EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

05 December 2024
EMA/8688/2025
Veterinary Medicines Division

Committee for Veterinary Medicinal Products (CVMP)

CVMP assessment report for Poulvac Procerta HVT-IBD-ND (EMEA/V/C/006306/0000)

Vaccine common name: Newcastle disease, infectious bursal disease and Marek's disease vaccine (live recombinant)

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

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

Introduction

The applicant Zoetis Belgium submitted on 3 October 2023 an application for a marketing authorisation (MA) to the European Medicines Agency (The Agency) for Poulvac Procerta HVT-IBD-ND, through the centralised procedure under Article 42(2)(a) of Regulation (EU) 2019/6 (mandatory scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 16 May 2023 as Poulvac Procerta HVT-IBD-ND has been developed by means of a biotechnological process, i.e. using recombinant DNA technology (Article 42(2)(a)(i)).

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

For active immunisation of one-day-old chickens and 18-19-day-old embryonated chicken eggs to

- reduce mortality, clinical signs and lesions caused by Marek's disease (MD) virus,
- reduce mortality, clinical signs and lesions caused by infectious bursal disease (IBD) virus and
- reduce mortality and clinical signs caused by Newcastle disease (ND) virus.

Onset of immunity:

- MD: 9 days post vaccination for *in ovo* and subcutaneous use
- IBD: 21 days post vaccination for *in ovo* and 14 days for subcutaneous use
- ND: 24 days post vaccination for *in ovo* and 21 days for subcutaneous use

Duration of immunity:

- MD: a single vaccination is sufficient to provide protection for the entire risk period
- IBD: 63 days of age
- ND: 63 days of age

The active substance of Poulvac Procerta HVT-IBD-ND is a cell-associated live recombinant turkey herpes virus (strain HVT-IBD-ND) expressing the VP2 protein gene of infectious bursal disease virus (IBDV) and the F protein gene of Newcastle disease virus (NDV). The vaccine induces active immunity against Marek's disease, infectious bursal disease (Gumboro disease) and Newcastle disease in chickens. The target species are chickens and embryonated chicken eggs.

Poulvac Procerta HVT-IBD-ND concentrate and solvent for suspension for injection contains 3,558 – 16,900 plaque forming units (PFU) of a deep-frozen suspension of cell-associated live recombinant turkey herpes virus (strain HVT-IBD-ND) expressing the VP2 protein gene of IBDV and the F protein gene of NDV per dose of 0.05 ml (*in ovo*) or 0.2 ml (subcutaneous use) and the concentrate is presented in packs containing one ampoule of 2,000 or 4,000 doses. The glass ampoules are stored in cryopreservation containers in a cane. Poulvac solvent is a sterile, watery solution presented in bags containing 200 ml, 400 ml, 800 ml or 1,000 ml of the solvent. The vaccine concentrate is mixed with the solvent prior to the subcutaneous (SC) injection in the neck or the *in ovo* injection.

The rapporteur appointed is Esther Werner and the co-rapporteur is Paul McNeill.

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

On 5 December 2024, the CVMP adopted an opinion and CVMP assessment report.

On 23 January 2025, the European Commission adopted a Commission Decision granting the marketing authorisation for Poulvac Procerta HVT-IBD-ND.

Scientific advice

The applicant received scientific advice (EMA/SA/0000094891) from the CVMP on 10 June 2022. The scientific advice pertained to the quality, safety and clinical development of the dossier.

The scientific advice mainly addressed the data requirements for application of the vaccine platform technology master file (vPTMF) and the possible data reduction for future marketing authorisation applications based on the vPTMF certificate.

Quality:

The advice concerning quality was followed by the applicant.

Safety:

The advice concerning safety was followed by the applicant.

Efficacy:

The advice concerning efficacy was followed by the applicant.

Vaccine Antigen Master file (VAMF) and vPTMF

The applicant submitted a Vaccine Platform Technology Master File (vPTMF) dossier simultaneously to the submission of the dossier for Poulvac Procerta HVT-IBD-ND. The reference for this vPTMF is EMEA/V/VPTMF/0002.

Part 1 – 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 is in line with legal requirements.

Part 2 - Quality

The provided data follows the data package agreed in the scientific advice and is in line with the guideline on data requirements for vaccine platform technology master files (vPTMF) (EMA/CVMP/IWP/283631/2021).

Vaccines produced based on the Poulvac Procerta vPTMF contain a cell-associated live recombinant HVT strain containing varying transgene(s) as active substance and freeze medium as stabiliser for cell-protection during storage at cryogenic temperatures. The vaccine presented as frozen concentrate needs to be diluted in Poulvac solvent before use by *in ovo* or subcutaneous administration. The qualitative and quantitative composition of the vaccine is satisfactorily described. The absolute quantity of virus to be contained in all platform-related vaccines will be within the same range. The specific specifications for each platform-related vaccine will be adjusted within this range based on the results of the safety and efficacy studies. The solvent has not been included in the vPTMF.

The overall manufacturing process of the viral antigen followed by vaccine blending has been adequately described. The viral strain is propagated on cells, and the harvest is immediately used for vaccine blending. Sufficient details on the filling and subsequent freezing process are provided.

Information on the starting materials to be included in the vPTMF has been provided and is acceptable.

Appropriate in-process control tests are in place.

The finished product control tests on the general characteristics, sterility testing, filling volume, and absence of mycoplasmas are satisfactorily described and validated, if necessary. Sufficient information on the basic principle of the potency and identity test has been included in the vPTMF. The test will be validated individually for each vaccine produced based on the vPTMF. The appropriate purity of the primary cells used for vaccine production has been confirmed and the omission of extraneous agents testing on the final vaccine is considered appropriately justified.

Supportive batch-to-batch consistency data from the first vaccine relevant for the vPTMF has been provided. The data is considered appropriate.

The proposed shelf life of the vaccine of 24 months at ≤ -150°C and the proposed in-use stability are considered sufficiently supported by supportive data from the first vaccine relevant for the vPTMF.

In conclusion, the data on quality of the Poulvac Procerta vPTMF is considered acceptable.

Part 3 - Safety

All studies and information provided in Part 3 of the dossier are considered relevant to support the warnings and advice proposed in the product information and the setting of the specifications for Poulvac Procerta HVT-IBD-ND. Due to the equivalences and similarities between Poulvac Procerta HVT-IBD-ND and other HVT vector vaccines using the same HVT vector platform as detailed in Part 2, all safety data generated for Poulvac Procerta HVT-IBD-ND are also considered relevant by the applicant for inclusion in the vPTMF.

The safety data consist of adequate studies regarding a tenfold overdose, spread to the target species and non-target species and reversion to virulence performed with Poulvac Procerta HVT-IBD-ND, as well as supportive studies for examination of immunological functions performed with the precursor product Poulvac Procerta HVT-IBD. Several additional only supportive US studies not performed under the requirements of European legislation are provided. It is noted that one non-target species study in ducks was terminated, which is not regarded as relevant to be included in the vPTMF.

A high-level summary of the main safety parameters for the pivotal studies conducted can be found in Part 3.G. conclusions on safety of the dossier. All studies are discussed in the relevant parts of the scientific overview and assessed.

The titre specification range for the vPTMF is determined by the data from the 10x overdose safety study of Poulvac Procerta HVT-IBD-ND. The specifications for the virus titres of each subsequent vaccine from the same product range have to remain inside the determined specification, as it has been indicated in the answers to the scientific advice request submitted before this MA application. The intended approach is regarded suitable and reasonable to support the safety for vaccines added to the vPTMF based on already generated data.

Part 4 - Efficacy

All studies and information provided in Part 4 of the marketing authorisation application (MAA) are considered relevant to support the proposed indications in the product information and setting of the specifications for Poulvac Procerta HVT-IBD-ND.

Due to the equivalences and similarities between Poulvac Procerta HVT-IBD-ND and other HVT vector vaccines using the same HVT vector platform as detailed in Part 2, the MD laboratory efficacy data generated for Poulvac Procerta HVT-IBD-ND are relevant for the HVT vector platform

technology and included in the vPTMF in Part 4.A and 4.B. Furthermore, the IBD and ND pre-clinical efficacy data are considered supportive data for the vPTMF, in order to apply for data reductions in case an IBD and/or ND insert is inserted in the HVT vector strain of the future HVT vector vaccines (using the vPTMF) - in line with the guideline on data requirements for vPTMF (EMA/CVMP/IWP/286631/2021), specifically section 6.2.2. The efficacy data consist of successful studies regarding OOI, DOI and MDA interference as well as of supportive studies provided for information only, which for example were used to determine the minimum infectious vaccine dose, the time of onset of immunity or to balance the concentration of the challenge virus.

The applicant's approach to provide all EU relevant efficacy data (OOI, MDA interference, DOI) generated for Poulvac Procerta HVT-IBD-ND, with the exception of DOI studies concerning the HVT antigen and efficacy field studies, as part of the vPTMF according to Guideline EMA/CVMP/IWP/286631/2021 section 5.2, is acceptable.

A high-level summary of the main efficacy parameters for the pivotal studies conducted can be found in Part 4.D Conclusion (part-4d-conclusion-on-efficacy) of the MAA. All studies are discussed in the relevant parts of the MA dossier and assessed below.

For future vaccines to be registered using the certified vPTMF, Part 4.A, Part 4.B and Part 4.C of the MAA for the subsequent HVT vector vaccines will include the efficacy data (if any) generated specifically for the subsequent HVT vectors, which is in line with Guideline EMA/CVMP/IWP/286631/2021 section 6.2.2 and where appropriate justifications/data to show that the addition, removal and/or change of an insert has no impact on the efficacy of the vaccine against MD (and/or ND and/or IBD).

Laboratory efficacy data collected in accordance with either European Pharmacopoeia (Ph. Eur.) or USDA 9CFR guidelines are suitable for demonstrating the lack of interference with efficacy when removing an insert, changing an insert and/or adding a new insert as per Guideline EMA/CVMP/IWP/286631/2021 section 6.2.2. With regard to future applications this means that laboratory efficacy data generated either according to Ph. Eur. or USDA 9CFR could be compared to the laboratory efficacy data generated for Poulvac Procerta HVT-IBD-ND to demonstrate the lack of interference with efficacy as discussed in the scientific advice.

Therefore, the vPTMF additionally contains all relevant efficacy data generated for Poulvac Procerta HVT-IBD-ND based on USDA 9CFR guidelines. Main design and outcome features of Ph. Eur. and USDA 9CFR guidelines for laboratory immunogenicity tests against the antigens of the platform vaccine are contrasted as recommended in the scientific advice. The detailed discussion of the comparability of the different study designs and outcomes is considered sufficient. The provided data are considered suitable to show similar efficacy and thus lack of interference for future HVT vector vaccines to be registered using the vPTMF.

The applicant's proposal for future platform products that the minimum end-of-shelf-life (EOSL) potency value should be the higher of the minimum EOSL potency specification of Poulvac Procerta HVT-IBD-ND and the highest potency of the batches used in the efficacy studies conducted in support of the new platform product, is considered acceptable, as for each product the higher titre used for the efficacy studies is used for the respective EOSL potency specification (worst-case scenario).

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 reference number PSMF-ZOETIS-BE1930-v1, has 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

The different sites involved in the manufacturing process of the vaccine are indicated. The corresponding manufacturing authorisations and GMP certificates are provided.

Manufacturers of the active substance and the finished product

Zoetis INC
2000 Rockford Road
Charles City, Iowa
50616-9101
United States

Activities performed: manufacture of active substance, in-process control testing (chemical, physical), primary packaging, secondary packaging, storage and/or distribution.

A GMP certificate issued by the Belgian Competent Authority (FAMHP) is available in EudraGMDP. The certificate was issued on 24/11/2022, referencing an inspection on 29/04/2022. EudraGMDP document reference number BE/GMP/2022/048.

A manufacturing authorisation from the competent US authority has been provided.

Zoetis Manufacturing & Research Spain S.L.
Carretera De Camprodon S/n
La Vall De Bianya
17813 Girona
Spain

Activities performed: finished product quality control testing (biological, microbiological sterility and chemical/physical), secondary packaging, batch release, storage and/or distribution.

A GMP certificate issued by the Spanish Competent Authority (AEMPS) is available in EudraGMDP. The certificate was issued on 08/05/2024, referencing an inspection on 05/04/2024. EudraGMDP document reference number ES/048HV/24.Manufacturing authorisation (No. 0730) was issued on 22/12/2021 and all activities performed at this production plant for this vaccine are allowed according to this certificate.

A declaration has been provided for the active substance manufacturer from the qualified person at the proposed EU batch release site stating that the active substance is manufactured in compliance with EU GMP. This was verified based on an audit performed from 28/02/2022 to 04/03/2022 by the Global Quality & Compliance Auditor from the EU batch release site (Zoetis Spain).

Manufacturer of the solvent

Zoetis Manufacturing & Research Spain S.L.
Carretera De Camprodon S/n
La Vall De Bianya
17813 Girona
Spain

Activities performed: manufacture of the solvent, quality control testing (chemical/physical, microbiological – sterility), batch release, primary packaging, secondary packaging, storage and distribution.

A GMP certificate issued by the Spanish Competent Authority (AEMPS) is available in EudraGMDP. The certificate was issued on 08/05/2024, referencing an inspection on 05/04/2024. EudraGMDP document reference number ES/048HV/24.

Manufacturing authorisation (No. 0730) was issued on 22/12/2021 and all activities performed at this production plant for the solvent are allowed according to this certificate.

Overall conclusions on administrative particulars

The summary of the pharmacovigilance system master file (PSMF) 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 is in line with legal requirements.

Part 2 - Quality

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

Qualitative and quantitative composition

The vaccine is presented as a frozen cell-associated concentrate for suspension for injection and contains a live recombinant turkey herpesvirus (HVT), strain HVT-IBD-ND, expressing the gene for the VP2 protein of IBDV and the gene for the fusion protein of NDV as active substance. The minimum titre per dose is 3,558 PFU. The vaccine contains neither an adjuvant nor a preservative. Other ingredients are L-glutamine, calf serum, dimethyl sulfoxide (DMSO) and Dulbecco's minimal essential medium (DMEM).

The product is available in multiple presentations containing 2,000 or 4,000 doses/ampoule.

The solvent for dilution is Poulvac solvent. The solvent contains sucrose, potassium dihydrogen phosphate, dipotassium phosphate, peptone (NZ Amine), phenol red and water for injections (WFI). The solvent is available in bags containing 200 ml, 400 ml, 800 ml or 1,000 ml.

The pack sizes are consistent with the dosage regimen and duration of use.

Container and closure system

The vaccine is filled into colourless type I glass ampoules in accordance with the Ph. Eur. 3.2.1. The ampoules are flame-sealed after filling.

The solvent is filled into a polyvinylchloride (PVC) bag that matches the filling volume. The following bag sizes are used: 250 ml (fill volume: 200 ml), 500 ml (fill volume: 400 ml) and 1,000 ml (fill volume: 800 or 1,000 ml). The bags are closed with a pharmaceutical grade polycarbonate stopper with a latex-free rubber disc.

Since the above-mentioned PVC bags are not allowed to be used from the 14th of December 2024 onwards, the applicant presented validation data for polypropylene (PP) bags with matching stoppers as additional primary packaging material for Poulvac solvent.

The specifications and certificates demonstrating Ph. Eur. compliance for the vaccine and solvent primary packaging materials are included in the dossier.

Product development

The different presentations of the vaccine and the solvent allow a flexible adaptation of the number of prepared vaccine doses to the preferred administration route (SC or in ovo) and to the number of chickens to b vaccinated. The method of manufacture and formulation of the vaccine and the solvent is the same as for other live MD vaccines routinely manufactured by the applicant. The selection and use of this recombinant vaccine are satisfactorily justified.

Poulvac Procerta HVT-IBD-ND is based on the apathogenic HVT FC-126 strain, which is commonly used as backbone for construction of HVT vector vaccines. The antigenic sequences of the inserts have been selected from virus strains with confirmed antigenicity and protection against circulating field strains of IBDV and NDV. The construction of the vaccine strain by homologous recombination using transfer plasmids with the insert of interest has been sufficiently explained.

The genetically modified organism (GMO) vaccine strain is cell-associated and, therefore, needs to be stored and transported frozen in liquid nitrogen. The handling, thawing and diluting have to be performed with care and by using protective equipment.

The production system used, and the pharmaceutical form of the vaccine are identical to other MD vaccines manufactured by the applicant.

The solvent used with the vaccine is the same as for the other live MD vaccines manufactured by the applicant. An indicator (phenol red) is included to enable a check on filling of the automated vaccination equipment in the field. The absence of a virucidal effect of the solvent was investigated by many solvent batch tests performed for batches used of already licensed live MD vaccines.

The difference in dose volume (0.05 ml for *in ovo* route and 0.2 ml for subcutaneous route) is linked with the volume of sterile solvent used to reconstitute the frozen cell concentrate before administration.

Description of the manufacturing method

The manufacturing process of the vaccine is a continuous process based on a seed lot system. The described manufacturing process of the HVT-IBD-ND antigen follows conventional processes for virus propagation on cells. Primary chicken embryo fibroblasts (CEFs) are prepared from embryos extracted from SPF embryonated eggs. After adjustment of the cell concentration, the cells are

immediately used for co-infection or the preparation of CEF monolayers. After CEF preparation, CEF monolayers or CEFs in suspension are infected with a defined amount of working seed virus (WSV).

The final cultures for antigen production are incubated until a characteristic cytopathogenic effect (CPE) is observed. The cells are harvested by trypsinisation, followed by centrifugation, and resuspension of the cell pellet in freeze medium. The cell count is determined, and the antigen batch is immediately used for vaccine blending.

For vaccine blending, the indicated number of cells in freeze medium are mixed under stirring with the excipients in the following order: DMSO, L-glutamine, calf serum and DMEM. After final homogenisation by stirring, the vaccine bulk is filled under stirring into 2 ml glass ampoules, which are subsequently sealed by flame-sealing.

The filled ampoules are frozen by a controlled freezing program and then transferred to final storage in liquid nitrogen. Samples for finished product testing are taken.

The key stages of the manufacturing process for the vaccine were considered in the validation study and are satisfactorily validated. In addition, consistency data on industrial scale batches of all proposed presentations complying with the proposed specifications were provided. The manufacturing process of the vaccine is considered sufficiently validated.

The solvent is prepared by consecutive dissolution. The pH is adjusted, and the solution is filter sterilised. After filtration through a membrane filter, the solvent is filled. The closed PVC bags are sterilised by steam sterilisation and placed in a secondary bag, whereas the proposed PP bags are placed in the overpouch before steam sterilisation. The process between formulation and terminal sterilisation is continuous and a maximum duration for the complete process has been validated. The solvent is finally stored at room temperature (RT) between 20 – 25°C. A validation study identifying critical measures, which are already implemented in the described process, as well as consistency data of three batches of each proposed presentation filled in PVC bags are provided. The described production process of the solvent filled in PVC bags has been satisfactorily validated.

With regard to solvent filled in PP bags, consistency data of R&D scale batches have been presented for validation of the manufacturing process. The validation of some parameters strictly linked to the automated filling process is still pending and will be addressed as post-authorisation measure. However, this is considered to have a negligible impact on the overall quality of the solvent. Thus, the manufacturing process of the solvent filled in PP bags is considered satisfactorily validated. Data of at least one batch of industrial scale manufactured as described should be provided as a post-authorisation measure.

Production and control of starting materials

Starting materials listed in pharmacopoeias

The following starting materials listed in a pharmacopoeia are used in the manufacture of the vaccine: DMSO, sodium hydrogen carbonate, hydrochloric acid, sodium phosphate monobasic monohydrate, sodium phosphate dibasic heptahydrate, anhydrous potassium phosphate monobasic, anhydrous dextrose, phenol red sodium salt, potassium chloride, gentamicin sulphate, sodium chloride, sodium hydroxide, purified water, water for injections, SPF embryonated eggs and calf serum (fortified bovine calf serum).

The following starting materials listed in a pharmacopoeia are used in the manufacture of the

solvent: sucrose, dipotassium phosphate, potassium dihydrogen phosphate, phenol red, sodium hydroxide and water for injections.

Example certificates of analysis (CoAs) have been provided, and all conform to the specifications of the referenced standard. If necessary, valid certificates of suitability issued by the EDQM were provided. The separate listing of the starting materials for the vaccine and for the solvent with reference to different standards is related to the different manufacturers. The equivalence of the mentioned standard to the quality standard as mentioned in the respective Ph. Eur. monograph has been confirmed. For example, phenol red is listed twice, referring to either the American Chemical Society (ACS) grade standard for phenol red sodium salt used in the manufacture of the vaccine, or the Ph. Eur. monograph for phenol red used in the manufacture of the solvent. The included reference for DMSO is also the ACS standard.

Starting materials not listed in a pharmacopoeia

Starting materials of biological origin

The following starting materials of biological origin are used at different stages of the production of the vaccine and the solvent: vaccine antigen HVT strain HVT-IBD-ND, porcine trypsin, and peptone (NZ Amine YT). The information on the virus strain is discussed in detail below. For all other starting materials of biological origin, satisfactory descriptions and CoAs were provided.

The active substance, cell-associated HVT-IBD-ND virus, is manufactured using cells and subsequently formulated and frozen in a continuous operation. MSV is produced in cells, filled in ampoules and stored in liquid nitrogen. Identity, sterility, absence of mycoplasma and absence of extraneous agents was tested on the master seed lot. Sterility, absence of mycoplasma and extraneous agents were tested in accordance with the relevant Ph. Eur. monographs 2.6.1, 2.6.7, 2.6.24 and Ph. Eur. 5.2.5.

A seed-lot system was satisfactorily established for the seed material. Details of source, passage history, controls and storage conditions for the MSV and WSV were provided and are considered appropriate.

A certificate of analysis for the MSV is provided. A transmissible spongiform encephalopathy (TSE) assessment for the MSV is submitted and confirms an extremely low risk of contamination for the MSV and the following WSV lots.

Risk of transmitting animal spongiform encephalopathy (TSE) agents

All starting materials of animal origin were considered in the TSE risk assessment. The risk assessment of all starting materials of biological origin complies with the current Ph. Eur. monograph 5.2.8 and the TSE Note for Guidance (EMEA/410/01 rev.3). The risk of transmitting TSE infectivity through the use of this vaccine is negligible.

Starting materials of non-biological origin

The starting materials of non-biological origin referred in this section are used as components of media at different stages of the manufacturing of the vaccine. For use in production, CoAs from supplier(s) must show compliance with the starting material specification. Specifications together with representative CoAs are provided in starting material monographs.

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

Detailed qualitative and quantitative composition, method of preparation and storage of the media and solutions prepared in-house are provided.

Antibiotics are used in cell growth media and solutions however, not more than minimal traces are to be expected in the final product.

Control tests during the manufacturing process

The proposed control tests during the manufacturing process of the antigen are considered adequate for the described continuous manufacturing process. The only proposed control test is the determination of the cell count of the CEF suspension before seeding and after harvest of each antigen batch. The method description, acceptance criteria and the validation of the cell count are considered satisfactory.

No in-process control tests are performed during the manufacture of the solvent, which is considered acceptable.

Control tests on the finished product

The general tests on the finished vaccine include a test on appearance. This test is satisfactorily described and the specified acceptance criteria are appropriate.

The potency assay used for identification and quantification of the HVT-IBD-ND strain contained in the vaccine is a viral plaque titration on CEFs followed by evaluation using indirect IF staining. The viral titre in PFU/dose is calculated from counting the HVT-positive plaques. Identity is confirmed by calculation of the HVT:IBD and HVT:ND ratio after dual staining with HVT-specific and IBD- or ND-specific antibodies. The test was satisfactorily validated according to the requirements of VICH GL1 and GL2 for quantitative assays.

Each batch of finished vaccine is tested on sterility in accordance with Ph. Eur. 2.6.1 and for absence of mycoplasmas in accordance with Ph. Eur. 2.6.7 and Ph. Eur. 2.6.21. Both tests have been satisfactorily validated.

A risk assessment for omission of extraneous agents testing on the finished vaccine considering the requirements of Ph. Eur. 5.2.5 has been provided and the omission of extraneous agents testing for the vaccine has been appropriately justified.

The control test on the filling volume on each batch of vaccine has been satisfactorily described and the specified acceptance criteria are appropriate.

The control tests on the finished solvent batches include a test on appearance, pH, sterility and fill volume. These tests are satisfactorily described, and the specified acceptance criteria are appropriate.

Batch-to-batch consistency

Data of three and additional four consecutive batches of vaccine, each blended with a different batch of antigen and of both presentations, were provided. All batches were in principle produced according to the described manufacturing process. The results complied with the proposed specifications for in-process control and finished product testing.

Data of three batches of each presentation of the solvent filled in PVC or PP bags were provided. The results complied with the proposed specifications for finished product testing. The applicant is requested to submit data of at least one industrial scale batch of the solvent filled in PP bags manufactured as described in the dossier as a post-authorisation measure.

Stability

The omission of the performance of stability studies for the antigen bulk is considered acceptable, since the antigen batches are immediately used for blending of the vaccine bulk.

A shelf life of 24 months during storage at cryogenic temperatures of ≤ -150°C is proposed for the vaccine in its final container. Real-time stability data are presented for seven batches stored in liquid nitrogen for 27 months. In conclusion, the proposed shelf life is considered acceptable.

A shelf life of 24 months during storage at ≤ 25°C is proposed for the solvent in its final container. Real-time stability data on six representative solvent batches filled in PVC bags of the lowest or the highest presentation stored as recommended over a period of 27 months complying with the proposed specifications was provided. The proposed shelf life of the solvent filled in PVC bags is considered acceptable.

Additional data of an ongoing stability study of the solvent filled in the newly proposed PP bags has been provided. The completed study report of the still ongoing real-time stability study should be provided as post-authorisation measure. Provided that all results are complying with the specifications, the shelf life of 24 months during storage at ≤ 25°C for the solvent filled in PP bags has been appropriately supported by data.

An in-use stability study of the diluted vaccine confirmed the proposed shelf life for the vaccine ready for use for two hours at room temperature.

Overall conclusions on quality

Poulvac Procerta HVT-IBD-ND contains a cell-associated live recombinant strain HVT-IBD-ND as active substance and freeze-medium as stabiliser for cell-protection during storage at cryogenic temperatures. The vaccine presented as frozen concentrate needs to be diluted in Poulvac solvent before use by *in ovo* or subcutaneous administration. The qualitative and quantitative composition of the vaccine and the solvent are satisfactorily described.

The overall manufacturing process of the viral antigen followed by vaccine blending as well as manufacture of the solvent are described. The viral strain is propagated on cells and the harvest is immediately used for vaccine blending. Sufficient details on the filling and subsequent freezing process are provided. The solvent is prepared by dissolution of all ingredients in water for injections, filling and final sterilisation and the overall process has been satisfactorily validated.

Information on the starting materials has been provided and is acceptable. A satisfactory TSE risk assessment in line with Note for Guidance on minimising the risk of TSE agents via human and veterinary medicinal products (EMA/410/01/Rev.3) was provided. The data on the preparation and characterisation of the viral seeds is appropriate.

Appropriate in-process control tests are in place.

The finished product control tests are satisfactorily described and validated, if necessary. Batch-to-batch consistency is considered to be sufficiently shown for the vaccine and the solvent.

The proposed shelf lives of the vaccine (24 months at ≤ -150°C) and the solvent filled in either PVC bags or PP bags (24 months at ≤ 25°C) are adequately supported by data. The in-use stability is satisfactorily supported by data.

The data on quality, manufacture and control of Poulvac Procerta HVT-IBD-ND and Poulvac solvent can be considered acceptable.

Post-authorisation measures:

- The applicant is requested to provide the completed report of the ongoing stability study for the solvent filled in PP bags.
- The applicant is requested to submit data of at least one industrial scale batch of the solvent filled in PP bags manufactured as described in the dossier.

Part 3 – Safety documentation (safety and residues tests)

General requirements

Poulvac Procerta HVT-IBD-ND is a trivalent, cell-associated, live recombinant virus vaccine for use in chickens containing live recombinant serotype 3 turkey herpesvirus (HVT) strain FC-126 with the VP2 gene of IBDV and the F gene of NDV inserted into the HVT genome. No adjuvant or preservative is included in the vaccine. The maximum antigen concentration per dose (0.05 ml *in ovo* or 0.2 ml subcutaneously) contains 16,900 PFU (Plaque Forming Units) of the recombinant HVT-IBD-ND strain.

A full safety dossier in accordance with Article 8(1)(b) has been provided. Studies to determine the safety of the vaccine were performed in accordance with Ph. Eur. monograph 0062 on vaccines for veterinary use, Ph. Eur. chapter 5.2.6 on evaluation of safety of veterinary vaccines and immunosera, Ph. Eur. Monograph 0589 "Marek's disease vaccine (live)", Regulation (EU) 2019/6 with Annex II, section IIIb, and VICH GL 44. Ph. Eur. monograph 0587 "Avian infectious bursal disease vaccine (live)" and Ph. Eur. monograph 0450 "Newcastle disease vaccine (live)" have also been considered.

This type of vector vaccine is eligible for the 'vaccine platform technology' concept as referred to in Annex II to Regulation (EU) 2019/6. A vPTMF for the Zoetis HVT vector platform has been submitted in parallel to this application.

Poulvac Procerta HVT-IBD-ND is presented as a frozen cell suspension in flame-sealed glass ampoules stored in liquid nitrogen, which is to be diluted before use in a sterile solvent (Poulvac solvent).

The vaccine is administered *in ovo* to 18-19-day-old embryonated chicken eggs (0.05 ml) or subcutaneously (SC) to 1-day-old chickens (0.2 ml) to stimulate active immunity against infection with Marek's disease virus (MDV), IBDV and NDV.

Safety documentation

Seventeen (17) safety studies were conducted to investigate the safety of the product. They include 14 pre-clinical studies investigating the safety of a 10-fold overdose, immunological functions, spread to non-target species (turkeys, ducks, pheasants, quails, and mice) and examination of

reversion to virulence as well as three clinical studies. The vaccine was administered by the *in ovo* and SC routes in the target species of minimum age, as recommended.

Most of the laboratory studies were reported to be GLP-compliant with the exception of part of the laboratory phase (back titration), which was performed at Zoetis Olot, Spain site (testing and batch release EU) or Zoetis VM RD Kalamazoo, US site (where the assay was developed and validated) (non-GLP site), two of the non-target species studies and one study on environmental survival. One production batch was used in the clinical trials conducted in Spain, France and Italy in compliance with the principles of good clinical practice (GCP).

Additionally, the applicant included seven non-GLP pre-clinical studies, and a summary report on three non-GCP clinical studies. All these studies were performed in the United States of America for registration of the product in non-European countries. In these additional and only supportive studies, the US solvent (Poulvac Sterile Diluent) was used, which has a slightly different composition compared with the EU solvent. Nonetheless, the pH range and most of the ingredients are the same.

Study title
Overdose and RTV
10x target animal safety
Bursal damage*
Immunosuppression*
Shed, spread, dissemination
Shed & spread
Spread from chicken to turkey
Spread from turkey to turkey
NTAS turkey
NTAS turkey
NTAS ducks
NTAS ducks (terminated)
NTAS quail
NTAS quail
NTAS pheasant
NTAS mice
NTAS mice
Dissemination
In vivo passages
Back passage and RTV
Environmental survival
Field study in Spain

Field study in France
Field study in Italy
Field safety in US
Field safety in US
Field safety in US

* Study was performed with Poulvac Procerta HVT-IBD
Studies in blue colour are only supportive because they were performed for registration purposes in other parts of the world and are not GLP- or GCP-conform.

Pre-clinical studies

Two breeds of SPF chickens were used in the EU pre-clinical studies (GD and VALO) and one further chicken line (Charles River) in the US studies. The susceptibility of these SPF chickens was shown by a challenge with a virulent MDV in two overdose studies. The susceptibility for the VALO line was not explicitly shown but is accepted as this breed has often been used before. For all EU studies, SPF certificates of the parent flocks are provided.

The applicant has included in the dossier a detailed overview over the clinical signs and lesion scoring recorded in the provided studies. Humane endpoints were determined to avoid unnecessary suffering and harm.

Safety of the administration of one dose

No single dose study is provided. A single dose safety testing is not required according to Ph. Eur. Monograph 0589 as an overdose study is requested here and therefore, no specific one-dose safety study was performed as suggested by Annex II to Regulation (EU) 2019/6. This approach also follows the scientific advice (SA) given previously on the submission of the MA application.

Safety of one administration of an overdose

One pivotal 10-fold overdose EU study and one supportive overdose US study were provided.

In the EU study, a 10-fold maximum overdose containing 169,033 PFU/0.2 ml of Poulvac Procerta HVT-IBD-ND was administered by the recommended routes (*in ovo* or SC) to 18-day-old embryonated SPF chicken eggs and 1-day-old SPF chicks (minimum age as required). The volume of 0.2 ml/egg was higher than the recommended volume of 0.05 ml/egg and was chosen in order to reach a sufficiently high virus titre per dose. Additionally, in this study a non-inoculated control group and one unvaccinated group that was challenged with a very virulent MDV (vvMDV) strain RB1B *in ovo* were included to demonstrate susceptibility of the chicken line used to infection with MDV.

The impact of the *in ovo* vaccination on hatchability was examined. The control group and both vaccinated groups were observed for 120 days for clinical signs and mortality; the group challenged for a maximum of 70 days. From the control group and both vaccinated groups, blood, organ samples and oropharyngeal/cloacal swabs were collected to recover the vaccine virus via cultivation on chicken embryo fibroblasts (CEFs; HVT plaque assay) and confirm the presence of the HVT, IBD and ND antigens via specific immunostaining (IFA). Presence of HVT-IBD-ND DNA in feather follicles by real-time HVT-PCR was assessed as well as bursa lesions. Additionally, body weight and bursa

weight were determined to calculate the bursa-to-body-weight ratio. Necropsies were performed on all chickens that died/were euthanised and at the end of the respective observation periods for examination of the presence of lesions related to MD and IBD.

No impact on hatchability was detected as hatch was over 90.4% in all groups. No clinical signs or deaths or specific lesions related to MD or IBD or ND were noted in the control group and the two vaccinated groups. 89.1% of the chicks challenged *in ovo* were diagnosed with MD, respectively, indicating the susceptibility of the chicken breed used. In both vaccinated groups, virus was recovered from all organs and feather follicles on nearly all days sampled. The swabs were negative on each day. No virus was recovered from the control group. Mean bursa lesion scores were similar in all groups and low at day 28 (maximum 0.2). Mean body weight, mean bursa weight and mean bursa-to-body-weight ratio were comparable in all groups.

In summary, the vaccine virus is considered safe for chickens when given at a 10-fold maximum dose to 18-day embryonated chicken eggs or 1-day-old chicks.

For the solely supportive US overdose study, Poulvac Procerta HVT-IBD-ND was inoculated at an approximately 4-fold maximum overdose *in ovo* in 18-day-old embryonated SPF chicken eggs (65,139 PFU/0.1 ml) and SC to 1-day-old SPF chicks (68,833 PFU/0.2 ml). A non-inoculated control group and a group challenged intraabdominally with vvMDV strain RB1B on the fifth day of life to prove susceptibility of the chicken line used to infection with MDV were also included in this study.

The impact of the *in ovo* vaccination on hatchability was examined. The control group and both vaccinated groups were observed for 120 days for clinical signs and mortality; the challenge group for a maximum of 50 days. Necropsies were performed at the end of the respective observation periods or earlier when chickens died or were euthanised. Detected tumours were examined by histology and via immunohistochemistry and PCR for confirmation. Body weights were determined.

No impact on hatchability was detected in the group inoculated *in ovo* (97% hatch). No clinical signs or deaths or specific lesions related to MD or IBD or ND were noted in the control group and the two vaccinated groups. In the group challenged, MD lesions were present in 98.31% of the chickens indicating the susceptibility of the chicken breed used. No differences in body weight were detected comparing groups.

In summary, the vaccine virus is considered safe when given at an approximately 4-fold maximum overdose to 18-day-old embryonated chicken eggs or to 1-day-old chicks.

Safety of the repeated administration of one dose

The vaccine is to be administered once via *in ovo* vaccination at 18-19 days of embryonation or via the subcutaneous route at one day of age. In accordance with Annex II to Regulation (EU) 2019/6, assessment of safety of repeated vaccination is therefore not required. This approach also follows the scientific advice (SA) given previously on the submission of the marketing authorisation (MA) application.

Examination of reproductive performance

No studies on the reproductive performance were provided as this product is not indicated for use in laying birds. In SPC section 3.7 Use during pregnancy, lactation or lay, the following adequate warning is included *'The safety of the veterinary medicinal product has not been established during lay.'* This approach also follows the SA given previously on the submission of the MA application.

Examination of immunological functions

Two supportive studies are provided, which were conducted to investigate the immunological functions in the precursor product Poulvac Procerta HVT-IBD.

The aim of the first EU study was to examine bursal damage after vaccination with an approx. 5-fold maximum overdose (126,080 PFU) to determine the appropriate timing for vaccination against ND to perform the subsequent immunosuppression study. Moreover, hatchability was examined as well as clinical signs, mortality and pathological lesions. Hatchability was over 93% in all groups. No clinical signs and mortality were observed. Considering bursa lesions scores and the development of bursas, the applicant decided to use day 14 after hatch as optimal time for vaccination with a ND vaccine.

In the subsequent EU study, 18-day-old embryonated SPF chicken eggs were vaccinated *in ovo* with the product; meanwhile another group remained naïve. Both groups were vaccinated at 14 days of life with a ND Hitchner B1 vaccine and challenged 14 days later IM with virulent NDV strain Herts to assess the influence of the vaccination with Poulvac Procerta HVT-IBD. Hatchability of the *in-ovo*-vaccinated group was only 77%; however, in all other studies provided, hatchability was over the 80% limit. No significant difference in seroconversion after vaccination against ND was noted. The protection rate was 100% in both groups; no clinical signs or mortality due to NDV were observed after NDV challenge, while all birds of a non-vaccinated control group succumbed to challenge.

Both studies can be considered as supportive for the MA application of Poulvac Procerta HVT-IBD-ND. There is no need to provide further data on this point as the insertion of another protein gene (F protein gene of NDV) is not considered to change the safety profile of the vaccine virus. This approach also follows the SA given previously on the submission of the MA application.

Special requirements for live vaccines

Spread of the vaccine strain

Spread in the target species

A GLP-compliant EU study examining shed, spread, and dissemination in the target species has been provided. SPF chickens vaccinated *in ovo* or SC either with the test product or with the parental HVT strain were mingled in various pens each including a naïve group of SPF chickens for a maximum observation period of 36 days. Clinical signs and mortality were observed. On specific days, randomly selected chickens per group and point in time were bled and euthanised for pharyngeal/cloacal swabs and tissues (bursa, spleen, kidney, lung, feather follicles) collection. At each point in time, a dust sample from each pen was taken.

No clinical signs or mortality related to the vaccination were observed. The vaccine strain and also its parental strain were found to disseminate to all tested tissues and were detectable in all tissues until the end of the observation period. In in-contact chickens only the blood, spleen and bursa from one chicken vaccinated with the parental strain was found positive on day 28.
On day 14, the vaccine strain was shed by the cloacal route from one of the chickens vaccinated *in ovo* with the vaccine strain. On day 22 cloacal swabs were negative. All oropharyngeal swabs remained negative. The presence of HVT antigen was detected in feather follicles from all vaccinated groups indicating shedding via dander as the dominant route of shedding. HVT was infrequently found in feather follicles from chickens in contact with HVT-vaccinated groups and, on a single occasion, from a chicken in contact with an HVT-IBD-ND-vaccinated group (SC, day 21/22). So,

spread to in-contact birds was limited.
HVT virus was detected in dust from all groups until the end of the study. However, the immunostaining of the samples remained negative indicating that non-infectious virus material was detected by the PCR. Feather dust samples were positive in PCR until day 35/36. The applicant noted that the inclusion of an NDV gene unlikely changes the characteristics of the construct and in the SPC of the similar product Poulvac Procerta HVT-IBD an excretion of a maximum of 6 weeks is mentioned. Therefore, a similar shedding behaviour would have been expected from this product. The wording was adapted to the wording in the SPC of the precursor product.

The supportive US study investigates the spread from vaccinated chickens to naïve chickens comparing also the HVT-IBD-ND vaccine strain to its parental HVT strain. Hatchability, clinical signs and mortality were assessed as well as virus recovery from blood, spleen, oropharyngeal/cloacal swabs, and debris/dust. Shedding via feather follicles and seroconversion to IBDV and NDV was also examined. No virus strain was isolated from dust samples at any point in time. HVT DNA was detectable in feather follicles of all vaccinated chickens. No spread to naïve chickens was observed.

Spread to the non-target species turkey (natural host of HVT)

In the first EU study, spread from vaccinated chickens to naïve turkeys was investigated. The second EU study (non-GLP) examined spread of the virus strain from vaccinated turkeys to naïve turkeys in comparison to its parental HVT strain. In both studies, it was shown that the vaccine virus is able to spread to in-contact turkeys. The pattern of spread was similar between the vaccine virus with its parental HVT virus.

The safety of an overdose of Poulvac Procerta HVT-IBD-ND for turkeys was investigated in a EU study (non-GLP). Approximately a 16-fold maximum overdose (277,111 PFU) was applied to 1-day-old turkeys. No clinical signs or deaths or specific lesions related to MD or IBD were noted. Therefore, the vaccine is regarded as safe for turkeys if contact with vaccinated chickens should occur.

The non-GLP status is acceptable for non-target species studies as GLP can be lifted for non-target animal safety studies according to Annex II of Regulation (EU) 2019/6.

Additionally, a US study is provided as supportive study, confirming the safety of the MSV in turkeys when given at an approximately 4.5-fold maximum overdose (73,667 PFU).

Spread to further non-target species

Five EU studies and two supportive US studies investigated the spread of the vaccine strain to further non-target species and foreign bird safety using an overdose of approximately 57,200 – 289,067 PFU/dose administered by the SC route. Two studies evaluated the safety of Poulvac Procerta HVT-IBD-ND in ducks (EU studies). The second study was terminated because of unexpected deaths due to husbandry problems and had to be repeated. Five more studies were performed in pheasants (EU study), quails (EU study + US study) and in mice (EU study + US study) showing that the vaccine strain is safe in all these species and that no spreading was detected.

In conclusion, the vaccine virus strain is able to spread from chickens to chickens (in EU study), chickens to turkeys and between turkeys. These findings are in line with the biological characteristics of the parental HVT strain, which were not changed by insertion of the VP2 and the F protein. Poulvac Procerta HVT-IBD-ND was shown to be safe in the relevant non-target species turkeys, ducks, pheasants, quails, and mice.

Dissemination in the vaccinated animal

A dedicated EU study examining shed, spread, and dissemination in the target species has been provided. The vaccine virus was detected in all tested tissues during the whole observation period. No differences compared to its parental strain were noted.

Dissemination of the vaccine strain in vaccinated animals was also investigated in the supportive US study. Samples from chickens vaccinated *in ovo* with a standard dose of HVT-IBD-ND or with the parental HVT strain were taken from blood, spleen, lung, thymus, feather follicles and filter dust, and were tested for virus by cultivation of isolated white blood cells (WBC) on CEFs and confirmed via IFA. Dust samples were stored for a maximum of 7 days after the end of the study to assess survivability of the virus. Presence of HVT DNA in feather pulps was assessed by quantitative real time PCR.

Virus could be re-isolated from all tested organ and blood samples of both vaccinated groups. Both viruses were detected in dust samples on D11 but not on D22 or later. Both viruses were recovered from feather pulp until D22.

The supportive study confirmed that the biological characteristics of the parental HVT strain were not changed by the insertion of two additional genes from IBDV and NDV, respectively.

Increase in virulence of attenuated vaccines

According to Ph. Eur. Monograph 0589 *"The test for increase in virulence is required for Marek's disease virus vaccine strains but not for turkey herpesvirus vaccine strains, which are naturally apathogenic."* Although the parent strain of HVT-IBD-ND is the naturally apathogenic HVT strain FC-126 and, therefore, no test is strictly necessary, the applicant has performed two studies according to Ph. Eur. Monograph 5.2.6 to demonstrate that the genetic modification did not affect the safety profile of the parental HVT strain and that the HVT-IBD-ND strain cannot become virulent through bird to bird passages. Additionally, one solely supportive US study is provided.

Reversion to virulence was investigated in two EU studies (back passages and comparison of MSV and its 5th passage) in accordance with the requirements of Ph. Eur. 5.2.6 and Ph. Eur. 0589, respectively.

Sequential passaging of the vaccine strain through five groups of SPF chickens was performed by *in ovo* inoculation of an approximately 2.5-fold maximum dose of the vaccine (40,867 PFU/dose-MSV) to the first group of 18-day-old embryonated SPF chicken eggs. At the following passages (2, 3, 4 and 5) 0.1 or 0.05 ml of pooled WBC suspension was prepared from blood and spleen samples and administered intraperitoneally (IP). The time between passages was seven days. Each passage group consisted of 30 animals except for the last group, which included 70 birds. The vaccine strain was recovered at all 5 passages. No clinical signs of disease were observed in any of the passage levels and no lesions of MD or IBD were found in the last group, where 20 chickens were observed for 21 days until necropsy.

The last passage of the MSV (P+5) was administered *in ovo* to 18-day-old embryonated SPF chicken eggs. Another group of 18-day-old embryonated SPF chicken eggs was inoculated with the unpassaged MSV in order to evaluate a possible increase in virulence during the *in vivo* passages. Unfortunately, this group was invalidated due to a bacterial contamination of the eggs. Only 50% of the inoculated eggs hatched and 14 out of 50 chicks developed clinical signs. In necropsies, omphalitis and yolk sac inflammation were found due to bacterial infection. The *in ovo* group was replaced by another group, which was vaccinated SC with the passaged virus. No abnormalities were found in the animals vaccinated with the MSV or the MSV P+5. Therefore, it is concluded that

no reversion to virulence occurred following five passages *in vivo*.

One supportive US study also investigated a possible increase in virulence due to *in vivo* passaging of the MSV. Additionally, re-isolated virus from each passage was characterised genotypically by PCR. No indication of a reversion to virulence was found. The PCR analysis showed that the full-length donor DNA remained stably inserted in the HVT genome and no gene deletion or rearrangement was observed.

Biological properties of the vaccine strain

One specific *in vitro* US study was conducted to determine the intrinsic biological properties of the vaccine strain regarding survivability of the virus in the environment. The vaccine strain was compared with its parental HVT strain, and it was found that the HVT-IBD-ND vaccine strain remained viable after the respective suspensions were dried at room temperature and subsequently incubated at 25°C or 30°C for 2 hours. The parental HVT strain remained viable for 5 hours. After that time, no viable virus could be detected anymore.

The results of all *in vivo* safety studies performed including the studies in non-target animals indicate that the biological properties of the apathogenic parental HVT strain are unaltered after insertion of the VP2 gene of IBDV and the F gene of NDV and some regulatory elements.

Based on the presented data, the safety profile of the vaccine strain can be considered acceptable.

Recombination or genomic reassortment of the strains

The chance of an *in vivo* recombination event is a theoretical possibility due to the similar replication cycle of HVT and other MDV serotypes, but to date no such events have been reported. The potential for recombination of the HVT-IBD-ND strain with virulent MDV is not greater than that of currently authorised vaccines containing HVT. The risk of such an event is considered low.

The genome of HVT is not segmented; therefore, genomic reassortment cannot occur.

The risk of recombination with IBDV or NDV strains is effectively zero, since both viruses are replicating in the cytoplasm and, therefore, recombination with a double-stranded DNA virus, such as the HVT-IBD-ND virus strain in Poulvac Procerta HVT-IBD-ND, which is presumably replicating in the nucleus just like the parental HVT strain, is not possible.

It can be concluded that the event of recombination or genomic reassortment is very unlikely. This assessment was made in compliance with the respective legal requirements.

User safety

The applicant presented a user safety risk assessment, which was conducted in accordance with CVMP "Guideline on user safety for immunological veterinary medicinal products" (EMEA/CVMP/IWP/54533/2006).

The main potential routes of accidental contact with the product were considered, and it was concluded that the most likely routes are those of accidental self-injection during SC application and dermal exposure by accidental spilling or when a glass ampoule explodes during the thawing process. Additionally, personnel involved in animal husbandry procedures may come into contact with excreted vaccine virus from feather follicles of vaccinated chickens.

In general, avian herpesviruses are not known to be a hazard to humans and HVT is not indicated as such in Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work. The genetic modification did not alter the biological properties of the strain as shown in the increase in virulence studies and studies on spread to the target species and non-target species.

The excipients and the constituents of the sterile solvent are commonly used in other vaccines and do not pose a risk for the user. The same applies to the traces of antibiotics present in the finished product.

None of the components of the vaccine (active ingredient or excipients) are expected to pose a risk to the user following accidental exposure. With regard to possible hazards of a vaccine stored in liquid nitrogen (risk of an ampoule exploding) and the recommended handling of the ampoules, an adequate warning is included in SPC section 3.5.

Based on the above risk assessment, it is concluded that the product does not pose an unacceptable risk to the user when used in accordance with the product information. Additionally, the risk of liquid nitrogen burns is negligible when the product is handled as recommended in the product information.

Study of residues

No residue depletion studies have been carried out for Poulvac Procerta HVT-IBD-ND.

All substances included in the composition of the vaccine are listed in Table 1 of the Annex to Commission Regulation (EU) 37/2010 or in the list of substances considered as not falling within the scope of Regulation (EU) 470/2009. Phenol red sodium salt has no pharmacological activity at the concentration used in the final product. The concentrations of residual antibiotics in the final product are far below the lowest established maximum residue limit (MRL) outlined in Table 1 of the Annex to Commission Regulation (EU) No 37/2010.

In conclusion, a withdrawal period of zero days is deemed appropriate for this vaccine.

Interactions

The applicant has not provided data investigating interactions of the vaccine with any other (immunological) veterinary medicinal product and therefore proposes to include the following statement in section 3.8 of the SPC: *'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 studies

Three combined safety and efficacy field trials in commercial broiler chickens were performed in Spain, France and Italy using a standard dose.

The following legal requirements were considered carrying out the clinical studies: VICH GL 9 (GCP) + GL 44 (target animal safety), Regulation (EU) 2019/6, EMEA/CVMP/852/99-FINAL, EMEA/CVMP/004/4-FINAL and Ph. Eur. Monograph 5.2.6.

Field trial 1 was performed in Spain on a commercial broiler farm in four houses (two per treatment). The performances of approximately 173,030 chickens vaccinated either *in ovo* with

Poulvac Procerta HVT-IBD-ND or *in ovo* with a comparator product were compared. The study was divided into two phases: rearing until early slaughter (thinning) on D30/33/34 and rearing until the final processing on days 42, 43 + 44. In house B (vaccinated with the control vaccine), there were some technical issues during the study resulting in the death of 449 birds. However, this is not considered relevant for the overall results of the study and mortality in all groups was in line with historical mortality data on this farm. Hatchability was satisfactory and comparable between groups. MDA against MD, IBD and NDV were confirmed, and mean titres were similar in the two treatment groups. No clinical signs or observations suggestive of MD, IBD and ND were observed during the entire study. No significant differences in body weight could be detected between the two groups and a similar zootechnical performance for the two treatment groups was calculated. Evaluation of necropsied birds on D28 showed no signs of IBD or MD lesions in gross pathology or in histopathology assessment.

Field trial 2 was performed in France also on a commercial broiler farm in four houses (one per treatment per farm). The performances of approximately 146,408 chickens vaccinated either *in ovo* with Poulvac Procerta HVT-IBD-ND or *in ovo* with a comparator product were monitored. There were two phases in this study, rearing until early slaughter (thinning) on D36 and D33, respectively, and final processing on D42 and D40/41, respectively. On one farm, the diagnosis of colibacillosis was made and in one of the houses, birds were treated with antibiotics. Both houses recovered within a few days without further consequences for the study. Hatchability was satisfactory and comparable between the two groups. MDA against MD, IBD and NDV were confirmed, and mean titres were similar in the two treatment groups. No clinical signs or observations suggestive of MD, IBD and ND were made during the entire study. Mortality in all groups was in line with historical mortality data on this farm. No significant differences in body weight could be detected between the two groups and a similar zootechnical performance for the two treatment groups was calculated. Evaluation of the necropsied birds on D28 showed no signs of typical MD, IBD or ND lesions in the gross pathology or in the histopathology assessment. Bursa weight was only obtained on one farm; however, during necropsies of the birds on the other farm, no lesions of the bursas were found. Statistical analysis demonstrated that there was no significant difference between condemnation rates in treatment groups. The number of seizures for tumours remained zero for both treatments.

Field trial 3 was performed in broiler chickens on a commercial broiler farm in four houses (one per treatment and sex) in Italy. The performances of approximately 44,000 chickens vaccinated either SC with Poulvac Procerta HVT-IBD-ND or SC with a comparator product were compared. Females were transported to the slaughterhouse on days 44/45 and males on days 49/51. All tested birds were positive for MDA against MD, IBD and ND on D0. No clinical signs or observations suggestive of MD, IBD and ND were made during the entire study. Mortality in all groups was in line with historical mortality data on the farm. No significant differences in body weight could be detected between treatment groups and similar zootechnical performance was calculated. During carcass assessment, no birds were condemned. Evaluation of the necropsied birds on D28 showed no signs of typical MD, IBD or ND lesions in gross pathology or in histopathology assessment.

Other histopathological lesions were observed in different tissues but were of similar frequency in the two treatment groups. Bursa-bodyweight ratio data were similar between treatment groups and consistent with other birds of similar age.

Additionally, the applicant provided a document summarising three field trials conducted in the US in commercial broiler chickens. In two studies, Poulvac Procerta HVT-IBD-ND was used to vaccinate *in ovo* on day 18 or day 19 of embryonation and in one further study, the vaccine was applied SC to 1-day-old chicks. As *in ovo* vaccination was conducted in one study in 19-day-incubated chicken eggs, the inclusion of this age group in the product information is considered as acceptable. In all

studies, a comparator vaccine or a combination thereof was used in parallel groups from the same breeding using the same route and age of vaccination. In one case, vaccination was only performed with half of a standard dose for the comparator vaccine and had no influence on the outcome of the study. Hatchability was acceptable in all groups. Daily observation of clinical signs and mortalities took place. No adverse events were noted. No clinical signs or deaths related to MD or IBD or ND were noted. Similar mortality rates were observed in all groups. At slaughter, production data were collected and all broiler chickens performed well. The results of the test vaccine and the comparator product were comparable. Results of these solely supportive studies give further assurance that vaccination of commercial broiler chickens with Poulvac Procerta HVT-IBD-ND is safe in the target species. However, these studies are of limited value as no complete study reports were provided.

It is noted that no field trials in chickens of the laying type were performed; however, as chickens of the laying type are not considered to be more sensitive as chickens of the meat type, this approach is accepted.

The field data provided show that the product is safe when used at a standard dose applied via *in ovo* to 18- to 19-day-old embryonated commercial chicken eggs or SC to 1-day-old commercial chicks.

Environmental risk assessment

An environmental risk assessment (ERA) according to Directive 2001/18/EC (Annex II, section D) was provided.

Based on the data provided, the ERA can stop at phase I. Poulvac Procerta HVT-IBD-ND is not expected to pose a risk for the environment when used according to the SPC.

Environmental risk assessment for products containing or consisting of genetically modified organisms

Poulvac Procerta HVT-IBD-ND is a live vector vaccine consisting of a live recombinant herpesvirus of turkeys (HVT) strain FC-126 and two chemically synthesized genes encoding for VP2 protein of IBDV strain 0 and for fusion (F) protein of NDV, which were inserted into the HVT backbone.

This vaccine falls within the scope of Directive 2011/18/EC on the deliberate release into the environment of genetically modified organisms. Detailed information on the possible risks for humans and the environment, the environmental risk assessment and a description of and justification for the proposed release according to the Notice to Applicants Vol. 6C has been provided.

Poulvac Procerta HVT-IBD-ND does not infect mammalians or humans and is restricted to the infection of *Galliformes* birds. The vaccine strain is cell-associated like its parental HVT strain and can only remain infectious as long as the host cells remains viable.

The vaccine virus was shed from vaccinated chickens via feather dander for a limited time span. Comingling of sentinels with vaccinated chickens led to an infrequent spread of the vaccine virus to naïve chickens and turkeys without causing disease. Accordingly, a biologically relevant spread of the vaccine virus into the environment could be detected. However, as HVT is apathogenic, it does not cause disease in in-contact turkeys or other non-target species and is self-limiting after spread to chickens. All relevant information on genetic stability and factors affecting it were included in the corresponding dossier sections.

Taken together, any risk emerging from the use of the attenuated vaccine is expected to be

negligible for humans and low for the environment.

Overall conclusions on the safety documentation

The applicant has provided one pivotal pre-clinical study to investigate the safety of a 10-fold maximum overdose in the target animal species of the minimum recommended age via the recommended routes *(in ovo* and SC). Batches used in these studies were pilot batches. A supportive study was provided performed at a 4-fold maximum overdose.

Based on the results, it was concluded that the safety for the chickens is acceptable when the vaccine is administered according to the recommended schedule and via the recommended routes. These findings were supported by results generated in three EU field trials (and three US trials) in commercial broiler chickens.

Tests on the immunological functions were carried out in the precursor product, which is not expected to adversely affect the immune response of the target animals. The data provided are considered relevant for this product as well as the insertion of another protein gene (F protein gene of NDV) is not considered to change the safety profile of the vaccine virus strain.

Reproduction safety was not investigated as the vaccine is not intended for use in laying birds.

As this is a live vaccine, the applicant has also conducted nine EU studies to establish the potential for shed, spread and dissemination of the vaccine strain and five supportive US studies. Shedding of the vaccine strain from vaccinated chickens was demonstrated for a maximum of 6 weeks after the vaccination. The wording of section 3.5 of the SPC was adapted to the SPC of the precursor product. It was concluded that the vaccine virus could spread from chickens to chickens, chickens to turkeys and between turkeys. Poulvac Procerta HVT-IBD-ND was shown to be safe in the relevant non-target species turkeys, ducks, pheasants, quails, and mice.

The biological properties of the vaccine strain were described adequately and found to be acceptable. As the vaccine strain is genetically modified, reversion to virulence and recombination or genomic reassortment of the strain were also assessed. The results showed that the potential risk is very low and acceptable. The final genetically modified organism (GMO) was shown to be genetically and phenotypically stable over five passages. The biological properties of the apathogenic parental HVT strain seem to be unaltered after insertion of the VP2 gene of IBDV and the F protein of NDV inclusively regulatory elements. The data presented are considered adequate to characterise the safety profile of the vaccine and the active substance.

A user safety assessment in line with the relevant guidance document has been presented. Based on the presented assessment, the product does not pose an unacceptable risk to the user when used in accordance with the product information. The worst-case scenario for user safety is considered to be risks associated with handling liquid nitrogen tanks and thawing of frozen glass vials (i.e. injuries due to exploding glass ampoules). Appropriate warnings for the user have been included in the product literature to indicate that liquid nitrogen containers and the vaccine should only be handled by properly trained personnel.

The vaccine is expected to pose a negligible risk to the environment when used as recommended. A recombination event between the vaccine virus strain and a field strain or another vaccine virus strain is unlikely.

Information concerning the release of genetically modified organisms was provided in the form of appropriate studies and through literature. Taken together, any risk emerging from the use of the attenuated vaccine is expected to be negligible for humans and low for the environment.

Poulvac Procerta HVT-IBD-ND is considered to be safe for the target species and non-target species, the user, the consumer and the environment.

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

General requirements

The vaccine consists of a genetically modified live herpesvirus of turkeys (HVT) strain F-126 vector with the gene encoding for the VP2 capsid protein of infectious bursal disease virus and the F gene of Newcastle disease virus, inserted into the HVT genome. The vaccine is filled into flame-sealed ampoules, to be reconstituted before use in a sterile diluent.

The vaccine is intended to:

- reduce mortality, clinical signs and lesions caused by Marek's disease (MD) virus,
- reduce mortality, clinical signs and lesions caused by infectious bursal disease (IBD) virus and
- reduce mortality and clinical signs caused by Newcastle disease (ND) virus.

when administered to chickens

- by the *in ovo* route at 18-19 days of embryonation at a dose of 0.05 ml or
- by the subcutaneous route at the day of hatch at a dose of 0.2 ml.

The vaccination scheme consists of one single injection; the minimum dose is 3,558 PFU/dose.

Immunity is intended to be established

- for MD from 9 days post vaccination for *in ovo* and subcutaneous use,
- for IBD from 21 days post vaccination for *in ovo* and 14 days for subcutaneous use,
- for ND from 24 days post vaccination for *in ovo* and 21 days for subcutaneous use.

A single vaccination is sufficient to provide protection for the entire risk period for MD and until 63 days of age for IBD and ND.

Efficacy was demonstrated in compliance with Regulation (EU) 2019/6, Ph. Eur. chapter 5.2.7 and Ph. Eur. monographs 0450: Newcastle disease vaccine (live), 0587: Avian infectious bursal disease vaccine (live) and 0589: Marek's disease vaccine (live).

Challenge model

Challenge strain of Marek's disease virus

The MDV challenge strain used in the laboratory efficacy studies is a classical virulent Marek's disease serotype 1 virus originally isolated from an ovarian tumour from a 9-week-old bird in USA. Based on percentage of mortality and lesions caused, the virus strain was classified as a virulent Marek's disease virus. The relationship between the challenge strain and strains collected in Europe has been described in literature (Murata et al., 2020) as there are relatively few amino acid differences in the *meq* sequence (virulence gene). The isolate was shown to be the US strain most closely related to the Eurasian virulent isolates (Trimpert et al., 2017; Mescolini et al., 2019). The

strain has been used as challenge material for several commercial vaccines in the EU and can be found in the same cluster as the European challenge isolate Md70. It is closely related to the RB1B challenge strain utilised in the MD challenge models of further commercial vaccines. Therefore, the strain is considered sufficiently similar to other virulent European isolates and is relevant for assessing vaccine efficacy in Europe. As the challenge strain is an MD serotype 1 isolate, it is heterologous to the MD serotype 3 HVT vaccine strain.

Two challenge model development studies demonstrate that validation criteria of Ph. Eur. monograph 0589 are met after an appropriate dilution of stock material administered at a dose of 0.2 ml to SPF chickens and after an appropriate dilution of stock material administered at a dose of 0.2 ml to broilers and SPF chickens. The MD challenge model which had been optimised using Poulvac Procerta HVT-IBD turned out to be quite severe in the initial studies with Poulvac Procerta HVT-IBD-ND. Moreover, for the new lot of challenge material used in some OOI studies, no explicit challenge model studies were performed. Therefore, multiple OOI studies were performed to achieve a balanced challenge with a sufficient number of susceptible non-vaccinated control chickens (≥ 70%) on the one hand. These requirements were met in all OOI studies. On the other hand, overchallenging the groups has to be avoided for a vaccine to pass the Ph. Eur. test of ≥ 80% relative protection.

Challenge strain of infectious bursal disease virus

The IBDV challenge material used in the laboratory efficacy studies is a very virulent infectious bursal disease strain isolated from brown layer pullets in USA, which showed approximately 26% (3,300 birds) mortality at 14 weeks of age with enlarged bursas (Jackwood *et al*., 2009). Based on mortality rates and histopathology results, the virus strain was classified as a very virulent infectious bursal disease virus (Jackwood *et al*., 2017). Molecular sequences and phylogenetic analysis confirm that the challenge strain is a very virulent IBDV isolate that probably was a descendant of the vvIBDV first observed in Europe and very similar to vvIBDV isolated from the EU. Therefore, the challenge strain is relevant for assessing vaccine efficacy in Europe. Following sequence analysis and phylogenetic assessment, the vvIBDV strain is considered heterologous to the IBDV strain present in the vaccine, which is a classical virulent IBDV isolate from Europe and from which the VP2 insert is derived.

The IBDV challenge studies performed with Poulvac Procerta HVT-IBD-ND as well as the performed calibration study demonstrate that the validation criteria of Ph. Eur. monograph 0587 are met after a relevant challenge dose administered at a dose of 0.1 ml to SPF chickens. As for MD, again multiple OOI studies were required to achieve a balanced challenge with a sufficient number of susceptible non-vaccinated control chickens (≥ 50% with characteristic signs and 100% of surviving chickens with at least degree 3 lesions of the bursa of Fabricius) on the one hand, and to avoid an overchallenging of vaccinated groups to pass the Ph. Eur. protection criteria (≥ 90%) on the other hand.

Challenge strain of Newcastle disease virus

The NDV challenge material Herts Weybridge 33/56 used in the laboratory efficacy studies is a virulent strain of avian paramyxovirus serotype 1, classified as a velogenic strain. The Herts Weybridge 33/56 strain is the standard challenge strain as described in Ph. Eur. monograph 0450 for live Newcastle disease vaccines and is heterologous to the vaccine strain. Therefore, it is considered relevant for the assessment of efficacy of Poulvac Procerta HVT-IBD-ND.

All NDV challenge studies performed with Poulvac Procerta HVT-IBD-ND demonstrate that the validation criteria of Ph. Eur. monograph 0450 are met after a relevant challenge dose administered at a dose of 0.1 ml to SPF and broiler chickens.

Efficacy parameters and tests

The efficacy parameters investigated in the efficacy studies were:

- Mortality, clinical signs (i.e. paralysis, severe depression) and lesions due to classical Marek's disease virus (MDV), HVT serology in the DOI and MDA studies (assessed using a validated in-house immunofluorescent assay), and MDV presence in feather follicles in the supportive DOI study (assessed using a validated in-house qPCR);
- Mortality, clinical signs (i.e. severe depression) and histological bursal lesions caused by IBDV, and IBDV serology in the DOI and MDA studies (assessed using commercial IDEXX and ProFlok IBD Plus ELISA);
- Mortality and clinical signs (i.e. severe depression) caused by NDV, and NDV serology in the OOI, DOI and MDA studies (assessed using commercial Biochek [NDV-F antibody test kit] and IDEXX ELISA, respectively).

The chosen parameters are in line with the requirements of Ph. Eur. monographs 0589, 0587 and 0450 and, therefore, considered appropriate for evaluating the efficacy of the product.

Efficacy documentation

Twenty-two pre-clinical studies were conducted to investigate the efficacy of the product. Laboratory studies were well documented and carried out in SPF chickens or SPF embryonated eggs as well as in MDA-positive commercial broiler birds or embryonated broiler eggs of the minimum age recommended for vaccination.

The batches used were representative pilot batches of the production method described in Part 2.B of this dossier, at the most attenuated passage level that will be present in the vaccine (MSV+5) with a dose not higher than the minimum titre. An MD DOI study considered as supportive only was conducted with Poulvac Procerta HVT-IBD vaccine at MSV+5. The method of manufacture for Poulvac Procerta HVT-IBD is also very similar to the production process described in Part 2.B of this dossier. The solvent utilised for the laboratory efficacy studies (also referred to as Poulvac Sterile Diluent in the studies) has negligible quantitative differences in the ingredient composition compared to the Poulvac Solvent described in the dossier. The solvent used in the ND laboratory efficacy studies (also referred to as Poulvac Marek Diluent in the studies) is the same as listed in this dossier (referred to as Poulvac Solvent).

The laboratory efficacy tests were conducted according to previously defined protocols.

Overview of the laboratory efficacy studies:

Evaluation of efficacy against MDV of Poulvac Procerta HVT-IBD-ND

Study title	**Vaccine dose (PFU/ds.)**
In ovo	
Establishment of minimum infectious dose (MID) and onset of immunity (**OOI**) for HVT-IBD-ND vaccine following ***in ovo*** vaccination against a virulent **Day 6 or Day 9 MDV**	3,165

challenge	
Establishment of minimum infectious dose (MID) and onset of immunity (**OOI**) for HVT-IBD-ND vaccine following ***in ovo*** vaccination against a virulent **Day 6 MDV** challenge	3,318
Establishment of minimum infectious dose (MID) and onset of immunity (**OOI**) for HVT-IBD-ND vaccine following ***in ovo*** vaccination against a virulent **Day 6 MDV** challenge	3,100
Establishment of minimum infectious dose (MID) and onset of immunity (**OOI**) for HVT-IBD-ND vaccine following ***in ovo*** vaccination against a virulent **Day 6 MDV** challenge	3,042
The efficacy of the HVT-IBD-ND vaccine against a vMDV challenge at **11 days** of age after ***in ovo*** or subcutaneous vaccination in chickens with **maternal antibodies**	3,467
Duration of Immunity (**DOI**) of HVT-IBD by assessment of vaccine presence and IBDV serology	3,161
Subcutaneous	
Establishment of minimum infectious dose (MID) and onset of immunity (**OOI**) for HVT-IBD-ND vaccine following **subcutaneous** vaccination against a virulent **Day 9 MDV** challenge	3,115
Establishment of minimum infectious dose (MID) and onset of immunity (**OOI**) for HVT-IBD-ND vaccine following **subcutaneous** vaccination against a virulent **Day 9 MDV** challenge	2,525
The efficacy of the HVT-IBD-ND vaccine against a vMDV challenge at **11 days** of age after *in ovo* or **subcutaneous** vaccination in chickens with **maternal antibodies**	3,558
Duration of Immunity (**DOI**) of HVT-IBD by assessment of vaccine presence and IBDV serology	3,434

*This study was done to support the registration of Poulvac Procerta HVT-IBD with an HVT-IBD vaccine batch and is included for information purposes only.

Evaluation of efficacy against IBDV of Poulvac Procerta HVT-IBD-ND

Study title	**Vaccine dose (PFU/ds.)**
In ovo	
Establishment of minimum infectious dose (MID) and onset of immunity (**OOI**) for HVT-IBD-ND vaccine following ***in ovo*** vaccination against a vvIBDV challenge at **Day 18**	3,110
Establishment of minimum infectious dose (MID) and onset of immunity (**OOI**) for HVT-IBD-ND vaccine following ***in ovo*** vaccination against a vvIBDV challenge at **Day 14**	3,306
Establishment of minimum infectious dose (MID) and onset of immunity (**OOI**) for HVT-IBD-ND vaccine following ***in ovo*** vaccination against a vvIBDV challenge at **Day 14**	3,050
Establishment of minimum infectious dose (MID) and onset of immunity (**OOI**) for HVT-IBD-ND vaccine following ***in ovo*** vaccination against a vvIBDV challenge at **Day 14**	3,193
The efficacy of HVT-IBD-ND against a vvIBDV Challenge at **35**, 42, or 49 Days after ***in ovo*** vaccination in chickens with **maternal antibodies**	3,290
The efficacy of HVT-IBD-ND against a vvIBDV challenge at **35 days** after ***in ovo*** or subcutaneous vaccination in chickens with **maternal antibodies**	3,056
Duration of immunity (**DOI**) for HVT-IBD-ND vaccine following vaccination against vvIBDV challenge at **Day 63**	2,819
Subcutaneous	
Establishment of minimum infectious dose (MID) and onset of immunity (**OOI**) for HVT-IBD-ND vaccine following **subcutaneous** vaccination against a vvIBDV challenge at **Day 14**	2,975
Establishment of minimum infectious dose (MID) and onset of immunity (**OOI**) following **subcutaneous** vaccination against a vvIBDV challenge at **Day 14**	3,400
The efficacy of HVT-IBD-ND against a vvIBDV	3,503

challenge at **35**, 42, or 49 Days after **subcutaneous** vaccination in chickens with **maternal antibodies**	
Assessment of potential **maternally derived antibodies** interference on the efficacy of HVT-IBD-ND after **subcutaneous** vaccination in seropositive chickens against a vvIBDV Challenge at **Day 49**	3,425
The efficacy of HVT-IBD-ND against a vvIBDV challenge at **35 days** after *in ovo* or **subcutaneous** vaccination in chickens with **maternal antibodies**	3,597
Duration of immunity (**DOI**) for HVT-IBD-ND vaccine following vaccination against vvIBDV challenge at **Day 63**	2,675

*Study included for information purposes only.

Evaluation of efficacy against NDV of Poulvac Procerta HVT-IBD-ND

Study title	**Vaccine dose (PFU/ds.)**
***In ovo* and subcutaneous**	
Onset of immunity (**OOI**) of HVT-IBD-ND vector vaccine administered ***in ovo*** and by **subcutaneous** route in SPF chickens, against ND challenge at **21 days** of age	*In ovo*: 2,305 s.c.: 2,864
Efficacy of HVT-IBD-ND vector vaccine administered ***in ovo*** and by **subcutaneous** route to **MDA** broilers, against ND challenge at **35 days** of age	*In ovo*: 2,115 s.c.: 2,812
Duration of immunity (**DOI**) of HVT-IBD-ND vector vaccine administered ***in ovo*** or by **subcutaneous** route in SPF chickens, against ND challenge at **63 days** of age	*In ovo*: 1,972 s.c.: 2,396

Pre-clinical studies

Dose determination

No explicit study on the determination of the vaccine dose has been performed, but all conducted OOI studies include the establishment of a minimum infectious dose (MID). In summary, sufficient efficacy for all three antigens was demonstrated for a minimum dose of 3,558 PFU.

Onset of immunity

MD:

Six studies designed and validated according to the requirements of Ph. Eur. monograph 0589, 2-3-3 Immunogenicity, were performed to determine the efficacy and onset of immunity against MD in SPF chickens, four including animals vaccinated *in ovo* and two using animals vaccinated via the SC route.

In summary, the animals were vaccinated at the minimum age, i.e. *in ovo* (18-day-old embryonated eggs) or SC at the day of hatch with a dose of the minimum titre or lower (i.e. ≤ 3,558 PFU/dose). Challenge was performed at 6 or 9 days of age (9 or 12 days post vaccination), as required by the Ph. Eur. for both routes of vaccination with an appropriate dilution of stock solution of the challenge strain (vMDV) via the SC route. The claimed onset of immunity (9 days post vaccination) for the MD component corresponds to the time of challenge mentioned in the Ph. Eur. for the immunogenicity test. The animals were observed for 70 days after challenge for mortality and clinical signs and post mortem examination was performed of all dead or euthanised animals and all remaining animals after the observation period. In case of inconclusive results, histology examination was conducted for clarification. According to Ph. Eur. monograph 0589, 2-3-3 Immunogenicity, the challenge is valid, if not less than 70% of the challenged control chickens died or showed severe clinical signs or macroscopic lesions of Marek's disease during the observation period following challenge. The vaccine complied with the test if the relative protection percentage (RPP) was not less than 80%. Hatchability was recorded for *in ovo* vaccination.

In study **1** (*in ovo* OOI against MDV [D6 or D9]), six groups of sixty 18-day-old embryonated SPF eggs were used. Two vaccine doses were administered to groups T03/T04 and T05/T06, by the *in ovo* route. Groups T01 and T02 remained unvaccinated. Thirty-five animals of the vaccinated group T03 and 36 animals of the unvaccinated group T01 were challenged with an appropriate dilution of vMDV at 6 days of age. The challenge was valid, as 71.4% of the non-vaccinated/challenged control group (T01) showed characteristic signs of Marek's disease. For the vaccinated/challenged group T03 at a dose below the minimum titre (i.e. ≤ 3,558 PFU/dose), the level of RPP after challenge was 84.4%, which is in line with the efficacy pass criteria mentioned in the Ph. Eur.
Thirty-six animals each of the vaccinated group T04 and of the unvaccinated group T02 were challenged with an appropriate dilution of vMDV at 9 days of age. The challenge was not valid, as only 36.1% of the non-vaccinated/challenged control group (T02) showed characteristic signs of Marek's disease. The differences in the challenge take could be explained by, in many instances, great variability of the biological material, which is considered acceptable.

In study **2** (SC OOI against MDV [D9]), three groups of 45 SPF chickens at the day of hatch were used. Two doses of vaccine below the minimum titre were administered to groups T02 and T03, by the subcutaneous route. Group T01 remained unvaccinated. Thirty-six animals each of the vaccinated group T03 and of the unvaccinated group T01 were challenged with an appropriate dilution of vMDV at 9 days of age. The challenge was valid, as 88.9% of the non-vaccinated/challenged control group (T01) showed characteristic signs of Marek's disease. For the vaccinated/challenged group T03, the level of RPP after challenge was 81.3%, which is in line with efficacy pass criteria mentioned in the Ph. Eur.

In three previous studies for the *in ovo* route and one previous study for the subcutaneous route, provided for informational purposes only, the relative protection rates were below the Ph. Eur. specification of ≥ 80% due to a vaccine dose far below the minimum efficacious titre and/or an overly virulent challenge of vMDV challenge virus. These studies were used to determine the minimum infective vaccine dose as well as to balance the concentration of the challenge virus.

It was concluded that vaccination with a dose less than the minimum content recommended in the product literature was efficacious and met the efficacy requirements:

- from 9 days post vaccination when administered by the *in ovo* route to 18-day-old embryonated eggs with a vaccine dose of 3,165 PFU and above;
- from 9 days post vaccination when administered by the subcutaneous route at day old with a vaccine dose of 3,115 PFU and above.

The proposed claim "reduction of mortality, clinical signs and lesions caused by Marek's disease virus" can be supported based on the results of the presented studies.

IBD:

Six studies designed and validated according to the requirements of Ph. Eur. monograph 0587, 2-3-5 Immunogenicity, were performed to determine the efficacy and onset of immunity against IBD in SPF chickens, four including animals vaccinated *in ovo* and two using animals vaccinated via the SC route.

In summary, the animals were vaccinated at the minimum age, i.e. *in ovo* (18-day-old embryonated eggs) or SC at the day of hatch with a dose of the minimum titre or lower (i.e. ≤ 3,558 PFU/dose). Challenge was performed at 14 days post vaccination as required by the Ph. Eur. for the SC route and 17 or 21 days post vaccination (14 or 18 days of life) for the *in ovo* route, with an appropriate dose of the challenge strain (vvIBDV), via the eye drop route. The time of challenge mentioned in the Ph. Eur. for the immunogenicity test is 14 days post vaccination. As the claimed onset of immunity for the IBD component is 14 days post vaccination for SC use and 21 days post vaccination for *in ovo* use, the time of challenge can be regarded as acceptable for both routes. The animals were observed for 10 days after challenge for mortality and clinical signs. Histological examination for lesions of the bursa of Fabricius of all surviving animals was carried out at the end of the observation period, with bursal damage scored as per Ph. Eur. monograph 0587. According to Ph. Eur. monograph 0587, 2-3-5 Immunogenicity, the challenge is valid, if not less than 50% of the challenged control chickens showed characteristic signs (clinical signs or mortality) of avian infectious bursal disease during the observation period following challenge and all of the surviving challenged control chickens showed ≥ degree 3 lesions of the bursa of Fabricius. The vaccine complied with the test if the dose provided ≥ 90% protection as assessed by notable clinical signs, mortality, and bursal lesion scores < 3.
Hatchability was recorded for *in ovo* vaccination.

In study **1** (*in ovo* OOI against IBDV [D18]), three groups of sixty 18-day-old embryonated SPF eggs were used. Two vaccine doses below the minimum titre were administered to groups T02 and T03, by the *in ovo* route. Group T01 remained unvaccinated. Forty animals each of the vaccinated group T03 and of the unvaccinated group T01 were challenged with an appropriate dose of vvIBDV at 18 days of age. The challenge was valid as in the non-vaccinated/challenged control group (T01) 75.0% of the birds showed characteristic signs of IBD and 100% of the surviving birds showed ≥ degree 3 lesions of the bursa of Fabricius. For the vaccinated/challenged group T03, the level of protection after challenge was 100%, which is in line with the efficacy pass criteria mentioned in the Ph. Eur.

In study **2** (SC OOI against IBDV [D14]), three groups of 44 SPF chickens at the day of hatch were used. Two vaccine doses below the minimum titre were administered to groups T02 and T03, by the subcutaneous route. Group T01 remained unvaccinated. Forty animals each of the vaccinated group T03 and of the unvaccinated group T01 were challenged with an appropriate dose vvIBDV at 14 days of age. The challenge was valid as 77.5% of the birds showed characteristic signs of IBD in the non-vaccinated/challenged control group (T01) and 100% of the surviving birds showed ≥ degree 3

lesions of the bursa of Fabricius. For the vaccinated/challenged group T03, the level of protection after challenge was 97.5%, which is in line with the efficacy pass criteria mentioned in the European Pharmacopoeia.

In three previous studies for the *in ovo* route and one previous study for the SC route, provided for informational purposes only, the protection rates were below the Ph. Eur. specification of ≥ 90% due to a vaccine dose below the minimum efficacious titre and/or an overly virulent challenge. These studies were used to determine the minimum infectious vaccine dose, the time of onset of immunity as well as to balance the concentration of the challenge virus in young chickens.

The *in ovo* administration was found to have a more variable efficacy against IBD than the subcutaneous administration in OOI studies. This difference between the administration routes was also observed, to a lesser degree, with the vaccine's efficacy against MD (MD OOI). The reason for this may be that *in ovo* administration does not always ensure that the animal/embryo receives the entire vaccine dose. Inoculation into the allantoic fluid sac or air cell (i.e. extra-embryonic) has been shown to be less efficacious than inoculation into the embryo (Islam *et al*, 2001). The mechanism behind this appears to be a reduced HVT viraemia in birds that were inoculated extra-embryonically instead of intra-embryonically (Islam *et al*, 2001). Since efficacy against IBD as well MD is dependent on the successful multiplication and dissemination of the HVT vaccine strain of Poulvac Procerta HVT-IBD-ND in the target species, variability in the site of *in ovo* inoculation is likely to impact the efficacy of the vaccine.

It was concluded that vaccination with a dose less than the minimum content recommended in the product literature was efficacious and met the efficacy requirements:

- from 21 days post vaccination when administered by the *in ovo* route to 18-day-old embryonated eggs with a vaccine dose of 3,110 PFU and above;
- from 14 days post vaccination when administered by the subcutaneous route at day old with a vaccine dose of 2,975 PFU and above.

The proposed claim "reduction of mortality, clinical signs and lesions caused by infectious bursal disease virus" can be supported based on the results of the presented studies.

ND:

One study designed and validated according to the requirements of Ph. Eur. monograph 0450, 2-3-5 Immunogenicity, was performed to determine the efficacy and onset of immunity against ND in SPF chickens, including animals vaccinated *in ovo* and via the SC route.

In summary, the animals were vaccinated at the minimum age, i.e. *in ovo* (18-day-old embryonated eggs) or SC at the day of hatch with a dose of the minimum titre or lower (i.e. ≤ 3,558 PFU/dose). Challenge was performed at 21 days post vaccination, as required by the Ph. Eur. for the SC route and 24 days post vaccination (21 days of life) for the *in ovo* route, with an appropriate dose of challenge strain vNDV Herts (Weybridge 33/56) via the intramuscular route as stipulated by the Ph. Eur. The time of challenge mentioned in the Ph. Eur. for the immunogenicity test is 21 days post vaccination. As the claimed onset of immunity for the ND component is 21 days post vaccination for SC use and 24 days post vaccination for *in ovo* use, the time of challenge can be regarded as acceptable for both routes. The animals were observed for mortality and clinical signs for 14 days after challenge. According to Ph. Eur. monograph 0450, 2-3-5 Immunogenicity, the challenge is valid, if not less than 100% of the challenged control chickens have died 6 days after the challenge. The vaccine complied with the test if the dose provided ≥ 90% protection as assessed by notable clinical signs of Newcastle disease and mortality.
Hatchability was recorded for *in ovo* vaccination.

In study **1** (*in ovo* and SC OOI against NDV [D21]), two groups (T04, T05) of fifty and one group (T01) of thirty-six 18-day-old embryonated SPF eggs were used. Additionally, two groups (T02, T03) of 26 SPF chickens at the day of hatch were used. Two vaccine doses below the minimum titre were administered to groups T04 and T05, respectively, by the *in ovo* route. At day of hatch, two vaccine doses below the minimum titre were administered to groups T02 and T03, respectively, by the SC route. Group T01 remained unvaccinated. Twenty-six animals each of the vaccinated groups T03 and T05 and fifteen animals of the unvaccinated group T01 were challenged with an appropriate dose vNDV at 21 days of age. The challenge was valid as 100% of chickens showed clinical signs and died between 2 to 3 days post challenge in the non-vaccinated/challenged control group (T01). For the SC vaccinated/challenged group T03, the level of protection after challenge was 92.0%, for the *in ovo* vaccinated/challenged group T05 the level of protection after challenge was 100%, which are both in line with the efficacy pass criteria mentioned in the Ph. Eur.

It was concluded that vaccination with a dose less than the minimum content recommended in the SPC was efficacious and met the efficacy requirements:

- from 24 days post vaccination when administered by the *in ovo* route to 18-day-old embryonated eggs with a vaccine dose of 2,864 PFU and above;
- from 21 days post vaccination when administered by the subcutaneous route at day old with a vaccine dose of 2,305 PFU and above.

The proposed claim "reduction of mortality and clinical signs caused by Newcastle disease virus" can be supported based on the results of the presented studies.

Duration of immunity

MD:

One study was performed to determine the duration of immunity against MD in SPF chickens, including animals vaccinated *in ovo* or via the SC route.

This study has been performed with Poulvac Procerta HVT-IBD and is considered supportive to establish the MD DOI for Poulvac Procerta HVT-IBD-ND as the HVT FC-126 backbone is the same in both vaccines.

In summary, the animals were vaccinated at the minimum age, i.e. *in ovo* (18-day-old embryonated eggs) or SC at the day of hatch with a dose of the minimum titre or lower (i.e. ≤ 3,558 PFU/dose). The animals were observed for 63 days for mortality and abnormal clinical signs and post-mortem examination was performed of all dead or euthanised animals. In case of inconclusive results, histology examination was conducted for clarification. Feather pulp and blood samples were taken at different timepoints from D21 until D63 after hatch in order to assess vaccine persistence through 63 days of age.
A group of unvaccinated SPF animals was included to validate the study. Hatchability was recorded.

In this study (*in ovo* and SC DOI for MDV), three groups of sixty 18-day-old embryonated SPF eggs were used. A vaccine dose below the minimum titre was administered to group T02 by the *in ovo* route. At hatch, a vaccine dose below the minimum titre was administered to group T03 by the subcutaneous route. Group T01 remained unvaccinated. The HVT and IBD serological antibodies (geometric mean titre - GMT) for the vaccinated groups increased from D21 at each subsequent timepoint throughout the study until D63 with 94.4% and 100% positive samples, respectively. IFA titres for MDV on D21 confirmed non-vaccinated birds had not been exposed to other Marek's disease viruses.

As discussed within the scientific advice dated 10/06/2022, Marek's disease vaccines have been generally accepted to induce lifelong immunity. A DOI claim for the entire risk period utilising a single dose is therefore justified for both administration routes against MD based on the known persistent infection of chickens with HVT and the serological response of the birds in the presented supportive study.

IBD:

One study, designed according to the Ph. Eur. monograph 0587, 2-3-5 Immunogenicity, was performed to determine the duration of immunity against IBD in SPF chickens, including animals vaccinated *in ovo* and via the SC route.

In summary, the animals were vaccinated with Poulvac Procerta HVT-IBD-ND at the minimum age, i.e. *in ovo* (18-day-old embryonated eggs) or SC at the day of hatch with a dose lower than the minimum dose (i.e. ≤ 3,558 PFU/dose). Challenge was performed at 63 days of age, as required by the Ph. Eur. for both routes of vaccination with an appropriate dose of the challenge strain (vvIBDV) via the eye drop route. The animals were observed for 10 days after challenge for mortality and clinical signs. Histological examination for lesions of the bursa of Fabricius of all surviving animals was carried out at the end of the observation period with bursal damage scored as per Ph. Eur. monograph 0587. According to Ph. Eur. monograph 0587, 2-3-5 Immunogenicity, the challenge is valid if not less than 50% of the challenged control chickens showed characteristic signs (clinical signs or mortality) of avian infectious bursal disease during the observation period following challenge, and all of the surviving challenged control chickens showed ≥ degree 3 lesions of the bursa of Fabricius. The vaccine complied with the test if the dose provided ≥ 90% protection as assessed by notable clinical signs, mortality, and bursal lesion scores < 3.
IBDV serology was performed on days 21, 42 (7 birds per pen culled and bled), and on days 52 and 62 (10 birds per pen) to consider seroconversion/absence of seroconversion after vaccination. Hatchability was recorded for *in ovo* vaccination.

In this study (*in ovo* and SC DOI against IBDV [D63]), two groups (T01 and T02) of one hundred and twenty 18-day-old embryonated SPF eggs and one group (T03) of 96 SPF chickens at the day of hatch were used. A vaccine dose below the minimum titre was administered to group T02 by the *in ovo* route. At hatch, a vaccine dose below the minimum titre was administered to group T03 by the subcutaneous route. Group T01 remained unvaccinated. Forty-five animals each of the vaccinated groups T02 and T03 and of the unvaccinated group T01 were challenged with an appropriate dose vvIBDV at 63 days of age. The challenge was not valid according to Ph. Eur. criteria, which are set specially to demonstrate the onset of immunity, as only 40.0% of the birds showed characteristic signs of IBD in the non-vaccinated/challenged control group (T01). Nevertheless, (100%) of the surviving birds displayed ≥ degree 3 lesions of the bursa of Fabricius and the comparisons between the treatment groups showed a significant difference (T02 with p=0.0008, T03 with p=0.0008) in characteristic signs of IBD (including mortality) between the vaccinated and non-vaccinated groups. The lower percentage of non-vaccinated chickens affected by post-challenge mortality and clinical signs is suspected to be representative of the older age of the birds (9 weeks) at a time when bursal involution has begun (from 3-6 weeks onwards, according to van den Berg et al., 2000). The level of protection after challenge was 100%. The vaccine induced the development of IBD-specific antibodies in vaccinated SPF birds within 21 days post-vaccination, which increased and were maintained until challenge. The geometric mean titre at D62 was 14,305 for T02 and 14,815 for T03.

In this challenge study at 63 days of age, which is the claimed duration of immunity, a significant difference in protection was demonstrated between vaccinated groups and controls, sufficiently supporting the proposed duration of immunity for both application routes.

ND:

One study, designed according to the Ph. Eur. monograph 0450, 2-3-5 Immunogenicity, was performed to determine the duration of immunity against ND in SPF chickens, including animals vaccinated *in ovo* and via the SC route.

In summary, the animals were vaccinated at the minimum age, i.e. *in ovo* (18-day-old embryonated eggs) or SC at the day of hatch with a dose lower than the minimum dose (i.e. ≤ 3,558 PFU/dose). Challenge was performed at 63 days of age, as required by the Ph. Eur. for both routes of vaccination with an appropriate dose of challenge strain vNDV Herts (Weybridge 33/56) via the intramuscular route as stipulated by the Ph. Eur. The animals were observed for 14 days after challenge for mortality and clinical signs. According to Ph. Eur. monograph 0450, 2-3-5 Immunogenicity, the challenge is valid if not less than 100% of the challenged control chickens have died 6 days after the challenge. The vaccine complied with the test if the dose provided ≥ 90% protection as assessed by notable clinical signs of Newcastle disease and mortality.
NDV serology was performed on days 14, 21, 28, 42, 56 and 62 using 10 chickens per group to consider seroconversion/absence of seroconversion after vaccination.
Hatchability was recorded for *in ovo* vaccination.

In this study (*in ovo* and SC DOI against NDV [D63]), two groups (T01 and T03) of sixty and eighty 18-day-old embryonated SPF eggs, respectively, and one group (T02) of 30 SPF chickens at the day of hatch were used. A vaccine dose below the minimum titre was administered to group T03 by the *in ovo* route. At hatch, a vaccine dose below the minimum titre was administered to group T02 by the subcutaneous route. Group T01 remained unvaccinated. Thirty animals each of the vaccinated groups T02 and T03 and of the unvaccinated group T01 were challenged with an appropriate dose vNDV at 63 days of age. The challenge was valid, as 100% of chickens showed clinical signs in the non-vaccinated/challenged control group (T01) and died between 3 to 4 days post challenge. For both administration routes, the level of protection after challenge was 100%. A significant difference ($p < 0.0001$) in protection between the vaccinated and non-vaccinated groups was shown in this study. The vaccine induced the development of NDV-specific antibodies in vaccinated SPF birds within 42 days post-vaccination, which increased in titre and were maintained until challenge. The geometric mean titre at D62 was 147 and 621 for T02 and T03, respectively. As it has been described, conventional ELISA NDV kits are not sensitive in detecting seroresponses induced by HVT-vectored ND vaccines, but still a good serological response was observed at Days 56 and 62, which is acceptable.

In this challenge study at 63 days of age, which is the claimed duration of immunity, efficacy requirements were met and a significant difference in protection was demonstrated between vaccinated groups and controls, sufficiently supporting the proposed duration of immunity for both application routes.

Maternally derived antibodies (MDA)

The studies were performed following the guidance of reflection paper EMA/CVMP/IWP/439467/2007:

- Three groups of animals (MDA+ non-vaccinated, MDA- vaccinated and MDA+ vaccinated at the minimum age recommended for use) were included in the studies;
- Challenge is supposed to be performed if MDA levels in control animals at the time of challenge are sufficiently low. Therefore, MD challenges were performed at 11 days of age, IBD challenges at 35 and 49 days of age and ND challenges at 35 days of age, when MDA levels had waned;
- It had to be shown whether the efficacy of the vaccine in animals vaccinated in the presence of

MDAs is, notwithstanding normal biological variation, similar to that obtained in animals of the same age but vaccinated in the absence of MDAs.

MD:

One study, designed according to the Ph. Eur. monograph 0589, 2-3-3 Immunogenicity, was performed to determine the efficacy against MD in commercial broiler chickens with maternal antibodies vaccinated *in ovo* and via the SC route.

In summary, the animals were vaccinated at the minimum age, i.e. *in ovo* (18-day-old embryonated eggs) or SC at the day of hatch with a dose of the minimum titre or lower (i.e. ≤ 3,580 PFU/dose). Challenge was performed at 11 days of age for both routes of vaccination with an appropriate dilution of stock solution of challenge strain (vMDV) via intra-abdominal injection. MDV and IBDV serology was performed on Days 0 and 10 for all treatment groups to determine the serological status of the animals used. After challenge infection, the animals were observed for 70 days for mortality and clinical signs and post-mortem examination of all dead or euthanised animals and all remaining animals was performed after the observation period. In case of inconclusive results, histology examination was conducted for clarification. According to Ph. Eur. monograph 0589, 2-3-3 Immunogenicity, the challenge is valid if not less than 70% of the challenged control (T01) chickens died or showed severe clinical signs or macroscopic lesions of Marek's disease during the observation period following challenge. The vaccine was considered immunogenic in the presence of MDA, if T02 and T03 (MDA+, vaccinated) percent protection, when compared to T01 (MDA+, non-vaccinated), was clinically relevant or significantly different ($p \leq 0.05$). Also, the percent susceptibility of T02 and T03 (MDA+, vaccinated) compared to T05 and T08 (SPF, vaccinated) was similar and within the range of normal biological variation based on a range of protection levels in OOI SPF studies.
Hatchability was recorded for *in ovo* vaccination.

In study **1** (*in ovo* and SC MDA against MDV [D11]), three groups of one hundred and thirty 18-day-old embryonated eggs with MDAs (T01, T02 and T06) and two groups of sixty 18-day-old embryonated SPF eggs (T04 and T08), were used. Additionally, at the day of hatch two groups of 99 chickens with MDAs (T03 and T07) and two groups of 45 SPF chickens (T05 and T09) were used. Four vaccine doses close to the minimum titre were administered by the *in ovo* route. Two vaccine doses close to the minimum titre were administered by the SC route. Group T01 remained unvaccinated. Eighty-three to eighty-five animals each of the vaccinated MDA+ groups T02 and T03 and of the unvaccinated MDA+ group T01, as well as 30 animals each of the vaccinated MDA- groups T05 and T08 were challenged with vMDV at 11 days of age. The challenge was valid, as 82.1% of the non-vaccinated/challenged control group (T01) showed characteristic signs of Marek's disease. The percent protection of the vaccinated/challenged MDA+ chickens (T02 and T03), when compared to the non-vaccinated/challenged MDA+ chickens (T01), was significantly different for each ($p < 0.0001$). The vaccinated MDA+ (T02 and T03) groups and vaccinated MDA- (T05 and T08) groups showed similar protection levels based on the range of protection levels in OOI SPF studies and considering the source/breeds differences between the MDA+ and MDA- animals.

Regarding serology, 100% of the broilers sampled at Day 0 had high levels of MDAs against MDV (GMT 239-256). A decrease in group GMT was observed with a reduction in titres to 220 (T01 non-vaccinated), 128 (T02 *in ovo* vaccinated) and 235 (T03 SC vaccinated) on D10.

It was concluded that vaccination by the recommended routes with a dose of the minimum content recommended in the product literature or below was efficacious and met the efficacy requirements including MDA+ animals.

The proposed claim "reduction of mortality, clinical signs and lesions caused by Marek's disease

virus" can be supported in vaccinated chickens with MDAs based on the results of the presented study.

IBD:

Three valid studies and one invalid study, designed according to the Ph. Eur. monograph 0587, 2-3-5 Immunogenicity, were performed to determine the efficacy against IBD in commercial broiler chickens with maternal antibodies, vaccinated via the *in ovo* and via the subcutaneous route.

In summary, the animals were vaccinated at the minimum age, i.e. *in ovo* (18-day-old embryonated eggs) or SC at the day of hatch with a dose of the minimum titre or lower (i.e. ≤ 3,580 PFU/dose). Challenge was performed at 35 days of age for both routes of vaccination and at 49 days of age for the SC route with an appropriate dose of the challenge strain (vvIBDV), via the eye drop route. IBD serology was performed on Day 0 and on Day 14, 26/27, afterwards weekly until challenge and at the end of the study to determine the serological status of the animals used. After challenge, the animals were observed for 10 days for mortality and clinical signs. Histological examination for lesions of the bursa of Fabricius of all surviving animals was carried out at the end of the observation period with bursal damage scored as per Ph. Eur. monograph 0587. According to Ph. Eur. monograph 0587, 2-3-5 Immunogenicity, the challenge is valid if not less than 50% of the challenged control (T01) chickens showed characteristic signs (clinical signs or mortality) of avian infectious bursal disease during the observation period following challenge and all of the surviving challenged control chickens (T01) showed ≥ degree 3 lesions of the bursa of Fabricius. The vaccine was considered immunogenic in the presence of MDAs if percent protection of vaccinated MDA+ animals, when compared to T01 (MDA+, non-vaccinated), was clinically relevant or significantly different ($p \leq 0.05$). Also, the percent susceptibility of vaccinated MDA+ animals compared to vaccinated SPF animals should be similar and within the range of normal biological variation, based on a range of protection levels in OOI SPF studies.
Hatchability was recorded for *in ovo* vaccination.

In study **1** (*in ovo* MDA against IBDV [D35]), five groups of sixty 18-day-old embryonated eggs, three groups with MDAs (T01, T02 and T03) and two SPF groups (T04 and T05), were used. Four vaccine doses close to the minimum titre (T02-T05) were administered by the *in ovo* route. Group T01 remained unvaccinated. Twenty-four animals each of the vaccinated groups T03 and T04 and of the unvaccinated group T01 were challenged with an appropriate dose vvIBDV at 35 days of age. The challenge was not valid according to Ph. Eur. criteria, which are set specially to demonstrate the onset of immunity in SPF birds, as only 4.2% of the birds in the non-vaccinated/challenged control group (T01, MDA+) showed characteristic signs of IBD and three birds did not show degree 3 lesions of the bursa of Fabricius. Nevertheless, the challenge is considered sufficiently validated in the absence of an unvaccinated SPF control group, as both findings are considered not unexpected due to the lower susceptibility in broilers compared to SPFs (LeGros et al., 2009). The percent protection of the vaccinated/challenged MDA+ chickens (T03: 66.7%), when compared to the non-vaccinated/challenged MDA+ chickens (T01: 12.5%), was significantly different ($p < 0.0005$). The protection percentage in the vaccinated MDA- (SPF) group T04 was 95.8%. The difference in percent protection for the vaccinated/challenged MDA+ group T03and the vaccinated/challenged MDA- group T04, was 29.2% which is somewhat above the range as set in the protocol. The normal biological variation of 15-20% as proposed was based on an acceptable range of protection levels in OOI SPF studies. This could be due to the higher dose of the vaccine used in the SPF group and/or associated to the presence of MDAs in 33.3% of the birds. Moreover, by using different bird types for MDA-positive and MDA-negative chickens, the biological variability is expected to be larger so that a range of 66.7% to 95.8% in percentage protection seems plausible.
Regarding serology, 100% of the broilers sampled at Day 0 had high levels of MDA against IBDV

(GMT T01: 7,841, T02: 8,584, T03: 7,703). A decrease in group GMT was observed with a reduction in titres to 83.2 (33.3% positive, non-vaccinated, T01) and 138.1 (vaccinated, T03) on D34.

In study **2** (SC MDA against IBDV [D35]), five groups of 30 chickens at the day of hatch, three groups with MDAs (T01, T02 and T03) and two SPF groups (T04 and T05), were used. Two vaccine doses close to the minimum titre (T02/T04 and T03/T05) were administered by the SC route. Group T01 remained unvaccinated. Twenty-four animals each of the vaccinated groups T03 and T05 and of the unvaccinated group T01 were challenged with an appropriate dose vvIBDV at 35 days of age. All birds in the non-vaccinated/challenged control group (T01, MDA+) showed at least degree 3 lesions of the bursa of Fabricius. The challenge was considered sufficiently validated as this finding is considered not unexpected due to the lower susceptibility in broilers compared to SPFs (LeGros et al. 2009). The percent protection of the vaccinated/challenged MDA+ chickens (T03), when compared to the non-vaccinated/challenged MDA+ chickens (T01), was significantly different ($p = 0.0075$). The difference in percent protection for the vaccinated/challenged MDA+ group T03 and the vaccinated/challenged MDA- group T05, was outside the range, as set in the protocol that was based on the variations within SPF birds. In addition to the difference of bird types for MDA-positive and MDA-negative chickens, it also appears that the Day 35 challenge was too early when more than 20% of unvaccinated birds were still seropositive. To show that, in line with EMA/CVMP/IWP/439467/2007, the protection rates in animals vaccinated in the presence of MDAs are similar to that obtained in animals of the same age but vaccinated in the absence of MDAs, a further study with challenge carried out at a later time when the MDAs had decreased to a greater extent, was conducted.
Regarding serology, 100% of the broilers sampled at Day 0 had high levels of MDAs against IBDV (GMT T01: 9441.6). A decrease in group GMT was observed with a reduction in titres to 99.3 (20.7% positive, non-vaccinated, T01) and 213.4 (vaccinated, T03) on D34.

In study **3** (SC MDA against IBDV [D49]), one group of 30 chickens with MDAs (T01) and two groups of 45 chickens with MDAs (T02 and T03) at the day of hatch were used. Two vaccine doses below the minimum titre (T02 and T03) were administered by the SC route. Group T01 remained unvaccinated. Twenty-four animals each of the vaccinated group T03 and of the unvaccinated group T01 were challenged with an appropriate dose vvIBDV at 49 days of age. All birds in the non-vaccinated/challenged control group (T01, MDA+) showed at least degree 3 lesions of the bursa of Fabricius. The challenge is considered sufficiently validated as this finding is considered not unexpected due to the lower susceptibility in broilers compared to SPFs (LeGros et al. 2009). The percent protection of the vaccinated/challenged MDA+ chickens (T03when compared to the non-vaccinated/challenged MDA+ chickens (T01was significantly different ($p = 0.0003$). The difference in percent protection for the vaccinated/challenged MDA+ group T03, when compared to historical data for vaccinated/challenged MDA- groups of similar age was similar. Regarding serology, 100% of the broilers sampled at Day 0 had high levels of MDAs against IBDV (GMT T01: 5663.4). A decrease in group GMT was observed with a reduction in titres to 18.1 (4.0% positive, non-vaccinated, T01) on day 48. In the vaccinated MDA+ group T03, 84.6% of the birds were positive for IBDV antibodies (GMT 941.3) on D48.

In one previous study, efficacy against a virulent heterologous IBD challenge could not be demonstrated due to low challenge susceptibility (10.5%) of the non-vaccinated control group. Chickens were considered still protected by MDAs at the time of challenge 35 days post vaccination. Even though the mean titres were considered to be low at the time of challenge (GMT 383), there were still 52% of birds (11 of 21) that were seropositive.

It was concluded that vaccination by the recommended routes with a dose less than the minimum content recommended in the product literature was efficacious and met the efficacy requirements

including MDA-positive animals.

In general, the proposed claim "reduction of mortality, clinical signs and lesions caused by infectious bursal disease virus" can be supported in vaccinated chickens with MDAs based on the results of the presented studies.

Nevertheless, it can be stated that in at least one study presented to demonstrate IBDV efficacy in commercial animals with very high levels of MDA against IBDV, there is some difference in protection seen in comparison to the results achieved with SPF animals, acknowledging different breeds of MDA+ and MDA- birds were used in the studies and could contribute to the differences in protection levels observed.

To reflect this possible difference in protection, a sentence has been added to the SPC (3.4.) that in presence of very high MDA levels against IBDV at the time of vaccination, chickens may have a reduced rate of protection against IBDV infection (in comparison with seronegative vaccinated chickens) during the period when MDAs are declining. Nevertheless, the protection rate remains statistically significantly higher in vaccinated compared to non-vaccinated chickens with MDAs.

ND:

One study, designed according to the Ph. Eur. monograph 0450, 2-3-5 Immunogenicity, was performed to determine the efficacy for ND in commercial broiler chickens with maternal antibodies vaccinated *in ovo* and via the SC route.

In summary, the animals were vaccinated at the minimum age, i.e. *in ovo* (18-day-old embryonated eggs) or SC at the day of hatch with a dose of the minimum titre or lower (i.e. ≤ 3,580 PFU/dose). Challenge was performed at 35 days of age for both routes of vaccination with an appropriate dose of challenge strain Herts (Weybridge 33/56) (vNDV) via the intramuscular route. NDV serology was performed on Days 0, 28 and 34 for all treatment groups to determine the serological status of the animals used. After challenge infection, the animals were observed for 11 days for mortality and clinical signs. As per Ph. Eur. monograph 0450, animals should have been observed for 14 days after challenge infection. This deviation from the study protocol, however, was unlikely to change the results and conclusions of the study because surviving non-vaccinated T02 chickens had recovered from the challenge eleven days post challenge indicating the NDV outbreak was considered finalised. According to Ph. Eur. monograph 0450, 2-3-5 Immunogenicity, the challenge is valid, if not less than 100% of the challenged control chickens have died 6 days after the challenge. The vaccine was considered immunogenic in the presence of MDAs, if T03 and T05 (MDA+, vaccinated) percent protection, when compared to T02 (MDA+, non-vaccinated), was significantly different ($p \leq 0.05$). Also, the percent susceptibility of T03 and T05 (MDA+, vaccinated) compared to T01 (SPF, vaccinated) was similar and within the range of normal biological variation. Hatchability was recorded for *in ovo* vaccination.

In study **1** (*in ovo* and SC MDA against NDV [D35]), two groups of eighty 18-day-old embryonated eggs with MDAs (T02 and T03) and one group of seventy-six 18-day-old embryonated SPF eggs (T01) were used. Additionally, at the day of hatch one group of 40 chickens with MDAs (T04) was used. A vaccine dose below the minimum titre was administered by the *in ovo* route to groups T01 and T03. A vaccine dose below the minimum titre was administered by the SC route to group T04. Group T02 remained unvaccinated. Thirty animals each of the vaccinated groups T01, T03 and T04 and of the unvaccinated group T02 were challenged with vNDV at 35 days of age. The challenge was not valid according to Ph. Eur. criteria, which are set specially to demonstrate the onset of immunity in SPF birds, as only 67% of the birds died within three to six days after challenge in the non-vaccinated/challenged control group (T02, MDA+) and 83% had died by the end of the study. However, 100% of the animals showed clinical signs. Nevertheless, the challenge is considered

sufficiently validated in the absence of an unvaccinated SPF control group, as the percentages of clinically sick and deceased T02 birds are sufficient evidence that the challenge was virulent. Based on clinical signs and mortality, the percent protection of the vaccinated/challenged MDA+ chickens (T03 and T04: 100%), when compared to the non-vaccinated/challenged MDA+ chickens (T02: 0.0%), was significantly different ($p < 0.0001$) for both vaccination routes. The protection percentage in the vaccinated MDA- (SPF) group T01 was 100%. No difference in % of protection for group T03 (*in ovo*) and group T04 (SC) versus the respective SPF vaccinated group (T01) was observed, considering the same parameters (mortality and clinical signs) for both SPF and MDA+ vaccinated animals.
Regarding serology, 100% of the broilers sampled at Day 0 had high levels of MDAs against NDV (GMT 21,454 for T02, 16,084 for T03 and 14,274 for T04). At Day 28 and Day 34, the % of positive birds decreased to 0% in the non-vaccinated MDA+ group (T02) with a GMT of 189 and 157, respectively. In the vaccinated MDA+ groups T03 and T04, the % of seropositive birds was 90% at Day 28 and 100% at Day 34, with GMTs at Day 28 between 4,689 and 6,815 and at Day 28 between 9,781 and 14,544.

It was concluded that vaccination by the recommended routes with a dose below the minimum content recommended in the product literature was efficacious and met the efficacy requirements including MDA-positive animals.

The proposed claim "reduction of mortality and clinical signs caused by Newcastle disease virus" can be supported in vaccinated chickens with MDAs based on the results of the presented study.

Interactions

No studies on interactions were performed. Therefore, the following statements are included in SPC section 3.8 and 5.1:

3.8 Interaction with other medicinal products and other forms of interaction:

"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."

5.1 Major incompatibilities

"Do not mix with any other veterinary medicinal product except the solvent recommended for use with the veterinary medicinal product."

Clinical trials

No specific clinical studies to examine the efficacy against MD, IBD and ND under field conditions are presented for Poulvac Procerta HVT-IBD-ND. As discussed within the scientific advice dated 10/06/2022, the omission of such studies is justified as for both routes of administration convincing laboratory data on efficacy against challenges with either vMDV, vvIBDV or vNDV representative for the EU field situation, have been generated in SPF chickens at 6 to 63 days of age as well as in MDA+ chickens at 11 to 49 days of age with representative MDA levels. Therefore, it is acceptable to consider the pre-clinical studies sufficient to ensure that no specific clinical efficacy trials are necessary (in line with Regulation (EU) 2019/6 and the Guideline on clinical trials with immunological veterinary medicinal products EMA/CVMP/IWP/260956).

The applicant performed three clinical studies in the EU to evaluate the safety of the vaccine under field conditions (Part 3.C of the dossier). These studies were performed with Poulvac

Procerta HVT-IBD-ND batches of intermediate titre and reference vaccines. All enrolled chicks had representative MDAs against HVT, IBDV and NDV at the time of hatch, which precluded serology to assess the immunogenicity of the vaccine. Production results of Poulvac Procerta HVT-IBD-ND-vaccinated flocks were comparable to those of flocks vaccinated with licensed vaccines of other MAHs. The health, necropsy and slaughter condemnation data of these studies provided no evidence that Poulvac Procerta HVT-IBD-ND lacked efficacy in the field. Together, the clinical data of these studies supported that there were no efficacy problems with Poulvac Procerta HVT-IBD-ND when administered *in ovo* to 18-19-day-old embryonated eggs or subcutaneously at hatch under commercial conditions.

Overall conclusion on efficacy

In general, the applicant adequately demonstrated the efficacy of the vaccine. However, some minor questions remain.

The results from 22 laboratory trials show that the product is effective for the active immunisation of one-day-old chickens and 18- to 19-day-old embryonated chicken eggs to:

- reduce mortality, clinical signs and lesions caused by Marek's disease (MD) virus,
- reduce mortality, clinical signs and lesions caused by infectious bursal disease (IBD) virus and
- reduce mortality and clinical signs caused by Newcastle disease (ND) virus

at the proposed dose of ≥ 3,558 PFU.

Onset of immunity

Onset of immunity has been demonstrated:

- at 9 days post vaccination for *in ovo* and subcutaneous use for MD,
- at 21 days post vaccination for *in ovo* and 14 days for subcutaneous use for IBD, and
- at 24 days post vaccination for *in ovo* and 21 days for subcutaneous use for ND.

Based on the results of the presented studies, the proposed claims concerning MD, IBD and ND could be adequately supported.

Duration of immunity

The duration of immunity has been adequately demonstrated for:

- MD: a single vaccination is sufficient to provide protection for the entire risk period,
- IBD: 63 days of age and
- ND: 63 days of age.

Maternally derived antibodies (MDA)

The proposed claims could be supported in vaccinated chickens with MDAs based on the results of the presented studies.

For MD, IBD and ND, it has been adequately demonstrated according to reflection paper EMA/CVMP/IWP/439467/2007 that MDAs did not interfere with vaccination.
Nevertheless, for IBD, there is some difference in protection seen between the results in commercial chickens and the results achieved with SPF animals in one study, which has been adequately reflected in the SPC.

Interactions

No studies on interactions were performed. Appropriate statements are included in SPC sections 3.8 and 5.1.

Clinical trials

No specific clinical studies to examine the efficacy of MD, IBD and ND under field conditions are presented for Poulvac Procerta HVT-IBD-ND. The omission of such studies was sufficiently justified.

Three clinical studies were performed to evaluate the safety of the vaccine under EU field conditions. Production results of Poulvac Procerta HVT-IBD-ND-vaccinated flocks as well as the health, necropsy and slaughter condemnation data of these studies provided no evidence that Poulvac Procerta HVT-IBD-ND lacked efficacy in the field.

Part 5 – Benefit-risk assessment

Introduction

Poulvac Procerta HVT-IBD-ND is a live recombinant vector vaccine containing a cell-associated live recombinant turkey herpesvirus (strain HVT-IBD-ND) expressing the VP2 protein gene of IBDV and the F protein gene of NDV. This product is considered a GMO under EU legislation. The vaccine is a frozen concentrate for suspension for injection stored in liquid nitrogen in freezing medium. No adjuvant or preservative is added. This frozen concentrate is to be diluted in sterile solvent before use. The pharmaceutical form of the final vaccine is a suspension for injection. Due to the nature of the product and concentration of excipients, the proposed withdrawal period is zero days.

Poulvac Procerta HVT-IBD-ND increases the range of available treatment possibilities because the recombinant vaccine strain induces immunity against three relevant poultry pathogens, Marek's disease virus (MDV), infectious bursal disease virus (IBDV) and Newcastle disease virus (NDV), which are frequently isolated in poultry stocks and they are of paramount importance in the poultry production industry. Furthermore, the vaccine can be applied at an early age of the birds (1-day-old) or even in embryonated chicken eggs (18-19 days-old) to provide protection against early replication of virulent MDV, IBDV and NDV in case of infection.

The vaccine is indicated for the active immunisation of chickens to reduce mortality, clinical signs and lesions caused by MDV, to reduce mortality, clinical signs and lesions caused by IBDV and to reduce mortality and clinical signs caused by NDV.

The vaccine is intended for use in chickens by the *in ovo* route at 18-19 days of embryonation at a dose of 0.05 ml or by the subcutaneous route at day of hatch at a dose of 0.2 ml.

The dossier was submitted in line with the requirements of Article 42(2)a of Regulation (EU) 2019/6.

Benefit assessment

Direct benefit

The proposed benefit of Poulvac Procerta HVT-IBD-ND is its efficacy in active immunisation of embryonated chicken eggs or one-day-old chickens:

- to reduce mortality, clinical signs and lesions caused by Marek's disease (MD) virus,
- to reduce mortality, clinical signs and lesions caused by infectious bursal disease (IBD) virus,
- to reduce mortality and clinical signs caused by Newcastle disease (ND) virus.

This benefit was shown in a large number of appropriately designed and well-executed pre-clinical studies.

The onset of immunity against MD was established at 9 days post vaccination for the *in ovo* route and for subcutaneous use; against IBD at 21 days post vaccination for the *in ovo* route and at 14 days for subcutaneous use; against ND at 24 days post vaccination for the *in ovo* route and at 21 days for subcutaneous use. The duration of protection is adequately demonstrated for MD, where a single vaccination is sufficient to provide protection for the entire risk period and for IBD and ND where the duration of protection is 63 days.

Clinical studies were mainly performed to evaluate the safety of the vaccine under EU field conditions. Production results of Poulvac Procerta HVT-IBD-ND-vaccinated flocks as well as the health, necropsy and slaughter condemnation data of these studies provided no evidence that Poulvac Procerta HVT-IBD-ND lacked efficacy in the field. The influence of maternally derived antibodies on the efficacy of the vaccine was investigated in well-designed laboratory studies, using commercial broiler chickens and eggs with confirmed MDAs against MDV, IBDV and NDV. It can be concluded that MDAs are not expected to interfere with vaccine efficacy. For IBD, there may be some difference in protection seen between results in commercial chickens with very high levels of MDAs in comparison to results achieved with SPF animals (on one study), which has been adequately reflected in the SPC.

Poulvac Procerta HVT-IBD was shown to be efficacious against MD, IBD and ND.

Additional benefits

Poulvac Procerta HVT-IBD-ND is easy to apply to embryonated chicken eggs by a single *in ovo* vaccination using an appropriate device/ applicator. The vaccine is also easy to apply to day-old chicks by a single subcutaneous vaccination. This limits the number of times the animals must be handled.

Poulvac Procerta HVT-IBD-ND can be applied at an early age of the birds (1-day-old) or even in embryonated chicken eggs (18-19-day-old) at the hatchery to provide protection against early replication of virulent MDV, IBDV and NDV and thus reduces clinical signs in case of infection. Consequently, the incidence of clinical MDV, IBDV and NDV outbreaks due to field contamination is reduced.

One single *in ovo* or subcutaneous vaccination is sufficient to stimulate immunity against three relevant poultry pathogens, MDV, IBDV and NDV. The vaccine strain was shown to be fully apathogenic to other avian species, limiting the risk to the environment.

Poulvac Procerta HVT-IBD-ND increases the range of available treatment possibilities for the active immunisation of chickens and embryonated chicken eggs against infections with MDV, IBDV and NDV.

Risk assessment

The main potential risks are identified as follows:

Quality

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. 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.

Safety

Risks for the target animal

The product is generally well tolerated in the target animal when administered in accordance with the product literature recommendations. No adverse reactions were observed after a tenfold overdose of Poulvac Procerta HVT-IBD-ND administered by the *in ovo* or subcutaneous route.

The vaccine strain was obtained by insertion of two additional genes into a naturally apathogenic vaccine strain, which is known to be safe for chickens. The biological properties (safety, dissemination, shed, spread) of the original strain were not changed by the genetic modification. Reversion to virulence could not be demonstrated. The chance of recombination with other strains or other viruses occurring is considered to be effectively zero.

Appropriate warnings are included in section 3.5 of the SPC regarding possible spreading of the vaccine.

Risk for the user

The avirulent parental HVT strain is non-pathogenic for humans and infects only avian hosts without causing clinical disease. There are no indications that the genetically modified virus strain HVT-IBD-ND behaves differently.

The vaccine is filled in glass ampoules and stored in liquid nitrogen; in exceptional cases, ampoules may explode upon warming up. Appropriate precautions and warnings for safe handling of the ampoules are included in the product literature. Only trained personnel should handle liquid nitrogen.

The user safety for this product is acceptable when used according to the recommendations in the product literature.

Risk for the environment

The vaccine virus is shed with feather dust and can remain infectious in the environment for some time. Spread to chickens and turkeys and between turkeys was observed. In general, HVT can infect avian species only and the vaccine strain contained in Poulvac Procerta HVT-IBD-ND was shown to be unable to infect mice. Appropriate measures mitigating the risk of spread of the vaccine strain to turkeys are included in the product literature.

Poulvac Procerta HVT-IBD-ND is not expected to pose a risk for the environment when used according to the recommendations in the product literature.

Risk for the consumer:

A residue study is not required. The withdrawal period is set at zero days.

Risk management or mitigation measures

The following measures are included in the product literature to minimise the above-mentioned risks:

- In the SPC is mentioned that '*The vaccine strain may be excreted by vaccinated chickens for a maximum of 6 weeks post-vaccination and has the potential to spread to turkeys and to a very limited extent to chickens*;
- The vaccine strain may spread. Appropriate veterinary and husbandry measures should be taken to avoid spread of the vaccine strain to unvaccinated chickens and turkeys;
- Detailed description of the handling of the vaccine ampoules stored in liquid nitrogen and a detailed description of the personal protection equipment. Liquid nitrogen should only be handled by trained personnel;
- The veterinary medicinal product is subject to a veterinary prescription.

Evaluation of the benefit-risk balance

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

For active immunisation of one day old chickens and 18-19 day old embryonated chicken eggs to
- reduce mortality, clinical signs and lesions caused by Marek's disease (MD) virus;
- reduce mortality, clinical signs and lesions caused by infectious bursal disease (IBD) virus and
- reduce mortality and clinical signs caused by Newcastle disease (ND) virus.

Onset of immunity:

- MD: 9 days post vaccination for *in ovo* and subcutaneous use
- IBD: 21 days post vaccination for *in ovo* and 14 days for subcutaneous use
- ND: 24 days post vaccination for *in ovo* and 21 days for subcutaneous use

Duration of immunity:

- MD: a single vaccination is sufficient to provide protection for the entire risk period
- IBD: 63 days of age
- ND: 63 days of age

Onset of immunity for MD, IBD and ND is adequately supported by data. Duration of immunity for MD is accepted to be life-long. The duration of immunity against IBD and ND is considered to be adequately supported by data at this point. The influence of maternal antibodies on the efficacy of the vaccine against MDV was studied using commercial broiler chickens with confirmed levels of MDA against MDV, IBDV and NDV. For IBD, there is some difference in protection seen between results in commercial chickens with very high MDA levels in comparison to results achieved with SPF animals in one study only, which has been adequately reflected in the SPC.

The formulation and manufacture of Poulvac Procerta HVT-IBD-ND is well described and the specifications set will ensure that a product of consistent quality will be produced. Currently, the claimed shelf life is considered fully supported by the available data.

Poulvac Procerta HVT-IBD-ND is well tolerated by the target animal and non-target animals and presents an acceptable risk for users and the environment when used as recommended. Appropriate precautionary measures have been included in the product literature.

Based on the data presented, the overall benefit-risk is considered positive.

The product information has been reviewed and is considered to be acceptable.

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 Poulvac Procerta HVT-IBD-ND is approvable since these data satisfy the requirements for an authorisation 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.