infectious bursal disease virus strain GB02, inactivated avian infectious bursal disease virus strain 89/03, inactivated avian reovirus strain ARV-1 and inactivated avian reovirus strain ARV-4 as active substances, and light liquid paraffin as adjuvant. The vaccine is presented in packs containing 1 bottle of 300 ml (1000 doses) or 600 ml (2000 doses).

The manufacturing method consists of blending of the different components followed by emulsification and can be considered as standard for this type of vaccine. For the 4 antigen components a Vaccine Antigen Master File is presented.

Procedures have been implemented to ensure the absence of extraneous agents in starting materials of animal origin. A TSE risk assessment for the starting materials used is provided. The risk that the final product may transmit TSE to the target animal is considered to be practically zero.

The production method, including appropriate in-process controls and quality control on the finished product together with control of the starting materials, generally ensure a consistent quality of batches of vaccine. The whole production process was evaluated at production scale and shown to be consistent.

The data provided support the proposed 24-month shelf life of the finished product. Stability data of broached product kept at elevated temperatures support the proposed 10 hours in-use shelf life at room-temperature.

In conclusion, the production process is adequately described and controls in place are generally appropriate to ensure the quality of the product at release and throughout the shelf life.

# Part 3 – Safety documentation (safety and residues tests)

## *General requirements*

Nobilis Multriva Gm+REOm is presented as an emulsion for injection for chickens containing inactivated avian infectious bursal disease virus and inactivated avian reovirus as active substances and liquid paraffin (mineral oil) as adjuvant. The vaccine is to be administered to chickens intramuscularly as a single dose of 0.3 ml in the breast or thigh region from 8 weeks of age onwards, but no later than 3 weeks before the onset of lay. The booster vaccination should be given at least 4 weeks after administration of the primary vaccination.

A full safety file in accordance with Article 8(1)(b) of regulation (EU) 2019/6 has been provided.

## *Safety documentation*

Five safety studies were conducted to investigate the safety of the product and included one pre-clinical study investigating the safety of the administration of one dose and four clinical trials. These studies were performed with the full-combination vaccine of the Nobilis Multriva range (Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS). The use of the full-combination vaccine is considered as a worst-case scenario for safety and is as such acceptable.

## *Pre-clinical studies*

One pivotal GLP safety study was provided. Safety of a single dose and of a double dose of the full-combination vaccine was studied in 7-week-old SPF birds. In order to achieve the 200% antigen content multiple vaccine blends had to be used (not all components of the full combination can be blended at 200% in one preparation, due to space limitations). Groups of 11 birds received one of the following: a single dose of the complete vaccine (0.3 ml), a double dose of the complete vaccine (0.6 ml), a single dose of a vaccine containing AMPV and EDSV antigens blended at 200% (0.3 ml), a single dose of a vaccine containing IBV, NDV, IBDV and ARV antigens blended at 200% (0.3 ml) or a 1:1 mix of the two 200% antigen vaccines (0.6 ml).

During the 14-day observation period, the birds were examined daily for clinical signs, intercurrent deaths and local reactions. None of the chickens showed abnormal signs of disease or died from causes attributable to vaccination during the observation period. No palpable local reactions were found in any of the birds. Post-mortem macroscopic and microscopic examinations were not performed.

On the basis of the results no safety concerns arose following the administration of the recommended dose or the double dose to chickens slightly below the minimum recommended age, providing therefore a valid demonstration of the safety of a single dose of the product. The absence of post-mortem data is considered justified, based on the high similarity of the composition of the product to the existing inactivated virus vaccines for poultry and the absence of palpable local reactions. The use of the full-combination vaccine is considered a worst-case and is acceptable.

## Safety of the administration of one dose

No overdose studies are required for inactivated vaccines.

## Safety of one administration of an overdose

No overdose studies are required for inactivated vaccines.

## Safety of the repeated administration of one dose

The vaccine is to be given once during a lifetime. No repeated dose safety studies are therefore required.

## Examination of reproductive performance

The vaccine is not intended for use during lay. Being an inactivated vaccine, the product is not considered a risk to the developing reproductive system. No specific studies were performed. In the field studies no differences were observed between vaccinated and control birds with respect to laying performance. A warning is included in the SPC not to use the product during lay or within 3 weeks before the onset of lay.

## Examination of immunological functions

The product is an inactivated vaccine and none of the components are considered a risk for the immune system, therefore no studies were performed. This is acceptable.

## User safety

The applicant has presented a user safety risk assessment which has been conducted in accordance with CVMP "Guideline on user safety for immunological veterinary medicinal products " (EMEA/CVMP/IWP/54533/2006) as well as the CVMP "Guideline on user safety for pharmaceutical veterinary medicinal products" (EMA/CVMP/543/03-Rev.01).

The main potential routes of accidental contact with the product have been considered and it was concluded that the most likely are those of accidental self-injection and dermal and/or oral exposure.

The active substances are inactivated viruses which are not infectious to humans. The excipients are commonly used in other vaccines and do not present a safety concern. However, the light mineral oil included in the formulation is of concern for the user since an accidental self-injection may have consequences that could, in the very worst-case, lead to loss of a digit due to blockage of blood vessels as a result of the pressure caused by inflammatory reactions.

As a result of the user safety assessment adequate information is included in section 3.5 of the SPC and since the product contains mineral oil, the standard warning for mineral oil-containing vaccines is appropriately included in the product literature.

Based on the above user risk assessment the CVMP concluded that the product does not pose an unacceptable risk to the user when used in accordance with the SPC.

## Study of residues

No studies of residues were performed. This is considered acceptable.

## MRLs

The active substances, being a principle of biological origin intended to produce active immunity, are not within the scope of Regulation (EC) No 470/2009.

The excipients, including adjuvants, listed in section 2 of the SPC are either allowed substances for which Table 1 of the Annex to Commission Regulation (EU) No 37/2010 indicates that no MRLs are required or are considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this product.

The antimicrobial substances used in the manufacturing process are present at low residual levels in the finished product. At these levels, they are devoid of pharmacological activity and are not considered to constitute a risk to the consumer.

## Withdrawal period

The withdrawal period is set at zero days.

## Interactions

The applicant has not provided data investigating interactions of the vaccine with any other veterinary medicinal product and therefore proposes to include a statement in Section 3.8 of the SPC that 'No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. A decision to use this vaccine before or after any other veterinary medicinal product therefore needs to be made on a case-by-case basis.' This is acceptable.

## *Clinical studies*

Tests with the largest combination of the Nobilis Multriva vaccine range (Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS) with the highest number of different antigens (9) are presented to substantiate the safety and efficacy of Nobilis Multriva Gm+REOm

Four single-centre, non-randomised, open, positive-controlled, two-armed GCP-compliant clinical studies with matched flocks were conducted to evaluate safety and efficacy in layer and broiler breeders. The studies were conducted in the Netherlands in well-managed farms.

All four studies were well designed and conducted and confirmed that the product is safe in both layer and broiler breeders when applied as part of the standard vaccination program.

| **Study 1: A field trial in the Netherlands to assess the safety and efficacy of a RT+IBm+ND+Gm+REOm+EDS vaccine in breeders** | |
|---|---|
| Study sites | Broiler breeder farm in the Netherlands, matched houses. |
| Study design | Open, positive controlled, two-armed (matched flocks). |
| Compliance with regulatory guidelines | GCP compliant |
| Animals | Conventional broiler breeders, originating from the same parent flocks and of the same age, were evenly distributed over the houses to obtain |

| | |
|---|---|
| | identical flocks of approximately 18,000 birds. |
| Test product | Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS, 0.3 ml, i.m. |
| Control product | Nobilis RT+IBmulti+G+ND 0.5 ml, i.m. plus Nobilis Reo inac. 0.5 ml i.m. |
| Vaccination scheme | Birds in the test group were vaccinated with the test product in the 14$^{th}$ week of life (right side breast muscle) while birds in the control group were vaccinated with the two control vaccines (in right and left side breast muscle). Prior to and after the 14$^{th}$ week, both flocks received all standard vaccinations for the farm. |
| Safety end points | Daily general health and feed intake for 2 weeks post vaccination (p.v.). Local reactions scored on 15 randomly selected birds in each group on days 1, 4, 7 and 14 p.v. and weekly thereafter until resolved. Egg production and hatchability was monitored for each group throughout the period of lay. Throughout the study, daily mortality, vaccinations and the use of medication were recorded. |
| Statistical method | Descriptive statistics (two groups) |
| ***Results*** | |
| Outcomes-Safety observations | General health and feed intake were scored as normal on all observation days.<br><br>A local reaction was observed in one bird (out of 15 tested) of the control group on day 7. In the test group on day 4, one bird showed a 2 cm long subcutaneous haemorrhage in the right breast, likely a result of mechanical trauma. On day 14 one bird had a large (10 x 3.5) subcutaneous reaction in the right breast. This concerned a hard swelling showing signs of inflammation (swollen and red). The bird was in good general condition. An additional scoring on day 20 revealed no further local reactions.<br><br>The mortality numbers were similar in both groups and comparable to normal mortality figures for this type of bird.<br><br>Egg production was good and highly comparable between groups. Fluctuations were due to general causes and detectable in both groups. Hatchability was highly similar. |
| Adverse events | Local reactions were observed both in the test and control groups. |
| ***Discussion*** | |
| Discussion/conclusions further to assessment | *The study was appropriately designed and conducted to an acceptable standard (GCP). While some local reactions were observed in the test group (but also in the control group), this did not lead to any changes in the performance of the flock when compared to the positive control group.*<br><br>*The study is considered to support the safety of the vaccine when applied under field conditions.* |

| **Study 2, A field trial in the Netherlands to assess the safety and efficacy of the RT+IBm+ND+Gm+REOm+EDS vaccine in layers** | |
|---|---|
| Study sites | Layer farm in the Netherlands, matched houses. |
| Study design | Open, positive controlled, two-armed (matched flocks). |
| Compliance with regulatory guidelines | GCP compliant |
| Animals | Conventional layers, originating from the same parent flocks and of the same age, were evenly distributed to obtain identical groups. Groups were housed in different rows of the rearing house and later placed in two houses to obtain identical flocks of approximately 10,000 birds. |
| Test product | Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS, 0.3 ml, i.m. |
| Control product | Nobilis RT+IBmulti+G+ND 0.5 ml, i.m. |
| Vaccination scheme | Birds in the test group were vaccinated with the test product in the 12$^{th}$ week of life (right side breast muscle) while birds in the control group were vaccinated with the control vaccine (right side breast muscle). Nobilis Salenvac T was applied at the same time as the study products, in the left breast muscle. Prior to and after the 12$^{th}$ week, both flocks received all standard vaccinations for the farm. |
| Safety end points | Daily general health and feed intake for 2 weeks post vaccination (p.v.). Local reactions scored on 15 randomly selected birds in each group on days 1, 4, 7 and 14 p.v. and weekly thereafter until resolved. Egg production was monitored for each group throughout the period of lay. Throughout the study, daily mortality, vaccinations and the use of medication were recorded. |
| Statistical method | Descriptive statistics (two groups) |
| ***Results*** | |
| Outcomes-Safety observations | General health and feed intake were scored as normal on all observation days.<br>Local reactions were not observed in control or test group.<br>The mortality numbers were similar in both groups and comparable to normal mortality figures for this type of bird.<br>Egg production was not correctly registered in the first weeks of the study. After the correction of the egg counter malfunction from week 27 egg production was comparable between groups. |
| Adverse events | No immediate or local reactions observed. |
| ***Discussion*** | |
| Discussion/conclusions further to assessment | *The study was appropriately designed and conducted to an acceptable standard (GCP). No general or local reactions were observed, and performance of the test and control groups was highly similar and within normal ranges. The study is considered to support the safety of the vaccine* |

| | |
|---|---|
| | *when applied in layers under field conditions.* |

| **Study 3, A field trial in the Netherlands to assess the safety and efficacy of the RT+IBm+ND+Gm+REOm+EDS vaccine in layers** | |
|---|---|
| Study sites | Layer farm in the Netherlands, matched houses. |
| Study design | Open, positive controlled, two-armed (matched flocks). |
| Compliance with regulatory guidelines | GCP compliant |
| Animals | Conventional layers, originating from the same parent flocks and of the same age, were evenly distributed to obtain identical groups. Groups were housed in different rows of the rearing house and later placed in two houses to obtain identical flocks of approximately 12,500 birds. |
| Test product | Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS, 0.3 ml, i.m. |
| Control product | Nobilis RT+IBmulti+G+ND 0.5 ml, i.m. |
| Vaccination scheme | Birds in the test group were vaccinated with the test product in the 12$^{th}$ week of life (right side breast muscle) while birds in the control group were vaccinated with the two control vaccines (in right and left side breast muscle). Avian encephalomyelitis/fowl pox vaccine was applied via wing web and Nobilis ILT via ocular route at the same time as the test and control vaccines.<br>Prior to and after the 12$^{th}$ week, both flocks received all standard vaccinations for the farm. |
| Safety endpoints | Daily general health and feed intake for 2 weeks post vaccination (p.v.). Local reactions scored on 15 randomly selected birds in each group on days 1, 4, 7 and 14 p.v. and weekly thereafter until resolved. Egg production was monitored for each group throughout the period of lay. Throughout the study, daily mortality, vaccinations and the use of medication was recorded. |
| Statistical method | Descriptive statistics (two groups) |
| ***Results*** | |
| Outcomes-Safety observations | General health and feed intake were scored as normal on all observation days.<br>Local reactions were not observed in the test or control groups.<br>Mortality was similar in both groups and somewhat higher than normal, which was attributed to feather pecking.<br>Egg production was within normal ranges and comparable between groups. |
| Adverse events | Immediate or local reactions were not observed. |

| ***Discussion*** | |
|---|---|
| Discussion/conclusions further to assessment | *The study was appropriately designed and conducted to an acceptable standard (GCP). No general or local reactions were observed, and performance of the test and control groups was highly similar and within normal ranges. The study is considered to support the safety of the vaccine when applied in layers under field conditions.* |

| **Study 4, A field trial in the Netherlands to assess the safety and efficacy of the RT+IBm+ND+Gm+REOm+EDS vaccine in broiler breeders primed with REOm** | |
|---|---|
| Study sites | Broiler breeder farm in the Netherlands, matched houses. |
| Study design | Open, positive controlled, two armed (matched flocks). |
| Compliance with regulatory guidelines | GCP compliant |
| Animals | Conventional broiler breeders, originating from the same parent flocks and of the same age, were evenly distributed over the houses to obtain identical flocks of approximately 12,000 birds. |
| Test product | Nobilis REOm 0.3 ml, i.m.<br>Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS, 0.3 ml, i.m. |
| Control product/ Placebo | Nobilis Reo 1133 (0.2 ml) plus Nobilis RT+IBmulti+G+ND 0.5 ml, i.m. plus Nobilis Reo inac. 0.5 ml i.m. |
| Vaccination scheme | Birds in the test group were vaccinated with the test product in the 8$^{th}$ week of life (Nobilis Multriva REOm) and 12$^{th}$ week of life (Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS). Birds in the control group were vaccinated in the 8$^{th}$ week of life with Nobilis Reo 1133 and in the 12$^{th}$ week with Nobilis RT+IBmulti+G+ND 0.5 ml, i.m. plus Nobilis Reo inac.<br>Concurrent with the vaccination at 8 weeks of age, birds were vaccinated via wingweb with Tremvac. Prior to and after these vaccinations, both flocks received all standard vaccinations for the farm. |
| Safety end points | Daily general health and feed intake for 2 weeks post vaccination (p.v.). Local reactions scored on 15 randomly selected birds in each group on days 1, 4, 7 and 14 p.v. and weekly thereafter until resolved. Egg production and hatchability was monitored for each group throughout the period of lay. Throughout the study, daily mortality, vaccinations and the use of medication was recorded. |
| Statistical method | Descriptive statistics (two groups) |
| ***Results*** | |
| Outcomes-Safety observations | Some data could not be collected as planned due to COVID-19 restrictions (hatchability data). |

| | |
|---|---|
| | General health and feed intake was scored as normal on all observation days.<br><br>A local reaction was observed in one bird (out of 15 tested) in the test group on day 7 (subcutaneous haemorrhage, Nobilis Multriva REOm) and in one bird on Day 15 (hard nodule in right breast 3x1 cm, Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS vaccination), the birds looked otherwise healthy. No local reactions were found one week later. Local reactions were found in one bird of the control group on Day 15 (mild subcutaneous inflammation in the right breast: Nobilis RT+IB multi+G+ND vaccination.<br><br>The mortality numbers were similar in both groups and comparable to normal mortality figures for this type of bird.<br><br>Egg production was good and highly comparable between groups. Hatchability was tested once and was similar. |
| Adverse events | Local reactions were observed both in the test and control groups. |
| ***Discussion*** | |
| Discussion/conclusions further to assessment | *The study was appropriately designed and conducted to an acceptable standard (GCP). While some local reactions were observed in the test group (but also in the control group), this did not lead to any changes in the performance of the flock when compared to the positive control group.*<br><br>*The study is considered to support the safety of the vaccine when applied to broiler breeders under field conditions.* |

## *Environmental risk assessment*

An environmental risk assessment has been performed in accordance with the "Note for guidance on environmental risk assessment for immunological veterinary medicinal products" (EMEA/CVMP/074/95-Final). It is concluded that the assessment can stop in phase I.

The product is a vaccine containing the inactivated viral poultry antigens and is adjuvanted with light liquid paraffin. Polysorbate 80, sorbitan oleate and PBS are included as excipients. The product is used in chickens and is administered intramuscularly. Therefore, direct exposure of the environment to the product does not take place. Any unused product or waste material does not pose an environmental risk. The product and waste should nevertheless be disposed by the appropriate channels and adequate advice is given in the product literature. As no live micro-organisms are present in the product, hazards and risks from the active ingredients are considered negligible. Excretion of any of the active compounds of the product or their metabolites by vaccinated animals, if occurring at all, will only be in minute amounts and does not pose a risk to the environment. In conclusion, this veterinary medicinal product is not expected to pose a risk for the environment when used according to the SPC.

## *Overall conclusions on the safety documentation*

By testing the largest combination of the Nobilis Multriva vaccine range (Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS) with the highest number of different antigens (9), the data found in the safety studies are considered applicable to Nobilis Multriva Gm+REOm.

The safety of a standard dose and a double dose of vaccine was tested in a GLP study. The vaccine

is blended to contain a standard dose of each antigen and thus there are no minimum or maximum potency vaccine batches. From the results it can be concluded that the vaccine is safe in birds from 7 weeks of age, when applied in accordance with the SPC.

The vaccine is to be applied once during the production lifetime of the birds. No repeated dose safety studies are therefore required.

The vaccine is not intended for use during lay. As an inactivated vaccine, the product is not considered a risk to the developing reproductive system. No specific studies were performed, and this is acceptable. A warning is included in the SPC to not use the product during lay or within 3 weeks before the onset of lay.

The product is an inactivated vaccine and none of the components are considered a risk for the immune system, therefore no studies were performed. The absence of specific studies or data on immunological functions is adequately justified.

The results of four field safety and efficacy field trials indicated no safety issues. The data are considered to support the safety of the vaccine when used in field conditions. In the field trials, no differences were observed between vaccinated and control birds with respect to laying performance, supporting the notion that the product does not pose a risk to the developing reproductive system when used as recommended.

A comprehensive user safety assessment has been provided by the applicant. Mineral oil was identified as major concern. Therefore, the standard warning for mineral-oil-containing vaccines is included in the product information, which is considered appropriate. The user safety has been adequately addressed and appropriate warnings are included in the SPC.

Based on the data provided, the ERA can stop at phase I. The product is not expected to pose a risk for the environment when used according to the SPC.

Residue studies are not required. The withdrawal period is set at zero days.

In conclusion, when used as recommended, the vaccine is considered to be generally safe for the target animal, the environment, the user and the consumer.

# Part 4 – Efficacy documentation (pre-clinical studies and clinical trials)

## *General requirements*

The following claims are made:

- Target species: Chickens
- Method of administration: Intramuscular injection
- Dose: 0.3 ml

Indication for use:

For the active immunisation of chickens for:

- passive immunisation of the progeny of the vaccinated chickens to:

- reduce mortality and clinical signs of disease caused by very virulent (CS89), classical (STC) strains of Infectious Bursal Disease virus.
- reduce viraemia and clinical signs of disease caused by Avian Reovirus genotypes 1 and 4.

Onset of immunity:

- IBDV and ARV: 4 weeks post-vaccination
- IBDV and ARV in progeny: 1 day of age

Duration of immunity:

- IBDV and ARV: 80 weeks of age
- IBDV and ARV in progeny: 3 weeks of age

Cross protection has been established for IBDV antigenic variant strains (variant E and GLS).
Cross protection has been established for ARV genotypes 2, 3 and 5.

The vaccine is positioned for use in chickens that have received primary vaccinations with live or inactivated vaccines against infectious bursal disease virus (e.g. Nobilis Gumboro D78, Innovax-ND-IBD) and avian reovirus (e.g. Nobilis Reo 1133, Nobilis Multriva REOm). This requirement is included in the SPC section 3.9. Therefore, data derived from studies in chickens that had received such prime-boost vaccinations were used to support onset and duration of efficacy.

## *Challenge models*

In order to demonstrate broad protection against very virulent, classical and antigenic variant strains of IBDV, the following IBDV challenge strains were used: Variant E challenge strain (Antigenic variant), GLS challenge strain (Antigenic variant), STC challenge strain (Classical) and CS89 challenge strain (Very virulent /Hyper- virulent), this is considered acceptable. Efficacy studies are set-up following EP monograph 0960 and 5.2.7 and are performed in the progeny of vaccinated chickens. The main clinical parameters to assess the efficacy of the vaccine are clinical signs, bursa histology, evidence of IBDV and mortality. As IBDV-specific antibodies are related to protection against IBD virus serological analysis was performed in vaccinated animals and progeny, this is acceptable as a correlate of protection.

In order to demonstrate broad protection against all circulating ARV strains, the following ARV challenge strains were used to assess vaccine efficacy: GA 96139: ARV genotype 1 (ARV-1), SL11A0249-12_FR: ARV genotype 2 (ARV-2), SL10A1581-32_ES: ARV genotype 3 (ARV-3) and GA 94594: ARV genotype 5 (ARV-5), this is acceptable. The challenge strains chosen are all different (heterologous) from the two strains in the vaccine. Efficacy studies are set-up following EP monograph 5.2.7 and are performed in the progeny of vaccinated chickens, mostly at one day of age, when birds are most susceptible to ARV. Some of the strains (ARV-2 and ARV-5) are expected to cause clinical signs of ARV disease very soon (within 2 days) after challenge of one-day-old birds. For the other strains, the efficacy read-out parameter used was viraemia. As ARV-specific antibodies are related to protection against ARV-induced viraemia and clinical signs, serological analysis was performed in vaccinated chickens in both pre-clinical and clinical studies as well as up to 21 days of the progeny of the vaccinated chickens. Serology is considered a correlate of a protective immune response.

## *Efficacy documentation*

Tests with the full-range vaccine out of the Multriva range (Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS) are presented to substantiate the efficacy of Nobilis Multriva

Gm+REOm.

A total of 27 studies were conducted to investigate the efficacy of the product and included 11 pre-clinical studies and 4 clinical trials that were further analysed in separately reported serological and challenge studies (12 in total). Laboratory studies were well documented and carried out in chickens of the minimum age recommended for vaccination, using pilot batches. Pilot batches were also used in the clinical trials.

## *Pre-clinical studies*

### Dose determination

Since the vaccine range Nobilis Multriva can be considered as an update and extension of the existing range of Nobilis inactivated vaccines, it is accepted that the vaccine (antigen) doses have been established based on prior experience.

### Onset of immunity

#### IBDV

Study 1 investigated the serological response to IBDV and ARV. A total of 230 day-old SPF hens and 26 roosters were included in the study. These were divided into 6 groups and treated as summarised in the table below:

| Treatment group | Number of birds | Prime vaccination at 1 day old | Prime vaccination at 2 weeks of age | Prime vaccination at 7 weeks of age | Boost vaccination at 16 weeks of age |
|---|---|---|---|---|---|
| 1 | 45 hens 5 roosters | none | none | none | none |
| 2 | 45 hens 5 roosters | none | none | none | Nobilis Multriva RT+IBm+ND+Gm + REOm+EDS |
| 3 | 45 hens 5 roosters | Innovax-ND-IBD | None | Nobilis Reo 1133 | Nobilis Multriva RT+IBm+ND+Gm + REOm+EDS |
| 4 | 45 hens 5 roosters | None | Nobilis Gumboro D78 | Nobilis Multriva REOm | Nobilis Multriva RT+IBm+ND+Gm + REOm+EDS |
| 5 | 25 hens 3 roosters | Innovax-ND-IBD | None | Nobilis Reo 1133 | Nobilis Multriva RT+IBm+ND+GB02 + REOm+EDS |
| 6 | 25 hens 3 roosters | None | Nobilis Gumboro D78 | Nobilis Multriva REOm | Nobilis Multriva RT+IBm+ND+Gm + REOm+EDS (25%) |

Antibodies to IBDV GB02 and 89/03 antigen were determined by virus neutralisation (VN) assay. Eggs collected from these birds were used in studies on the protection of progeny.
Antibody titres to IBDV (GB02 and 89/03) in hatch mates at day of age were below the detection limit (<5 $\log_2$) of the VN test. IBDV responses were consistently lower in group 2 (test vaccine only) compared to primed groups, but in all groups a strong response (12-16 $\log_2$) was observed at 20 weeks of age (4 weeks post vaccination with the test vaccine) and titres were maintained at this level to the last sampling point at 84 weeks post vaccination.

Study 2 evaluated the serology for IBDV and ARV in progeny from vaccinated birds. Eggs were collected at 26 weeks of age (10 weeks post-vaccination (w.p.v.) from the 5 groups of vaccinated birds in study

1. After hatching, blood samples to determine MDA were taken at Day 0, 8, 15, 22 and 29. All offspring had detectable levels of MDAs to IBDV GB02 and 89/03 up to 29 days of age. At day 29 the titres were clearly higher in the primed groups (6-8 $log_2$) compared to the non-primed group (3-4 $log_2$).

In study 3, the efficacy against IBDV CS89 challenge was determined in the offspring of vaccinated birds. The study was designed in accordance with Ph. Eur. 0960 requirements. Eggs were taken at 10 weeks post vaccination from birds in 1; groups of 25 hatched chicks were included in the study. In addition, a group of 10 MDA-chicks was included. At 21 days, all birds were challenged with IBDV CS89 and observed (scored) daily for 4 days. At Day 4 post challenge, birds were euthanised and bursa lesions were scored. The study can be considered valid since all controls were IBDV positive. Groups coming from 2 and 3 of study 1 complied with the test, whereas for the group coming from group 4 of study 1 the requirement (not more than 3 birds IBDV positive) was not met. It can be concluded that at 10 weeks post vaccination, Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS provides adequate protection to offspring against vvIBDV challenge in accordance with Ph. Eur. 0960 requirements, either with or without prior priming with Innovax-ND-IBD, with a DOI of 21 days in progeny.

In study 4, the efficacy against IBDV CS89 challenge was determined in the offspring of vaccinated birds. The study was designed in accordance with Ph.Eur.0960 requirements. Eggs were taken at 69 weeks post vaccination from birds in study 1; groups of 22 hatched chicks were included in the study. In addition, a group of 10 MDA-chicks was included. At 21 days, all birds were challenged with IBDV CS89 and observed (scored) daily for 4 days. At Day 4 post challenge, birds were euthanised and bursa lesions were scored. The study can be considered valid since all controls were IBDV positive. All vaccinated animals were fully protected. It can be concluded that at 69 weeks post vaccination, Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS provides adequate protection to offspring against vvIBDV challenge in accordance with Ph. Eur. 0960 requirements, either with or without prior priming with Innovax-ND-IBD, with a DOI of 21 days in progeny.

Several challenge studies were performed on offspring from birds in the field trials. These birds were all primed with Nobilis Gumboro D78 and boosted with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS. Protection against Variant E, STC and GLS strains was observed in offspring up to 21 days of age, when eggs were collected up to 66 weeks post vaccination.
In conclusion, based on the data provided, protection against vvIBDV is considered supported, with an OOI of 4 weeks. The claimed OOI in offspring of vaccinated birds (1 day of age) is considered supported for vvIBDV. Together the data is considered adequate to support the claimed protection against classical, very virulent and antigenic variant strains of IBDV, after vaccination according to a prime-boost schedule.

**ARV**

In study 1, described above in the section on IBDV, serological responses to ARV-1 and ARV-4 were determined by ELISA. The study can be considered valid, since control birds remained seronegative throughout the study. Birds vaccinated with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS at 16 weeks of age developed detectable antibodies to ARV-1 and ARV-4 by 4 weeks post vaccination, which remained at similar levels (7-8 $log_2$ for ARV-1 and 6-7 $log_2$ for ARV-4) between 30 and 100 weeks of age (84 weeks post vaccination).

In study 2, described above in the section on IBDV, serological responses to ARV-1 and ARV-4 in progeny (eggs taken at peak of lay, 10 w.p.v.) of vaccinated birds were investigated. MDAs against ARV-1 were detectable in all chicks at day 1, however there were a few birds (10-20%) with non-detectable antibodies to ARV-4. At 29 days, most of the MDA levels for ARV-1 and ARV-4 had decreased below the detection limit of their respective tests. In conclusion, since antibodies are required to confer protection

to progeny, the serological studies support an onset of immunity of 4 weeks post vaccination in principle.

The actual protection of progeny after vaccination of parents with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS after priming with Nobilis Reo 1133 or Nobilis Multriva REOm was shown in a number of studies. Chicks hatched from eggs taken at 44 weeks post vaccination were challenged at one day of age with ARV-1, ARV-2, ARV-3 and ARV-5 and were found to be protected from viraemia and clinical signs. In a second study in progeny taken at 63 weeks post vaccination, chicks were protected at day of age from ARV-5 challenge as detected by viraemia. In a further study in progeny taken at 35 weeks post vaccination, chicks were protected at day of age from ARV-2 and ARV-5 challenge as detected by viraemia and clinical signs. In a study in progeny taken at 63 weeks post vaccination, chicks at day of age had reduced clinical signs after ARV-2 challenge. Lastly, progeny taken at 70 weeks post vaccination had significantly reduced clinical signs after ARV-1 challenge at 14 days of age. Overall, based on the studies performed, it can be concluded that vaccination with the vaccine provides passive immunization of the progeny of vaccinated chickens to reduce mortality and clinical signs of disease caused by ARV with an onset of immunity of 4 weeks in the parent and 1-day-of-age in the offspring.

**Duration of immunity**

**IBDV**

Study 4 was set up similarly to study 3 above (section OOI, IBDV). However, eggs were collected when parents were 85 weeks old (69 weeks p.v.). All MDA- birds were found IBDV positive. None of the MDA+ chicks were IBDV positive. Based on these results, an average MDA HI titre of 6.3 $\log_2$ (IBDV GB02) and 6.2 $\log_2$ (IBDV 89/03) can be considered as a protective level. It can be concluded that Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS provides adequate protection of offspring against vvIBDV challenge either with or without prior priming with Innovax-ND-IBD or Nobilis Gumboro D78, with a duration of immunity of 69 weeks post vaccination in the parent flock and 21 days in the progeny.

Study 1 was performed in SPF layers to evaluate the serological response to the IBDV component of Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS vaccine. The study involved a prime-boost vaccination schedule. The antibody titres for anti-IBDV GB02 and anti-IBDV 89/03 antibodies were measured up to 84 weeks post-vaccination and found to be very stable. Eggs were collected from chickens vaccinated with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS alone or in combination with Innovax-ND-IBD or Nobilis Gumboro D78 when the chickens were 85 weeks of age. It was observed that all progeny derived from vaccinated chickens were 100% protected against vvIBDV (CS89) challenge at 3 weeks of age.

In conclusion, based on the data provided, in birds vaccinated according to a prime-boost schedule, a DOI of 80 weeks post vaccination against IBDV is considered supported. The claimed DOI in offspring of vaccinated birds (21 days of age) is also considered supported.

**ARV**

In study 1, described above in the section on IBDV, serological responses to ARV-1 and ARV-4 were determined by ELISA. Birds vaccinated with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS at 16 weeks of age developed detectable antibodies to ARV-1 and ARV-4 by 4 weeks post vaccination, which remained at similar levels up to 84 weeks post vaccination.

Study 2 was performed in the progeny of birds vaccinated in study 1 (described above (section OOI, IBDV). Chicks were derived from eggs collected at 44 weeks of age from groups 1, 3 and 6 (MDA-, Nobilis Reo 1133 + test vaccine and Nobilis Multriva REOm + Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS at 25%, respectively), and assigned to groups of 10 chicks challenged at one day of age with ARV-1, ARV-2, ARV-3 or ARV-5. Viraemia was tested at 3 days post challenge. In

the control groups, 60, 90, 78 and 50% of birds were positive for ARV, respectively. In the vaccinated groups, all birds were protected, with the exception of one bird in the Nobilis Reo 1133+Multriva group challenged with ARV-5 (90% protection, non-significant difference with controls). It can be concluded that for prime-boost regimens protection of progeny at one day of age against viraemia after genotype 1, 2, 3 and 5 challenge was adequate for up to 44 weeks post vaccination.

In study 3, progeny from birds in study 1 (refer to table in section OOI, IBDV) was used to test efficacy against ARV-5 challenge. Eggs were collected at 63 weeks of age from groups 1, 2, 3 or 4 (MDA-; Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS; Nobilis Reo 1133+ Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS; Nobilis Multriva REOm+ Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS ) and groups of 15 chicks were challenged at one day of age with ARV-5 virus. At day 3 post challenge, viraemia was determined in all birds. The study can be considered valid since, in the MDA- control birds, no antibodies were detected, and the level of infection was adequate (87%). A statistically significant reduction of the number of birds with viraemia after challenge was observed in all three vaccinated groups (protection 100%, 100% and 93%, respectively). A mean antibody titre of 5.9 $\log_2$ for ARV-1 and 4.7 $\log_2$ for ARV-4 was found in the progeny of layer birds that were only vaccinated with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS. It can be concluded that protection of progeny at one day of age against ARV genotype 5 challenge was adequate for up to 63 weeks post vaccination with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS with or without prior priming (Nobilis Reo 1133 or Nobilis Multriva REOm).

Study 4 included chicks derived from vaccinated parents (study 1, refer to section on OOI, IBDV). Eggs were collected at 51-52 weeks of age from birds in groups 1, 3 and 6 (MDA-, Nobilis Reo 1133 + Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS, Nobilis Multriva REOm + Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS blended at 25% antigens). Per parent group, 30 chicks were assigned to each of two groups and challenged at one day of age with ARV-2 or ARV-5. Birds were observed for clinical signs twice daily until 21 days post-challenge. Efficacy evaluation was based on clinical signs until Day 21, body weight at Day 21 and macroscopic and histological examinations of the hocks. The study can be considered valid since no antibodies were detected in MDA- control birds and the level of infection in the control birds was adequate with clinical signs, reduced weight gain (ARV-2 only) and histopathological lesions in the hocks. Progeny from birds in group 3 (Nobilis Reo 1133 + Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS) showed a reduction of clinical signs caused by either ARV-2 or ARV-5 challenge. Progeny from birds in group 6 (Nobilis Multriva REOm + Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS at 25%) showed a reduction of clinical signs caused by ARV-5 challenge and had significantly lower histopathological lesion scores after ARV-5 challenge. No significant effects of vaccination were observed after ARV-2 challenge in this group. This appears to indicate that the protection induced by this vaccination schedule is lower than for the Nobilis Reo 1133 + Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS combination, possibly due to the use of the 25% batch. The study provides support of efficacy, with a reduction of clinical signs due to ARV-2 and ARV-5 in progeny of birds primed with Nobilis Reo 1133 and boosted with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS with a DOI of 35 weeks post vaccination.

Study 5 was performed in progeny from parents vaccinated in study 1 (refer to section on OOI, IBDV). Eggs were collected when parents were 62-63 weeks of age. The parents were either non-vaccinated (offspring group A and C) or primed at 2 weeks of age with Nobilis Gumboro D78 and at 7 weeks of age with Nobilis Multriva REOm and vaccinated at 16 weeks of age with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS (offspring group B). Group A and B were challenged with ARV serotype 2, group C was mock-challenged. After challenge until the end of the study at 21 days post-challenge, birds were observed for clinical signs twice daily. Efficacy evaluation was based on weights, clinical signs (mortality and leg malfunction) and necropsy findings. Combined leg malfunctioning ARV scores were significantly lower in offspring from ARV vaccinated birds when compared to non-vaccinated birds: 73.3

% vs. 30%. The maximum clinical score observed was significantly lower in group B compared to group A. No difference in macroscopical necropsy scores of histology scores was observed. No difference in survival was observed since few birds reached the humane endpoint. It can be concluded that protection of progeny at day of age against clinical signs induced by serotype 2 challenge was adequate for up to 63 weeks post vaccination with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS with prior priming with Nobilis Multriva REOm.

Study 6 was performed in progeny from parents vaccinated in study 1 (refer to section on OOI, IBDV). Eggs were collected when parents were 86-88 weeks of age from groups 1, 3 and 4 (MDA-; Nobilis Reo 1133+ Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS; Nobilis Multriva REOm+ Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS). Chicks (30 per group) were challenged with ARV-1 in the footpad at 14 days of age. An additional MDA- group was mock-challenged. Birds were weighed and footpad and tendon measurements were collected at the time of challenge. Footpads were scored at 5 days post-challenge. At 19 and 28 days of age, body weights and footpad/tendon measurements were conducted. The tendon or footpad to body weight ratios were calculated. At the end of the study, birds underwent post-mortem examination to evaluate macroscopical lesions on the legs, hock-joint, tendons and footpads. In addition, samples were collected for histology. None of the MDA- birds showed detectable antibodies in the ARV ELISA. All of the MDA- challenged birds had swollen or reddened footpads at day 5 post challenge; the score was significantly higher compared to the mock-challenged group. Vaccinated birds were significantly protected against ARV-1-induced footpad swelling/discoloration when compared to MDA- birds. The tendon/body weight ratio was lower in the Nobilis Multriva REOm+Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS vaccine group compared to the MDA- birds, showing protection against tendon thickening. ARV-1 challenge did not lead to significant differences in macroscopic observations at necropsy compared to the mock-treated group. Thus, progeny from eggs collected at 70 weeks p.v. from birds primed with Nobilis Reo 1133 or Nobilis Multriva REOm and vaccinated with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS showed a significant reduction of footpad swelling (and tendon swelling) caused by ARV-1 challenge at 14 days of age.

In study 7, the same set-up was applied. Eggs were collected when parents were 93-95 weeks of age and chicks were challenged with ARV-1 in the footpad at 21 days of age. Significant protection of vaccinated birds was observed, supporting a DOI of 21 days in the progeny and a DOI of 79 weeks post-vaccination in the parent birds.

In study 8, eggs were collected from parent birds vaccinated at 16 weeks of age with Nobilis Multriva RT+IBm+ND+Gm+REOm (group 2) or at 7 weeks of age with Nobilis Reo 1133 and subsequently at 16 weeks of age with Nobilis Multriva RT+IBm+ND+Gm+REOm (group 3) while group 1 remained unvaccinated. Eggs were collected when parents were 37-39 weeks of age and chickens were challenged or mock-challenged at 14 or 21 days of age via footpad injection with ARV-5. Significant protection against footpad swelling was observed in progeny from group 2 challenged at day 14 and in progeny from group 3 challenged at day 14 or day 21.

In conclusion, birds vaccinated with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS at 16 weeks of age developed detectable antibodies to ARV-1 and ARV-4 by 4 weeks post vaccination, which remained stable until 84 weeks post vaccination. Progeny from vaccinated birds were shown to be MDA+ until 21 days of age, which is considered adequate to support the claimed DOI in progeny. Protection against ARV challenge was investigated in the progeny of vaccinated birds at one day of age since this is the time of the highest susceptibility of the chicks, which is acceptable. The use of viraemia as a read-out in most of these studies is considered acceptable, considering the rather complicated nature of the clinical disease. Protection of progeny after vaccination of parents with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS after priming with Nobilis Reo 1133 or Nobilis Multriva REOm was shown in a number of studies. Birds were challenged at one day of age with ARV-1, ARV-2, ARV-3 and ARV-5 and were found to be protected from viraemia and clinical signs.

**Maternally derived antibodies (MDA)**

No studies were performed. This is accepted since at the age of vaccination MDAs are no longer relevant.

**Interactions**

The applicant has not provided data investigating interactions of the vaccine with any other veterinary medicinal product and therefore proposes to include a statement in Section 3.8 of the SPC that 'No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. A decision to use this vaccine before or after any other veterinary medicinal product therefore needs to be made on a case-by-case basis.

## *Clinical trials*

Four clinical trials were performed in the Netherlands. In these experiments the safety and efficacy of Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS was evaluated under clinical conditions. The general set-up of the studies is described in the safety part of the dossier. In these four studies blood samples were collected and serological data were generated in nine studies. In order to substantiate efficacy claims, seven challenge experiments were performed in the laboratory with animals vaccinated in the field. The set-up of these studies and the results are discussed below for each virus.

**IBDV**

In study 1 serology was performed on samples taken during the first field study. Commercial layers in the two groups were vaccinated with various vaccines as per the farm vaccination scheme.

Blood samples were collected from at least 20 birds per flock at 12, 16, 18 and 22 weeks of age and then every 10 weeks until the end of the production period (approximately 86 weeks of age). Approximately 100 hens from the test flock were kept until 102 weeks of age and additional bleeds were taken from 20 birds at 92 and 102 weeks of age. Average antibody titres for the test flock increased after booster with the test vaccine, for both IBDV antigens, compared to the control flock, and remained high up to 102 weeks of age.

In study 2, serology was performed on samples taken during the second field study. Commercial layers in the two groups were vaccinated with various vaccines as per the farm vaccination scheme.

Blood samples were collected from 20 birds per flock at 12, 16, 18 and 22 weeks of age and then every 10 weeks until 92 weeks and at 100 weeks of age. Average antibody titres for the test flock increased after booster with the test vaccine, for both IBDV antigens, compared to the control flock, and remained high up to 100 weeks of age.

In study 3, serology was performed on samples taken during the third field study. Breeders in the two groups were vaccinated with various vaccines as per the farm vaccination scheme.

At 14, 19, 21, 25, 35, 45, 56, and 60 weeks of age blood samples were taken from 20 randomly selected hens in each group. Before the birds went to slaughter in week 60, approximately 100 birds from each flock were transferred to a research centre and were kept until they were approximately 84 weeks of age (study 4). Blood sampling from 20 selected tagged hens per flock was carried out at approximately 5-week intervals. Antibody profiles for test and control breeder flocks were similar for both IBDV antigens.

In study 5 serology was performed on samples taken during the fourth field study. Breeders in the

two groups were vaccinated with various vaccines as per the farm vaccination scheme.

Antibody profiles for test and control breeder flocks were similar for both IBDV antigens from 4 weeks post-vaccination to 100 weeks of age.

In study 6, IBDV and ARV MDA levels in progeny of broiler breeders were determined. The parents were vaccinated in another study with priming live vaccines against ARV and IBDV and with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS vaccine or Nobilis RT+IB multi+G+ND & Nobilis Reo inactivated vaccines. When the vaccinated parent birds were 44.5 weeks of age, eggs were collected, and birds were hatched for evaluation of MDA levels. From both groups, 30 progeny birds were placed in isolators and blood samples were taken on Days 1, 7, 14, 21 and 28. Samples were analysed for ARV-1, ARV-4, IBDV 89/02 and IBDV GB02. MDA antibody titres to IBDV were substantial up to 14 days of age in both groups and declined gradually thereafter.

Study 7 included 135 one-day-old progeny birds from broiler breeders that were vaccinated in the third field study with Nobilis Gumboro D78 at 27 days of age and with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS at 14 weeks of age. These chicks were hatched from eggs taken at 53 weeks of age. Control groups consisting of MDA- SPF layer chicks were also included. Birds were allocated to 8 challenge groups, 4 non-challenged pathology control groups and 4 serology groups. Challenge was performed at 7 or 14 days of age, with IBDV variant E or IBDV CS89 via ocular route. At all three time points tested, the mean VN titres for both IBDV GB02 and IBDV 89/03 in the MDA- birds were below the detection limit of the assay. In MDA+ birds, anti-IBDV titres showed a steady reduction over time, with mean anti-IBDV GB02 VN titres of 9.4 $\log_2$ and 6.1 $\log_2$ and mean anti-IBDV 89/03 VN titres of 9.8 $\log_2$ and 6.3 $\log_2$ at 7 and 14 days of age, respectively. Controls were all IBDV positive after VarE or CS89 challenge. Vaccinated birds were 100% and 91.3% protected from VarE challenge and 96% and 100% protected from CS89 challenge at 7 and 14 days of age, respectively. The study was appropriately designed except for the timing of the challenges (at one and two weeks of age), which is not in accordance with Ph. Eur. 0960 (3 weeks of age). Adequate protection against both variant E and CS89 strains was observed when chicks were challenged at 14 days of age. This indicates a duration of immunity of 40 weeks post vaccination after priming with Nobilis Gumboro D78 and vaccination with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS in commercial breeders.

Study 8 included 80 one-day-old progeny birds from broiler breeders vaccinated in the third field study with Nobilis Gumboro D78 at 27 days of age and with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS at 14 weeks of age. These chicks were hatched from eggs taken at 68 weeks of age. Control groups consisting of MDA- SPF layer chicks were also included. Birds were allocated to 6 challenge groups, 2 non-challenged pathology control groups and 4 serology groups. Challenge was performed at 21 days of age, with IBDV variant E or IBDV CS89 via ocular route. The VN titres in the MDA- animals were below the detection limit of both assays at all four time points. In MDA+ birds anti-IBDV titres showed a steady decline over time, with mean anti-IBDV GB02 VN titres gradually falling from a titre of 9.7 $\log_2$ at 1 day of age to 4.0 $\log_2$ at the time of challenge and the mean anti-IBDV 89/03 titres falling from 9.6 $\log_2$ at 1 day of age to 4.5 $\log_2$ at 21 days of age, with 3/10 birds having titres at or below the detection limit of the assay. All controls were IBDV positive, in the vaccinates 62.5% was protected from VarE and 100% from CS89 challenge. The study was appropriately designed, fully in accordance with Ph. Eur. 0960 requirements. Adequate protection against both variant E and CS89 strains was observed. This indicates a duration of immunity of 54 weeks post vaccination after priming with Nobilis Gumboro D78 before vaccination with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS.

Study 9 was performed using progeny of broiler breeders vaccinated in the third field study with Nobilis Gumboro D78 at 27 days of age and with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS at 14 weeks of age. These chicks were hatched from eggs taken at 80 weeks of age. Control groups consisting of MDA- SPF layer chicks were also included. Birds were allocated to 4 challenge groups, 2 non-challenged

pathology control groups and 4 serology groups. Challenge was performed at 21 days of age, with IBDV GLS strain or IBDV STC strain. At the time of challenge (21 days of age) the mean IBDV GB02 and IBDV 89/03 VN antibody titres were 4.2 $log_2$ and 5.4 $log_2$, respectively for the MDA+ test group, whilst antibody levels were below the detection limit in the MDA- control group. All control birds were IBDV positive. Vaccinated birds were 8% protected from IBDV GLS challenge and 91.7% protected from IBDV STC challenge. The study was designed fully in accordance with Ph. Eur. 0960 requirements. Adequate protection against STC, but not GLS, strain was observed. This indicates a duration of immunity of 66 weeks post vaccination after priming with Nobilis Gumboro D78 before vaccination with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS.

Study 10 included progeny from broiler breeders vaccinated in the fourth field study with Nobilis Gumboro D78 at 27 days of age and with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS at 15 weeks of age (test group) or with Nobilis Gumboro D78 at 27 days of age and with Nobilis RT+IBmulti+G+ND and Nobilis Reo inac at 15 weeks of age (control group). Offspring were hatched from eggs taken at 71 weeks of age. A challenge control group consisting of MDA- SPF layer chicks was included. Birds were allocated to 3 challenge groups and 4 serology groups. Challenge was performed at 14 days of age, with IBDV GLS strain via ocular route. In general, similar levels of anti-IBDV GB02 VN antibody were seen in the offspring of both vaccinated parent groups. The levels of anti-IBDV 89/03 VN antibodies tended to be slightly higher in the offspring of the test vaccine-vaccinated parents. At the time of challenge test vs control antibody levels were for anti-IBDV GB02: 7.3 $log_2$, vs 6.8 $log_2$; anti-IBDV 89/03: 5.4 $log_2$, vs 4.6 $log_2$. MDA- birds were seronegative at the time of challenge. All non-vaccinated control birds were IBDV positive. Test birds were 90% protected, control birds 100%. The study was appropriately designed, largely in accordance with Ph. Eur. 0960 requirements, except for the timing of the challenges. Adequate protection against IBDV GLS strain was observed when chicks were challenged at 14 days of age. This level of protection was achieved at 56 weeks post vaccination after priming with Nobilis Gumboro D78 before vaccination with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS.

Study 11 included progeny from broiler breeders vaccinated in the fourth field study with Nobilis Gumboro D78 at 27 days of age and with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS at 15 weeks of age. Offspring were hatched from eggs taken at 63 weeks of age. A challenge control group consisting of MDA- SPF layer chicks was included. Challenge was performed at 21 days of age, with IBDV GLS strain via ocular route. In MDA- animals, VN neutralisation levels were below detection levels. In the MDA+ group, a mean VN titre of 5.4 $log_2$ IBDV GB02 was found, whereas 6/10 birds were seronegative for IBDV 89/03 VN with a mean titre of 2.2. $log_2$. All MDA- birds were IBDV positive. The test group was 42% protected; this was statistically significant. The study was appropriately designed, fully in accordance with Ph. Eur. 0960 requirements. Protection against GLS strain was observed at 48 weeks post vaccination after priming with Nobilis Gumboro D78 before vaccination with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS.

In conclusion, high antibody titres to IBDV GB02 and IBDV 89/03 were found up to 102 weeks of age in layers and breeders vaccinated in the frame of a commercial vaccination programme, including Innovax-ND-IBD or Gumboro D78 priming. Protection was observed in progeny of such (primed) birds, against variant E and CS89 strains at 54 weeks post vaccination, against STC strain at 66 weeks post vaccination and against GLS strain at up to 56 weeks post vaccination. Since no clear decrease of antibody titres was observed in the serological studies, these prime-boost regimes are considered to provide protection of progeny against IBDV for the claimed 80 weeks post vaccination. The results of clinical studies support the claimed protection against IBDV.

**ARV**

Study 1: birds in the first field study were treated as summarised above for IBDV. Serological

evaluation was performed using an ARV-1 and an ARV-4 specific antibody ELISA. Birds were not primed with ARV antigen. In the test group (very) low titres specific for ARV-4 were observed. ARV-1 specific titres were medium already before vaccination, which is indicative of a field infection. After vaccination, ARV-1 titres increased to high levels in the vaccinated birds: a booster effect appears to have occurred. No conclusion can be drawn on ARV-1 titres in birds vaccinated with the test vaccine without priming vaccination. The results indicate very little effect of vaccination with the test vaccine alone on ARV-4 titres.

Study 2: birds in the second field study were treated as summarised above for IBDV. Serological evaluation was performed using an ARV-1 and an ARV-4 specific antibody ELISA. Birds were not primed with ARV antigen. In the test group, titres specific for ARV-4 increased after vaccination and remained at medium levels until 100 weeks. ARV-1 specific titres were medium already before vaccination, which is indicative of a field infection. After vaccination, ARV-1 titres increased to high levels in the vaccinated birds: a booster effect appears to have occurred. No conclusion can be drawn on ARV-1 titres in birds vaccinated with the test vaccine without priming vaccination. The results indicate some effect of vaccination with the test vaccine alone on ARV-4 titres.

Study 3: birds in the third field study were treated as summarised above for IBDV. As part of the routine vaccination schedule for the farm, all birds had been primed against ARV using Nobilis Reo 1133 (genotype ARV-1-like) at 8 weeks of age. Serological evaluation was performed using an ARV-1 and an ARV-4 specific antibody ELISA. In the test group, low titres specific for ARV-4 were initially observed after priming, which increased after vaccination with the test vaccine to medium-high and remained until 84 weeks of age. However, ARV-4 titres increased steeply between 56 and 62 weeks of age, which is suggestive of a field challenge and may well have helped maintain titres against ARV-4. ARV-1 specific titres were medium after priming and increased to high levels after vaccination with the test vaccine. The ARV-1-specific titres remained high for up to 84 weeks, however also here a (small) increase in titre was observed between weeks 56 and 62, suggestive of a field challenge.

Study 4: birds in the fourth field study were treated as summarised above for IBDV. Birds in the test group had been primed against ARV using Nobilis Multriva REOm, while the control flock had been primed with Nobilis Reo 1133 (genotype ARV-1-like) at 8 weeks of age. Serological evaluation was performed using an ARV-1 and an ARV-4 specific antibody ELISA. In the test group low titres specific for ARV-4 were initially observed; after priming the titre increased to medium high and after vaccination with the test vaccine these remained at medium high to medium levels until 100 weeks. ARV-1-specific titres were medium after priming and increased to high after vaccination with the test vaccine. The ARV-1-specific titres gradually decreased to medium levels at 100 weeks of age. Data are supportive of a DOI of 84 weeks post vaccination, after priming with Nobilis Multriva REOm.

In study 5, the levels of maternal derived antibodies (MDA) in the progeny of vaccinated broiler breeders from field study 3 were studied. The broiler breeders were vaccinated with live prime vaccines against Reo and Gumboro and with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS vaccine or Nobilis RT+IB multi+G+ND & Nobilis Reo inac vaccines at 15 weeks of age. When the vaccinated parent birds were 44.5 week of age, eggs were collected, and birds were hatched for evaluation of MDA levels. After hatching the progeny had substantial antibody titers for ARV1 and ARV4. These titres remained high until 7 days of age (doa) and decreased gradually over time; the detection limit was reached at 28 doa.

In study 6, progeny from broiler breeders in the test group in field study 3 was taken at 53 weeks of age to determine levels of MDA (for set-up refer section on IBDV above). The test group had been vaccinated with Nobilis Reo 1133 at 8 weeks and Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS at 14 weeks of age. The day-1 titre for ARV-1 was 5.6, decreasing to 2.2 Log2 at 14 doa, and for ARV-

4 this was 2.3 and 1.7 Log2 at 1 and 14 doa, respectively.

In study 7, progeny from broiler breeders in the test group in field study 3 was taken at 68 weeks of age to determine levels of MDA (for set-up refer section on IBDV above). The test group had been vaccinated with Nobilis Reo 1133 at 8 weeks and Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS at 14 weeks of age. Moderate levels of anti-ARV antibodies were seen in day-old birds which showed a steady reduction over time, with mean anti ARV-1 ELISA titres falling to 1.43 Log2 at 21 days-of-age and mean anti ARV-4 ELISA titres falling below the detection limit at 21 days-of-age.

Study 8: progeny from broiler breeders vaccinated in the third field study with Nobilis Reo 1133 at 8 weeks of age and with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS at 14 weeks of age were hatched from eggs taken at 80 weeks of age (n=80). Blood samples were taken at 1, 7, 15 and 21 days of age and analysed by ARV-1 and ARV-4 antibody ELISA. Anti-ARV titres showed a steady reduction over time, with mean anti ARV-1 ELISA titres falling from 4.3 (day 1) to 1.6 log2 at 21 days-of-age and mean anti ARV-4 ELISA titres falling from 2.9 (day 1) to 1,0 log2 at 21 days-of-age (titers at day 14 and 21 were below the detection limit of the assay).

Study 9: progeny from broiler breeders vaccinated in the fourth field study with Nobilis Multriva REOm at 8 weeks of age and with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS at 15 weeks of age (test group) or with Nobilis Reo 1133 at 8 weeks of age and with Nobilis RT+IBmulti+G+ND and Nobilis Reo inac at 15 weeks of age (control group) were hatched from eggs taken at 71 weeks of age. Blood samples were taken at 1, 7 and 14 days of age and analysed by ARV-1 and ARV-4 antibody ELISA for MDA. Antibody titres to ARV-1 were of similar, moderate levels at day-of-age, in progeny of control or test birds, gradually declining to non-detectable levels at 21 days of age. Anti-ARV-4 titres were only detected in progeny from test birds, falling below detection level between day 14 and 21.

In study 10, progeny from broiler breeders in field study 4 was challenged with ARV strains. The test group was vaccinated with Nobilis Multriva REOm vaccine at 8 weeks of age followed by a booster vaccination at 14 weeks of age with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS vaccine. The control group was vaccinated with live Nobilis Reo 1133 at 8 weeks of age followed by booster vaccination at 14 weeks of age with Nobilis Reo inac. Eggs were collected when birds were 44 weeks of age, MDA- control groups consisted of day-old SPF chicks. Challenge was performed at day of age with ARV-1, ARV-2, ARV-3 or ARV-5 challenge strain (i.m.). After challenge birds were scored for clinical signs twice daily. Blood was collected from hatch mates at t=0 and at t=3 all birds were euthanised and blood was collected to screen for ARV viraemia. The MDA+ test group showed mean antibody titers for ARV-1 of 5.7 $\log_2$ and ARV-4 of 3.2 $\log_2$. The MDA+ control group showed an antibody titre for ARV-1 of 4.9 $\log_2$ but no ARV-4 titre was observed. After challenge with 4 different ARV genotype (ARV-1, -2, -3 and -5) strains there was no viraemia detected in any of the progeny of both vaccinated MDA+ broiler groups. On the other hand, the majority of progeny of the MDA- control group (70-100%) were tested positive for the presence of viraemia. It is noted that it is unclear whether broilers and (SPF) layers have the same susceptibility to viraemia, MDA- broilers were not available. Based on the data, protection against clinical signs and viraemia after ARV-1, -2, -3 and -5 challenge can be expected in the progeny of vaccinated birds at day-of-age.

In study 11, progeny from broiler breeders included in field trial 3 was challenged with ARV strains. The test group was vaccinated with Nobilis Multriva REOm vaccine at 8 weeks of age followed by a booster vaccination at 15 weeks of age with inactivated Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS vaccine. Eggs were collected when birds were 61 weeks of age, MDA- control groups consisted of day-old SPF chicks. Challenge was performed at day of age with ARV-1 or ARV-2 challenge strain (i.m.) or mock-challenge with PBS was given. After challenge birds were scored for clinical signs for 21 days. Cloacal swabs were taken daily from day 1 to 6 and on day

10, to determine shedding. Blood was collected from hatch mates at t=0 and at t=3 blood was collected in the viraemia groups to screen for ARV viraemia. Macroscopic and histopathological changes in hock joints were evaluation at necropsy (21 days p.c.), bodyweight was recorded on day 1 and 21. Shedding in MDA+ was significantly lower at day 1 and 2 after ARV-1 challenge and on days 3 to 5 after ARV-2 challenge. It is possible that this could be due to different infection dynamics in broilers versus layers rather than an effect of MDA. After ARV-2 challenge, clinical signs occurred in both MDA+ and MDA- birds, these were significantly higher compared to non-challenged birds. The results of the study appear to support the notion that broilers are more susceptible to clinicals signs of ARV (except effects on growth) compared to layer type birds. The absence of an MDA- broiler group makes it difficult to draw (any) conclusions from the study.

In conclusion, efficacy against challenge with ARV-1, 2, 3 and 5 strains was shown in progeny from birds at 30 weeks post vaccination. A reduction of clinical signs (ARV-2 and ARV-3) and viraemia was achieved. These results support the results of the pre-clinical studies.

## *Overall conclusion on efficacy*

**IBDV**

The proposed indication for IBDV is passive immunisation of the progeny of the vaccinated chickens to reduce mortality and clinical signs of disease caused by very virulent (CS89) and classical (STC) strains of infectious bursal disease virus and cross-protection against antigenic variants (variant E and GLS). The vaccine is to be used as a booster vaccination following priming with live or inactivated vaccines against infectious bursal disease virus (e.g. Nobilis Gumboro D78, Innovax-ND-IBD). The claimed onset of immunity 4 weeks post vaccination and the duration of immunity 80 weeks post-vaccination. In progeny, an OOI of 1 day of age and a DOI of 3 weeks of age is claimed. A total of 4 pre-clinical studies was performed in support of the efficacy against IBDV: a serological study in vaccinated birds, a serological study in progeny and two challenge studies in progeny.

Birds vaccinated with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS at 16 weeks of age developed detectable antibodies to IBDV GB02 and IBDV 89/03 by 4 weeks post vaccination, which remained at similar levels until 67 weeks of age (51 weeks post vaccination). Antibodies in progeny of vaccinated birds (MDA) could be detected at least up to 15 days of age. Eggs were collected at 10 weeks and at 69 weeks post vaccination. When challenged with vvIBDV at 21 days of age, the progeny was adequately protected. This result was achieved with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS vaccination either with or without prior priming with Innovax-ND-IBD. In another study, birds were primed with Nobilis Gumboro D78 or Innovax-ND-IBD and boostered with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS. The antibody titres for anti-IBDV GB02 and anti-IBDV 89/03 antibodies were measured up to 84 weeks post-vaccination and found to be very stable. Eggs were collected from vaccinates at 85 weeks of age. It was observed that all progeny derived from vaccinated chickens were 100% protected against vvIBDV (CS89) challenge at 3 weeks of age.

Based on the data provided, protection against vvIBDV is considered supported, with an OOI of 4 weeks and a DOI of 80 weeks post vaccination. The claimed onset (1 day of age) and duration (21 days of age) of immunity in offspring of vaccinated birds is considered supported for vvIBDV.

Five serological studies were performed to evaluate IBDV efficacy in the field studies. IBDV antibodies were detected by Gumboro GB02 and Gumboro 89/03 virus neutralisation assay. Average titres in test and control flocks were similar. In addition, five laboratory challenge studies were performed using birds derived from the clinical trials. In one study the MDA titres in progeny from test and control group broiler breeders were determined at 30 weeks post vaccination. IBDV MDA titres were substantial until 14 days of age.  Several challenge studies were performed on offspring from birds in the field trials.

These birds were all primed with Nobilis Gumboro D78 and boostered with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS. Protection against Variant E, STC and GLS strains was observed in offspring up to 21 days of age, when eggs were collected up to 66 weeks post vaccination. These studies support the claimed DOI of 21 days in progeny as well as the claimed cross protection against variant strains.

**ARV**

The vaccine is intended to induce passive immunity in the progeny of vaccinated chickens, to reduce viraemia and clinical signs of disease caused by avian reovirus (genotypes 1 and 4) and to provide cross-protection against ARV genotypes 2, 3 and 5. The vaccine is to be used as a booster vaccination following priming with either live or inactivated vaccines (Nobilis Reo 1133, Nobilis Multriva REOm). The claimed onset of immunity is 4 weeks post-vaccination and 1 day of age in the progeny, the duration of immunity is 80 weeks post-vaccination in the vaccinated chickens and 3 weeks in the progeny. A total of 6 pre-clinical studies were performed in support of the efficacy against ARV: a serological study in vaccinated birds, a serological study in progeny and four challenge studies in progeny.

Birds vaccinated with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS at 16 weeks of age developed detectable antibodies to ARV-1 and ARV-4 by 4 weeks post vaccination which remained at similar levels until 100 weeks of age (84 weeks post vaccination). Offspring of SPF birds vaccinated with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS alone or after priming with Reo vaccines showed levels of MDA that persisted until 28 days of age. Protection of progeny after vaccination of parents with Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS after priming with Nobilis Reo 1133 or Multriva REOm was shown in a number of studies. Birds were challenged at one day of age with ARV-1, ARV-2, ARV-3 and ARV-5 and were found to be protected from viraemia and clinical signs.

The onset of immunity of 4 weeks p.v. is considered sufficiently supported by the serological data. Cross-protection against serotype 2, 3 and 5 is considered sufficiently supported by the data provided. The claimed DOI of 80 weeks post vaccination is sufficiently supported by the data. Protection against ARV-4 is assumed based on presence of anti-ARV 4 antibodies and the cross-protection shown for the closely related ARV-5.

Four serological studies were performed on sera taken from the clinical trials. In addition, five serological studies in progeny of vaccinated birds were performed, as well as two (laboratory) challenge studies in progeny from field-vaccinated animals. Antibody titres to ARV-1 and ARV-4 were of similar, moderate levels at day-of-age, in progeny of birds primed using Nobilis Reo 1133 or Nobilis Multriva REOm and boostered using Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS, in the different field studies. In the progeny the antibody titres gradually declined, disappearing by 21 days post vaccination. Antibody levels were similar in progeny from eggs collected at 68, 71 or 80 weeks of age of the parent flock. A challenge study was performed in progeny (broilers) derived from eggs collected at 30 weeks post vaccination from birds vaccinated with Nobilis Multriva REOm and Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS. An MDA- control groups consisted of day-old SPF chicks. Challenge was performed at day of age with ARV-1, ARV-2, ARV-3 or ARV-5 challenge strains. The results support protection against clinical signs (ARV-2 and ARV-3) and viraemia for all challenge strains. A second study in progeny derived from eggs taken at 46 weeks post vaccination from breeders vaccinated in the field with Nobilis Multriva REOm and Nobilis Multriva RT+IBm+ND+Gm+REOm+EDS suggests a reduction of viraemia but not clinical signs after ARV-1 or ARV-2 challenge. In general, results from clinical trials support the efficacy of the prime-boost regimens against ARV-1, -2, -3, -4 and -5.

# Part 5 – Benefit-risk assessment

## *Introduction*

Nobilis Multriva Gm+REOm is presented as an emulsion for injection for chickens containing inactivated infectious bursal disease virus, strain GB02, inactivated infectious bursal disease virus, strain 89/03, inactivated avian reovirus, strain ARV-1 and inactivated avian reovirus, strain ARV-4 as active substances and light liquid paraffin as adjuvant. The target species is chickens.

This vaccine is intended for use as a booster vaccination following priming with either live or inactivated vaccines in the vaccination schedule. The vaccine is to be administered intramuscularly as a single dose of 0.3 ml in the breast or thigh region from 8 weeks of age onwards, but no later than 3 weeks before the onset of lay.

At the time of submission, the applicant applied for the following indications:

For the active immunisation of chickens for passive immunisation of the progeny of the vaccinated chickens to reduce mortality and clinical signs of disease caused by very virulent (CS89) and classical (STC) strains of infectious bursal disease virus (IBDV), and to reduce viraemia and clinical signs of disease caused by avian reovirus (ARV) genotypes 1 and 4.

Onset of immunity: - IBDV and ARV: 4 weeks post-vaccination.

- IBDV and ARV in progeny: 1 day of age

Duration of immunity: - IBDV and ARV: 80 weeks post-vaccination.

- IBDV and ARV in progeny: 3 weeks of age.

Cross protection has been established for IBDV antigenic variant strains (variant E and GLS).

Cross protection has been established for ARV genotypes 2, 3 and 5.

The proposed withdrawal period is zero days.

The vaccine is presented in packs containing 1 bottle of 300 ml (1000 doses) or 600 ml (2000 doses).

The dossier has been submitted in line with the requirements for submissions under Article 8 of Regulation (EU) 2019/6 – full application.

## *Benefit assessment*

### Direct benefit

The proposed benefit of Nobilis Multriva Gm+REOm is its efficacy against IBDV and ARV, which was investigated in a large number of well-designed pre-clinical and clinical studies conducted to an acceptable standard. The studies were performed with the full-combination vaccine, results are considered applicable for fall-out vaccines like Nobilis Multriva Gm+REOm.

The following indication is considered supported by the data provided:

For the active immunisation of chickens for passive immunisation of the progeny of the vaccinated chickens to reduce mortality and clinical signs of disease caused by very virulent (CS89) and classical (STC) strains of infectious bursal disease virus (IBDV), and to reduce viraemia and clinical signs of disease caused by avian reovirus (ARV) genotypes 1 and 4.

Onset of immunity: - 4 weeks post-vaccination.

- in progeny: 1 day of age

Duration of immunity: - 80 weeks post-vaccination.

- in progeny: 3 weeks of age.

Cross protection has been established for IBDV antigenic variant strains (variant E and GLS).

Cross protection has been established for ARV genotypes 2, 3 and 5.

**Additional benefits**

Nobilis Multriva Gm+REOm is a combination of inactivated viral components, reducing the need for the application (injection) of different vaccines within a short timeframe. Compared to existing inactivated viral vaccine combinations, the dose volume is smaller which is an advantage with respect to injection-site safety and animal welfare.

## *Risk assessment*

*Quality*

Information on development, manufacture and control of the finished product has been presented in a satisfactory manner. Quality data for each of the antigens is included in the respective vaccine antigen master files. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use. The whole production process was evaluated at production scale and shown to be consistent. The data provided support the proposed 24-month shelf life.

*Safety*

Measures to manage the risks identified below are included in the risk management section.

*Risks for the target animal*

Administration of Nobilis Multriva Gm+REOm in accordance with SPC recommendations is generally well tolerated.

The safety of the vaccine in chickens at 7 weeks of age was confirmed in a GLP safety study and four clinical trials. The main reported adverse reaction is injection site swelling that was observed in some animals after being administered the standard dose. However, the effects were mild and transient.

*Risk for the user*

The CVMP concluded that user safety for this product is acceptable when used according to the SPC recommendations. Standard safety advice for veterinary medicinal products containing mineral oil is included in the SPC.

*Risk for the environment*

Nobilis Multriva Gm+REOm is not expected to pose a risk for the environment when used according to the SPC recommendations. Standard advice on waste disposal is included in the SPC.

*Risk for the consumer:*

The product is not considered to pose a risk to consumer safety. Based on the components, residue

studies are not required. The withdrawal period is set at zero days.

*Special risks*

None identified.

## *Risk management or mitigation measures*

Appropriate information has been included in the SPC to inform on the potential risks of this product relevant to the target animal, user and environment and to provide advice on how to prevent or reduce these risks.

*User safety*

User safety risks have been identified. These risks have been addressed by the safety warnings included in the SPC.

*Environmental safety*

No specific environmental safety risks have been identified. Standard advice on waste disposal is included in the SPC.

Conditions or restrictions as regards the supply or safe and effective use of the VMP concerned, including the classification (prescription status)

The veterinary medicinal product is subject to a veterinary prescription.

## *Evaluation of the benefit-risk balance*

The product has been shown to be efficacious for the active immunisation of chickens for passive immunisation of the progeny of the vaccinated chickens to reduce mortality and clinical signs of disease caused by very virulent (CS89) and classical (STC) strains of infectious bursal disease virus (IBDV), and to reduce viraemia and clinical signs of disease caused by avian reovirus (ARV) genotypes 1 and 4. Cross protection has been established for IBDV antigenic variant strains (variant E and GLS) and for ARV genotypes 2, 3 and 5.

Information on development, manufacture and control of the active substance and finished product has been presented and lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use. It is well tolerated by the target animals and presents an acceptable risk for users, the environment and consumers, when used as recommended. Appropriate precautionary measures, including withdrawal period, have been included in the SPC and other product information.

Based on the data presented, the overall benefit-risk balance is considered positive.
The product information has been reviewed and is considered to be satisfactory and in line with the assessment.

## *Conclusion*

Based on the original and complementary data presented on quality, safety and efficacy, the Committee for Veterinary Medicinal Products (CVMP) considers that the application for Nobilis Multriva Gm+REOm is approvable since these data satisfy the requirements for an authorisation as set out in the legislation (Regulation (EU) 2019/6).

The CVMP considers that the benefit-risk balance is positive and, therefore, recommends the granting of the marketing authorisation for the above mentioned veterinary medicinal product.