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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for Ultifend ND IBD (EMEA/V/C/005347/0000)

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.

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Introduction

The applicant CEVA-Phylaxia Veterinary Biologicals Co. Ltd. submitted on 31 October 2019 an application for a marketing authorisation to the European Medicines Agency (the Agency) for Ultifend ND IBD, through the centralised procedure under Article 3(1) of Regulation (EC) No 726/2004 (mandatory scope).

On 17 February 2021, the CVMP adopted an opinion and CVMP assessment report.

On 20 April 2021, the European Commission adopted a Commission Decision granting the marketing authorisation for Ultifend ND IBD.

The eligibility to the centralised procedure was agreed upon by the CVMP on 16 April 2019 as Ultifend ND IBD has been developed by recombinant DNA technology.

The applicant applied for the following indications:

For the active immunisation of chickens and chicken embryonated eggs:

- To reduce mortality, clinical signs and lesions as well as to reduce virus shedding caused by Newcastle disease virus (NDV).
- To reduce mortality, clinical signs and bursa lesions caused by very virulent infectious bursal disease virus (IBDV).
- To reduce the mortality, clinical signs and lesions caused by classical Marek's disease virus (MDV).
- To reduce the loss of daily weight gain caused by IBDV infection.

The final proposals for the indications are:

For the active immunisation of one-day-old chicks or 18-day-old chicken embryonated eggs:

- To reduce mortality, clinical signs and lesions, and virus shedding caused by NDV.
- To reduce mortality, clinical signs and bursa lesions caused by very virulent IBDV.
- To reduce mortality, clinical signs and lesions caused by classical MDV.

The active substance of Ultifend ND IBD is a live, cell-associated recombinant rHVT-ND-IBD virus strain, with a minimum titre of 4,000 and a maximum titre of 12,000 plaque forming units per dose (PFU/dose). The vaccine, a recombinant turkey herpesvirus (HVT, serotype 3 MDV; FC-126 strain) expresses the fusion gene (F-gene) of the D26 NDV strain and the virion protein 2 gene (VP2-gene) of the serotype 1 NVSL STC (Edgar strain) IBDV strain. The recombinant HVT vaccine strain rHVT-ND-IBD is intended to protect against three major poultry diseases at the same time when administrated *in ovo* as a single dose of 0.05 ml to 18-day-old chicken embryonated eggs or administrated subcutaneously as a single dose of 0.2 ml to one-day-old chicks. The target species are chickens and chicken embryonated eggs.

Ultifend ND IBD consists of a frozen cell concentrate fraction stored in liquid nitrogen and a solvent for suspension for injection. The vaccine is presented in 2-ml sealed glass ampoules containing 1,000, 2,000 or 4,000 doses. The solvent is presented in plastic bags (PVC) of 400, 800, 1,000, 1,200 and 1,600 ml, respectively.

The rapporteur appointed is Esther Werner and the co-rapporteur is Petra Falb.

The dossier has been submitted in line with the requirements for submissions under Article 12(3) of Directive 2001/82/EC – full application.

Marketing authorisation under exceptional circumstances

Not applicable.

Scientific advice

Not applicable.

MUMS/limited market status

Not applicable.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

A detailed description of the pharmacovigilance system was provided (version 07/2017) which fulfils the requirements of Directive 2001/82/EC. Based on the information provided, it is accepted that the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country.

It is concluded that the pharmacovigilance system is adequate and will permit the applicant to discharge their pharmacovigilance responsibilities in accordance with regulatory requirements.

Manufacturing authorisations and inspection status

Manufacture of the final product takes place in the European Union at three sites, two sites of the CEVA Company, CEVA-Phylaxia Veterinary Biologicals Co. Ltd. located in Budapest, Hungary, and CEVA SANTÉ ANIMALE located in Libourne, France. The third manufacturing site is INFOMED FLUIDS SRL, located in Bucharest, Romania.

The manufacture of the active substance takes place at the CEVA-Phylaxia Veterinary Biologicals Co. Ltd. site, Hungary. Manufacture of the final product including batch release is carried out at the CEVA-Phylaxia Co. Ltd. site, Hungary.

The manufacture of the solvent is carried out at the CEVA-Phylaxia Co. Ltd. site, Hungary and the INFOMED FLUIDS SRL site, Romania, whereas finished product batch release of the solvent is only carried out at the CEVA-Phylaxia Veterinary Biologicals Co. Ltd. site, Hungary.

Both CEVA sites have a manufacturing authorisation. INFOMED FLUIDS SRL has a manufacturing authorisation.

GMP certificates, which confirm the date of the last inspection, have been provided.

A GMP declaration for the active substance manufacturing site CEVA-Phylaxia Veterinary Biologicals Co. Ltd., Budapest, Hungary was provided.

Overall conclusions on administrative particulars

The detailed description of the pharmacovigilance system was considered in line with legal requirements.

The GMP status of the active substance and of the finished product manufacturing sites has been satisfactorily established and is in line with legal requirements. A list of organisms handled in the same building where the Ultifend ND IBD vaccine is intended to be produced has been provided.

Part 2 – Quality

Chemical, pharmaceutical and biological/microbiological information (quality)

Qualitative and quantitative particulars of the constituents

Ultifend ND IBD is a trivalent, cell-associated, live recombinant vector vaccine for chickens. The active substance is a genetically-modified live recombinant herpesvirus of turkeys, strain rHVT-ND-IBD expressing the fusion protein (F-gene) of NDV and the capsid virion protein 2 (VP2-gene) of IBDV at a titre between 4,000 and 12,000 plaque forming units (PFU) per dose of 0.05 ml (*in ovo* dose) or 0.2 ml (subcutaneous dose). Other ingredients are included as excipients in the formulation. However, trace amounts of gentamicin remain in a single vaccine dose (not more than 150 ng/ dose) which is considered acceptable. The applicant's manufacturing approach is considered acceptable, as the residual level of gentamicin per final dose (not more than 150 ng) is very low. The frozen cell concentrate fraction is to be reconstituted with the sterile solvent provided before administration to chickens or chicken embryonated eggs.

The solvent consists of stabilisers, nitrogen source, buffers, pH indicator and water for injections.

The product is available as a frozen cell concentrate for injection in 2-ml flame sealed hydrolytic type I glass ampoules with 1,000, 2,000 or 4,000 doses.

The liquid sterile solvent is available in plastic bags of polyvinylchloride (PVC) which contain 400 ml, 800 ml, 1,000 ml, 1,200 ml or 1,600 ml.

Container and closure

The open or closed type I glass ampoules comply with European Pharmacopoeia (Ph. Eur.) chapter 3.2.1. The glass ampoules are flame-sealed. Closed ampoules are purchased sterile and pyrogen free. The sterilisation processes applied for the open ampoules are considered suitable to ensure sufficient innocuousness with respect to the risk of contamination due to container material. Both chosen containers are suitable for storage in liquid nitrogen and are typical for storage of cell-associated vaccines. The ampoules are labelled with liquid nitrogen-proof plastic labels. The labelling of the ampoules is done prior to filling or immediately after filling.

The liquid sterile solvent is filled in plastic bags of polyvinylchloride (PVC) with an infusion port which has a twist-off cap and contain 400 ml, 800 ml, 1,000 ml, 1,200 ml or 1,600 ml. Solvent bags are filled, closed/sealed and placed in an overpouch and heat-sealed with two connecting tubes. The content is accessible through a septum. Certificates of analysis demonstrating Ph. Eur. compliance for plastic bags (PVC) were provided. Bags are autoclaved as part of the terminal heat sterilisation. The filled solvent is autoclaved in its final container in a continuous process in accordance with Ph. Eur. 5.1.1 "steam sterilisation" chapter and 5.1.5 "Application of the F0 concept to steam sterilisation of aqueous preparations" chapter. Satisfactory validation of the terminal heat sterilisation is provided and

acceptable. Based on the above conditions, parametric release for sterility testing of the solvent can be approved.

The pack/container sizes for the frozen cell-associated fraction and sterile solvent are consistent with the vaccination schedule and intended use. Reasonable justification regarding residual amounts of cryoprotectant excipients has been given. Residual amounts of cryoprotectant excipients are not expected to affect the efficacy and safety of the vaccine.

Product development

The applicant has provided adequate information on the choice of antigens, excipients, container-closure system and overages as well as the vaccine production. A reasonable justification regarding the suitability and relevance of the chosen recombinant vaccine strain rHVT-ND-IBD for the European field has been provided.

A naturally-occurring, non-pathogenic turkey herpesvirus coded as FC-126 HVT strain, which was isolated in 1970 in the USA by R. L. Witter from a 23-week-old turkey flock that was experiencing losses from lymphoid tumours, was chosen as parental strain to obtain the recombinant strain able to express NDV F-gene and IBDV VP2-gene. The recombinant strain, rHVT-ND-IBD is able to express nucleotide sequences coding for the MDV, NDV and IBDV antigens at the same time.

The method of manufacture, formulation and storage is the same as for other live Marek's disease vaccines routinely manufactured by the applicant. There is no adjuvant or any preservative present in the vaccine. Trace amounts of gentamicin remain in a single vaccine dose (not more than 150 ng/dose) which is considered acceptable.

The vaccine can be administered via the *in ovo* route (0.05 ml) or by the subcutaneous route (0.2 ml). The active ingredient is identical and the difference in dose volume is due to the amount of sterile solvent used to reconstitute the frozen cell concentrate.

The vaccine is presented in two fractions; a frozen cell-associated concentrate containing the active substance and a liquid sterile solvent. The cell concentrate is filled into 2-ml flame-sealed glass ampoules and labelled with liquid nitrogen-proof plastic labels prior to filling or immediately after filling. The solvent is filled in plastic bags (PVC) which are sterilised by heat as part of the terminal sterilisation of the finished product. The chosen glass containers are suitable for storage in liquid nitrogen and are typical for ampoules used for the storage of cell-associated vaccines against MDV. The chosen PVC plastic bags are clear and colourless which permits the visual inspection of the content and are considered suitable; the same system has been used for other CEVA-Phylaxia vaccines.

The potency and identity test of the active ingredient are carried out in a combined test.

Description of the manufacturing method

The production process is considered as standard manufacture for other live Marek's disease vaccines routinely manufactured by the applicant. The manufacturing process consists of the following main steps: cells are counted and planted into roller bottles in growth media and incubated. The working seed virus (WSV) is thawed and diluted in growth media; cell cultures are inoculated with WSV and incubated. The development of cytopathogenic effect (CPE) formation is monitored. When monolayer cells show characteristic CPE, the cells are harvested. The cell suspension is aseptically collected into sterile centrifuge tubes. Cells are spun; supernatant is discarded. Then, cells are pooled and resuspended in cryoprotectant 1 (contains gentamicin). The resuspended cells are then filtered and collected into a sterile collection container. After harvest and before blending, the cells may be stored

refrigerated in sterile glass or disposable plastic containers until further processing. Based on the cell count, the bulk is supplemented with the required quantity of cool cryoprotectant 1 and cool cryoprotectant 2 to prepare the final vaccine bulk. The use of additional gentamicin along with cryoprotectant 1 solution to adjust / dilute the pooled cell suspension (virus harvest) in order to reach the target cell count / concentration for the blending process of the bulk vaccine is acceptable as this results in very low residual gentamicin levels (150 ng/dose) in a final vaccine dose. Moreover, formulation ranges of the target cell count for the blending of the bulk vaccine have been reconsidered and revised to allow flexibility while ensuring batch to batch consistency and titre of the final product. After the addition of cryoprotectant 2 and DMSO the bulk vaccine is filled immediately into sterilised pre-labelled glass ampoules using a filling-sealing machine. The bulk vaccine may be stored cooled in a large, sterile laboratory glass, plastic vessel or stainless-steel mixer tank with cooling jacket up to one hour until filling. The filled ampoules are flame sealed. After filling and closing the ampoules are frozen. Filled ampoules may be kept cooled before freezing or immediately placed in the freezing machine. The freezing process takes the ampoules to at least -50 °C. The frozen ampoules are then placed into liquid nitrogen or nitrogen vapour for storage.

The whole production process is based on the seed-lot principle and carried out under aseptic conditions, using sterile materials, containers and equipment. Batches of finished product are made within seven passages from the master seed. Laboratory efficacy and field studies as well as the genetic comparison of MSV and MSV+7 passages demonstrated that the MSV remains stable and efficient.

The results of four consecutive vaccine batches demonstrate that the manufacturing process is capable of producing the finished product of the intended quality in a reproducible manner. The in-process controls established are considered adequate for this type of manufacturing process.

In general, the production processes of the active substance and the finished product are described adequately.

The solvent is manufactured by mixing the required ingredients with water for injection in a batch assembly container until all the ingredients are dissolved. The pH is adjusted. The solvent is dispensed into final containers using a hand fill or an automatic filling and sealing machine. The product is filled into plastic bags of varying sizes. The finished solvent products are tested for appearance, pH, osmolarity, sterility and fill volume. The solvent bags are terminally sterilised in their final containers by a validated method in accordance with Ph. Eur. 5.1.1., therefore parametric release for sterility testing of the solvent is supported.

The consistency of solvent production is shown by the results for three representative batches of each presentation (400 ml, 1,000 ml, 1,200 ml and 1,600 ml). The production process of the solvent is described adequately. Equivalence in production of the solvent between the two manufacturing sites has been demonstrated.

Production and control of starting materials

Active substance

The active substance, cell-associated rHVT-ND-IBD virus, is manufactured using cells and subsequently formulated and deep frozen in a continuous operation. Holding times are kept very minimal to guarantee cell viability and are indicated for each operating step. 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.

WSV is prepared as described for the finished product. WSV is tested for appearance, identity, sterility and absence of mycoplasma in accordance with the relevant Ph. Eur. monographs 2.6.1 and 2.6.7. However, freedom from contamination with extraneous agents was not provided as MSV and each final product batch is tested in accordance with relevant Ph. Eur. monographs.

Details on production and control of MSV and WSV, as well as information on the passage history of the MSV, have been provided. Stability and efficacy of MSV at passage level 7 has been demonstrated by laboratory efficacy and field studies, and by genetic comparison at MSV and MSV+7 level. Thus, the applicant's approach to use MSV passages higher than level 5 is acceptable.

Excipients

Specifications of excipients and other starting materials (e.g. materials of biological and non-biological origin, media) are defined and analytical methods are provided.

Starting materials listed in a pharmacopoeia are compliant with the relevant pharmacopoeial monographs.

For starting materials not listed in pharmacopoeia example certificates of analysis are provided.

The documentation provided for all materials of animal origin demonstrates their compliance with the Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev.3) and Commission Directive 1999/104/EEC.

Moreover, the product is intended for poultry, a non-TSE-susceptible species.

It is concluded that, the risk of transmitting TSE infectivity through the use of this vaccine is negligible.

Starting materials listed in pharmacopoeias

Internal specifications and/or representative certificates of analysis (CoA) were provided and all conform to Ph. Eur. required specifications.

Specific materials not listed in a pharmacopoeia

Starting materials of biological origin

A seed-lot system was satisfactorily established for the active ingredient. Details of source, passage history, controls, storage conditions MSV and WSV were provided and are considered appropriate. Certificates of analysis for the MSV and WSV were provided. Based on the risk assessment provided, the seed materials do not pose a risk for TSE transmission.

During the construction and passages of the recombinant virus, no materials posing a TSE infectivity risk were used, except for one material used in cell culture media and during transfection. Representative and valid certificates of suitability issued by the EDQM (CEP) for these materials were provided. It must also be noted that the used cell culture is not susceptible to ruminant prion infection.

For materials of avian or porcine origin, no TSE risk can be deduced from these starting materials as the material is sourced from none TSE relevant species.

Acceptable information was provided on starting materials of animal origin in compliance with the NfG on TSE/BSE risk (EMEA/410/01 Rev. 3) and it is concluded that there is no evidence for any risk that the seed material or the manufacturing process would be contaminated with TSE infectivity.

Starting materials of non-biological origin

For starting materials of non-biological origin not listed in a pharmacopoeia representative certificates of analysis and a supplier's statement with respect to the source of the ingredients were provided.

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

Information regarding the qualitative and quantitative composition of all culture media, gentamicin solution, freezing solutions, their treatment processes and their storage conditions is provided in the dossier. All components are either tested or treated to ensure that there are no contaminants or further assurance is given that there is no potential risk.

Specific measures concerning the prevention of the transmission of animal spongiform encephalopathies

A BSE/ TSE risk assessment was made in accordance with the current Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMEA/410/01 Rev. 3).

The documentation provided for all materials of animal origin demonstrated their compliance with the Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev.3) and Commission Directive 1999/104/EEC.

Moreover, the product is intended for poultry, a non-TSE susceptible species.

It is concluded that, the risk of transmitting TSE infectivity through the use of this vaccine is negligible.

Control tests during the manufacturing process

The applicant presented in-process data for the manufacture of four production batches of virus harvest. During manufacture, the following in process control tests are carried out to ensure the quality parameters: cell count (determination of viable cells) and check of proper sealing of the filled ampoules. The bulk vaccine will be controlled for bacterial and fungal sterility. The filling volume is checked at regular intervals during filling by a graduated measuring device. The correct sealing of the ampoules is regularly monitored during filling on a representative number of ampoules by visual inspection. The tops of the ampoules must be round without enclosed bubbles. Test descriptions and the limits of acceptance are presented.

The in-process controls are adequately described and deemed sufficient to control all critical steps in the manufacturing process. Validation of the key test (cell count) is provided. The cell count results of four consecutive production runs of cell-associated virus are provided, which demonstrated consistency of the manufacturing of the virus harvest.

During the production of the solvent a pH control after all components are dissolved is carried out and, if necessary, pH is adjusted. A fill-volume control is performed during filling of the PVC bags and limits

of acceptance for each solvent presentation are established. Test descriptions and the limits of acceptance are presented. The in-process controls for the solvent are adequately described and deemed sufficient to control all critical steps in the manufacturing process of the solvent. Information on validation batches of the solvent for both manufacturing sites have been provided. In addition, detailed information for the control tests of the filling procedure for both filling processes have been provided. Furthermore, results of the fill volume control test for validation batches were presented. Equivalence in production of the solvent between the two manufacturing sites has been demonstrated for both manufacturing sites.

Control tests on the finished product

Finished product controls performed on the finished frozen vaccine fraction are appearance, pH, sterility, mycoplasma control, identification of HVT L78 protein, NDV F protein and IBDV VP2 protein as well as potency of the recombinant vaccine strain rHVT-ND-IBD and extraneous agents.

The descriptions of the methods used for the control of the finished product and the specifications were provided. The relevant test methods, i.e. sterility test, mycoplasma control, potency, identity and extraneous agents testing are satisfactorily validated.

Identity of active substance and potency tests are combined in one virus titration assay, which is performed on each batch of final product. Different product dilutions are incubated on a cell line. The tests are performed for HVT 78 protein, NDV F protein and IBDV VP2 protein, confirming the identity of the host virus and the presence of both inserted genes. Validation of the test was performed and considered acceptable.

A sterility test is performed by direct inoculation technique according to Ph. Eur. 2.6.1 and 0062 methods. The suitability of the test method was appropriately validated.

The pH value of the finished product is tested in accordance with Ph. Eur. 2.2.3 methods. Appropriate limits were reconsidered as more experience through the production of a similar product was gained.

A test for absence of mycoplasmas is performed in accordance with Ph. Eur. 2.6.7. Two methods can be used (culture and PCR method). The suitability of the PCR method was appropriately validated. An inhibitory effect on indicator mycoplasma growth in the presence of the vaccine (trace amounts of gentamicin) could not be observed. The cultural test method can be applied for routine release testing.

A test for absence of extraneous agents is performed on each batch of vaccine in accordance with Ph. Eur. 2.6.25 or using a validated PCR method. The suitability of the alternative PCR methods for testing the absence of extraneous agents was sufficiently validated. The methods were adequately described and are considered acceptable.

Finished product tests are performed on the solvent: pH value (in accordance with Ph. Eur. 2.2.3), osmolarity (Ph. Eur. 2.2.35), sterility (Ph. Eur. 2.6.1), appearance (colour) and extractable volume. Appropriate limits were set for each of these tests. Sterility of the solvent is tested in accordance with Ph. Eur. 2.6.1 and 0062 methods. A routine virucidal test on each batch of solvent is not deemed necessary as the applicant has given a reasonable justification for his approach. Parametric release for sterility in line with Ph. Eur. 5.1.1 can be accepted as the solvent is sterilised in its final container (terminal sterilisation) in a continuous process.

In general, the proposed tests on the finished product and sterile solvent, as well as their proposed specifications are considered adequate to control the quality of the finished product and solvent. Equivalence of production between the two solvent manufacturing sites has been demonstrated.

Batch-to-batch consistency

Results of finished product tests for four consecutive vaccine batches are provided; one batch at 1,000 doses per vial, two batches at 2,000 doses per vial and one batch at 4,000 doses per vial were presented. All batches conform to the release requirements. Results of finished product tests for three 400 ml, three 800 ml, three 1,000 ml, three 1,200 ml and three 1,600 ml batches of the solvent filled into PVC bags and manufactured at Infomed Fluids SRL, Romania are presented and conform to the release requirements (Part 2 F). The provided batch data demonstrate consistency of production at the Infomed Fluids SRL manufacturing site. Furthermore, results from finished product tests for three 400 ml and three 800 ml batches of solvent filled into PVC bags and manufactured at CEVA-Phylaxia Co. Ltd. site, Hungary have also been provided, which confirm equivalence in production between the two solvent production sites and batch-to-batch consistency at the CEVA-Phylaxia manufacturing site for the two smaller solvent pack sizes.

Stability

Four batches of the vaccine (include vials of each dose) stored in the final container in liquid nitrogen for a maximum of 21 months were tested for real time stability. The product was tested on each time point for identity and rHVT-ND-IBD titre (PFU/ dose), appearance and pH, since sterility testing was done at time point 0 and will be repeated after 21 months of shelf life. All parameters met the specifications at all time points. The potency test results show no significant loss of titre supporting a shelf life of 18 months. Because vials from two suppliers are proposed, stability data including batches of the minimum number of doses and batches on the maximum number of doses from each supplier are required. However, in the stability study only vaccine batches filled in closed glass ampoules were used. Nevertheless, as the glass type (hydrolytic type I glass) is the same for all vials (open and closed ampoules), no difference in stability is expected and the stability data on the presented four batches are considered sufficient.

In-use stability of the vaccine was studied by dissolving three ampoules each of two batches of finished product in solvent. Samples were taken after dilution and 2 hours of storage at room temperature. Virus titres (PFU/ dose) were measured. Between T0 and T2 an overall drop in titre of 13.7% and an average drop of 14.36% for the subcutaneous application and an average drop of 12.93% for the *in ovo* application was seen for two batches over the two-hour storage. Nevertheless, in any case the PFU/ dose remained above the minimum efficacious dose of 4,000 PFU/ dose in all tests. Based on these data, the minimum release titre is justified to guarantee the minimum protective dose within a two-hour in use period.

The solvent is stored at room temperature. In order to assess the stability of the solvent, a stability study was performed using three consecutive batches of each solvent presentation (400, 800, 1,000, 1,200 and 1,600 ml) produced at Infomed Fluids SRL, Romania. Appearance, sterility, pH, osmolality and fill volume were tested at regular intervals (three months). Only solvent bags (PVC) of the 400- and 800-ml presentation were stored for a maximum of 30 months. All parameters remained within specifications throughout the storage period except for a few pH measurements; however, the applicant provided an explanation, which is acceptable. Sterility was not tested at the end of shelf life (30 months). Originally, a stability study up to 36 months was planned, however, the water loss dropped under the nominal value at 36 months, whereas at 30 months the filling volume stayed above the nominal value. Nevertheless, all solvent batches of these two smaller pack sizes were found to be sterile at 36 months of storage. From this result, it can be assumed that these batches were also sterile at 30 months. Thus, the stability of the 400- and 800-ml presentations is deemed fully demonstrated. The stability study for the 1,000-, 1,200- and 1,600-ml presentations is still ongoing, and 24 months results were provided; all results comply with the set specifications. A shelf life of 30

months for solvent in PVC bags is proposed. Based on the current data, a shelf life of 30 months for all solvent presentations is supported. The outstanding data of the stability study of the solvent should be provided post authorisation. In relation to the few lower pH measurements in case of smaller solvent pack sizes (400 and 800 ml), the applicant clarified that this difference comes from a lower pH value during bulk preparation than the one used for the larger pack sizes, which is considered acceptable.

Overall conclusions on quality

Ultifend ND IBD is a recombinant trivalent vaccine for chickens and chicken embryonated eggs consisting of a live recombinant herpesvirus of turkeys, strain rHVT-ND-IBD expressing the fusion protein gene of NDV and the capsid VP2 protein gene of IBDV. The vaccine is available frozen in glass ampoules containing 1,000, 2,000 or 4,000 doses and is diluted before use in sterile solvent. The pharmaceutical form of the final vaccine after reconstitution is a suspension for injection. One *in ovo* dose consists of 0.05 ml and one subcutaneous dose consists of 0.2 ml.

Acceptable information regarding the qualitative and quantitative composition is provided. At release, one dose of vaccine contains 4,000 to 12,000 PFU/dose of Ultifend ND IBD virus strain rHVT-ND-IBD as active ingredient. The applicant has provided a comprehensive description of the development of the product including the choice of antigens, excipients, container-closure system and overages as well as the vaccine production and of the validation of the production process. Reasonable justification has been given regarding the suitability of the chosen recombinant vaccine strain rHVT-ND-IBD and the development of test methods to test the identity and potency of batches. Furthermore, relevance of the vaccine virus for the European field was provided.

The production process of the antigen is considered as standard manufacture for this type of vaccines. The virus is grown on a cell line. The production process of the active ingredient is described adequately.

In general, the starting materials are properly described. For the starting materials of animal origin further assurance was provided that they are either tested for or treated to ensure that there are no contaminants or that the treatment process ensures the removal of any potential risk of extraneous agents. Furthermore, compliance of starting materials of animal origin with TSE regulation was shown. The risk that the final product may transmit TSE to the target animal was estimated as negligible.

In principle, the in-process tests are deemed sufficient to control all critical steps in the manufacturing process.

The proposed tests on the finished product are adequate to control the product quality. Validation studies were provided for all key tests, i.e. potency and identity, absence of mycoplasma, sterility and extraneous agents tests. Based on safety and efficacy considerations the specifications set for the potency tests were adequately justified. However, the applicant reconsidered the specification criterion for the appearance test of the final product (yellowish brown homogenous suspension). In addition, a new pH range has been set for the final product. Test results of four consecutive production runs including in-process data for the manufacture of four pilot scale vaccine bulks, conforming to the in-process and final product specifications are provided. Results of four final vaccine batches demonstrate that final product remains stable for 18 months when stored frozen under the recommended conditions. It is recommended to use the reconstituted vaccine within two hours. In use stability data for *in ovo* and subcutaneous use for two reconstituted final product batches were available and show that the product meets the proposed specifications when reconstituted according to the directions. Based on the stability data, a shelf life of 18 months for the final vaccine batches and an in-use shelf life of two hours for the reconstituted product are supported.

Infomed Fluids SRL, Romania, produced all solvent batches presented in the original dossier. Results of three 400 ml and three 800 ml final solvent batches demonstrate that solvent remains stable for 30 months when stored below 25 °C in plastic bags. However, sterility has not been tested at the end of shelf life (30 months) and a reasonable justification for this has been provided by the applicant. A proposed shelf life of 30 months for the smaller solvent pack sizes (400 and 800 ml) is considered acceptable. Stability data for the larger solvent pack sizes (1,000 to 1,600 ml) are available for up to 24 months but the study is still ongoing. Overall, the trend in stability data collected so far support a shelf life of 30 months for all solvent pack sizes (400 – 1,600 ml). However, final stability data (30 months testing time point) for the larger solvent pack sizes (9 batches) are still outstanding and need to be addressed adequately by the applicant post authorisation.

Different presentations of final product (1,000, 2,000 and 4,000 doses) and solvent (400, 800, 1,000, 1,200 and 1,600 ml) are available which allow the user to adjust the vial size and application route to the number of animals or eggs to be vaccinated in one vaccination course. The omission of adjuvants and preservatives is considered desirable to exclude potentially toxic excipients from veterinary medicinal products, wherever possible.

Based on the review of data on quality, the manufacture and control of Ultifend ND IBD are considered acceptable. A recommendation to submit the final stability data of the larger solvent pack sizes post authorisation is also considered acceptable.

Part 3 – Safety

Introduction and general requirements

Ultifend ND IBD is a trivalent, cell-associated, live recombinant virus vaccine for use in chickens containing live recombinant serotype 3 turkey herpes virus (HVT) strain FC-126 expressing a surface glycoprotein from Newcastle disease virus (NDV) and a capsid protein from infectious bursal disease virus (IBDV). No adjuvant or preservative is included. The maximum antigen concentration per dose (0.05 ml *in ovo* or 0.2 ml subcutaneous in chicken) contains 12,000 PFU (plaque-forming units) of cell-associated live recombinant rHVT-ND-IBD.

A full safety file in accordance with Article 12(3)(j) 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)', Directive 2001/82/EC and VICH GL 44.

Ultifend ND IBD is presented as a frozen suspension in flame-sealed glass ampoules stored in liquid nitrogen, which is to be diluted in a sterile solvent before use.

The vaccine is administered *in ovo* (*i.o.*) to 18-day-old chicken embryonated eggs (0.05 ml) or subcutaneously (s.c.) to one-day-old chicken (0.2 ml) to stimulate active immunity against MDV, NDV and IBDV.

Safety documentation

Fourteen safety studies were conducted to investigate the safety of the product and included ten laboratory studies investigating the safety of the administration of one dose, a 10-fold overdose, shedding, spread and dissemination in the target species, spread to non-target species (turkeys, pigeons and pheasants), reversion to virulence as well as four field trials. The vaccine was administered by the s.c. and *i.o.* routes, as recommended.

Laboratory studies were reported to be GLP-compliant, with the exception of a part of the laboratory phase which was performed at Autopsy Kkt./Autopsy Path Kft. (non-GLP-compliant site) and carried out in target animals of the minimum age recommended for vaccination using MSV and MSV+2. MSV+2 was used in the overdose studies because this passage had a higher titre than the MSV, making dilution and administration more convenient. Two production batches were used in the field trials, which were conducted in Hungary in compliance with the principles of GCP.

Studies applicable to live vaccines and GMO products were conducted to investigate the dissemination of an overdose of the vaccine strain, the spread from vaccinated animals to non-vaccinated contacts and reversion to virulence.

Study title
Laboratory studies
Safety of one dose (80,000 PFU) test in SPF chickens after *in ovo* inoculation
Safety of one dose (80,000 PFU) test in SPF chickens after *subcutaneous* inoculation
Overdose safety test of the MSV+2 of R051 vaccine (planned dose: 120,000 PFU) in SPF chickens after *in ovo* inoculation
Overdose safety test of the MSV+2 of R051 vaccine (planned dose: 120,000 PFU) in SPF chickens after *subcutaneous* inoculation
Shed, spread and dissemination test of the MSV of R051 vaccine (80,000 PFU) in SPF chickens after *subcutaneous* inoculation
Spread test of the MSV of R051 vaccine (80,000 PFU) from subcutaneously inoculated SPF chickens to non-treated turkeys
Foreign bird safety test of the MSV of R051 vaccine (80,000 PFU) in turkeys after *subcutaneous* inoculation
Foreign bird safety test of the MSV of R051 vaccine in pigeons after *subcutaneous* inoculation
Foreign bird safety test of the MSV of R051 vaccine in pheasants after *subcutaneous* inoculation
Increase in virulence test of the MSV of R051 vaccine (8,000 PFU) in SPF chickens after *in ovo* inoculation
Field safety and efficacy trial of R051 vaccine in broiler chickens in Balmazújváros-Nagyhát
Field safety and efficacy trial of R051 vaccine in broiler chickens in Pusztadobos
Field safety and efficacy trial of R051 vaccine in layer chickens in Szerencs
Field safety and efficacy trial of R051 vaccine in layer chickens in Szendrő

Laboratory tests

Two breeds of SPF chicken were used in the laboratory studies (Babcock and Novogen). The susceptibility of these chickens was shown by a challenge with a virulent MDV in one 80,000 PFU/dose study (Babcock; s.c. inoculation) and one overdose study (Novogen, *i.o.* inoculation). For all studies,

SPF certificates of the parent flocks have been provided.

The randomisation method used in the studies investigating the safety of s.c. administration is not optimal. The chickens were assigned to the groups as they came to hand. The main reason to use this simplified method of randomisation was that repeated handling is too much stress for the one-day-old chickens. The argumentation is acceptable, but for future studies it is proposed to use computerised randomisation (e.g. random distribution of the wing tag numbers before wing tagging) and combine the wing tagging with the randomisation so that no additional handling of the chickens is necessary.

For the assessment of the results of the observation (and the decision for euthanasia), no clinical scoring system seems to have been used and no humane endpoint to avoid unnecessary suffering of the study animals seems to have been implemented. In line with the 3Rs principles, the applicant should include information about fixed endpoints for euthanasia in future study reports.

Furthermore, the CVMP questioned the practice of debeaking of one-day-old chickens as it may be expected to cause unnecessary pain and stress to the study animals. The CVMP also questioned the need to breed surplus animals for the particular laboratory studies conducted. The applicant took note of the CVMP concerns.

Safety of the administration of one dose

A single dose safety testing is not required according to Ph. Eur. monograph 0589 (Marek's disease vaccines [live]), as an overdose study is requested. Originally, the two studies mentioned in this section were intended as overdose studies. However, in a later development stage, the maximum release titre was increased from the originally planned 8,000 PFU/dose to 12,000 PFU/dose. Therefore, the studies conducted with 80,000 PFU/dose (MSV) were no longer acceptable as overdose studies and are now provided as safety studies of one dose. In one of the studies the vaccine was administered *i.o.* to 18-day-old chicken embryonated eggs and s.c. to one-day-old chickens in another study.

The vaccine did not cause clinical signs of Marek's disease in any of the vaccinated chickens. No macroscopic or microscopic lesions attributable to the vaccine were observed. No statistically significant differences regarding hatchability (≥ 80%) and mortality could be detected. No visible or palpable local reactions were observed after s.c. administration of Ultifend ND IBD.

Based on these results, no major safety concerns arose following the administration of 80,000 PFU/dose to the target species of the minimum recommended age, thus providing a valid demonstration of the safety of a single dose of the primary vaccination of the product. The applicant has provided satisfactory answers/justifications to all identified other concerns.

Safety of one administration of an overdose

The safety of an overdose was evaluated in two laboratory studies for both routes of administration (s.c. and *i.o.*). The birds were vaccinated with a 10-fold overdose (120,000 PFU/dose) in accordance with the requirements of Ph. Eur. 0589. MSV+2 was used because this passage had a higher titre than the MSV, making dilution and administration more convenient. In one study, the vaccine was administered *i.o.* to 18-day-old chicken embryonated eggs and s.c. to one-day-old chickens in another study.

The vaccine did not cause clinical signs of Marek's disease in any of the vaccinated chickens. No macroscopic or microscopic lesions attributable to the vaccine were observed. No statistically significant differences regarding hatchability (≥ 80%) and mortality could be detected. No visible or palpable local reactions were observed after s.c. administration of Ultifend ND IBD.

In one overdose study, the bodyweight gain from D3 to D31 was significantly greater in the control group compared to the vaccinated birds with a difference of 23.3 g. The applicant argues that the reason for this is the characteristics of the chickens because they originated from a young parent flock, which means the hatching weight was below the expected average. The difference in bodyweight gain between vaccinated animals and controls is an isolated result in this overdose study. It was not confirmed in the other overdose and one-dose studies or in the field trials. Therefore, it is not regarded necessary to include a statement under SPC section 4.10.

Based on these results, no safety concerns arose following the administration of an overdose 10 times higher than the recommended dose to the target species of the minimum recommended age.

Safety of the repeated administration of one dose

No repeated-dose studies are required for this vaccine, taking into account that the vaccination schedule consists of a single lifetime dose.

Examination of reproductive performance

No reproductive safety studies were provided, as the product is not indicated for use in breeding birds. The following warning was therefore included in section 4.7 of the SPC: "Laying birds: Do not use in birds in lay and within 4 weeks before the start of the laying period."

Nevertheless, in four laboratory studies, ovary and testis samples were taken at the end of the study and investigated macroscopically and by histology. All samples were negative for the presence of MD, even at a 10-fold overdose. It can be concluded that the vaccine has no negative impact on reproductive performance. This is not directly reflected in the product literature, which is supported because the product is not indicated for use in birds in lay. Any hint to impacts on reproductive performance may lead to confusion in this regard.

Examination of immunological functions

No further studies were conducted to investigate the effects of the product on immunological functions. No adverse effects were observed in any of the safety or efficacy studies. It is therefore unlikely that this vaccine will have an adverse effect on immunological functions, as the non-pathogenic parent HVT virus is not known to be immunosuppressive in chickens. Based on the safety studies conducted with this vaccine, the genetic modification did not change the safety profile of the virus.

Special requirements for live vaccines

Spread of the vaccine strain

The spread of the vaccine strain from vaccinated to unvaccinated chickens was investigated in one study in which SPF chickens of one day of age were vaccinated with an overdose of 80,000 PFU according to the recommended vaccination schedule and by the recommended route (s.c.) and left in contact with unvaccinated sentinels for up to 120 days. Samples were taken from vaccinated and unvaccinated birds on study days 7, 14, 28, 35, 42 and 49. Shedding of the vaccine strain from vaccinated chickens was demonstrated for 42 days after the vaccination. On day 49, none of the samples (tracheal swab, cloacal swab, feather tip) was tested positive using a quantitative PCR (qPCR) for detection of HVT and a non-quantitative gel-based PCR for detection of rHVT-ND. This is adequately reflected in section 4.5 of the SPC. It was not possible to isolate the virus from the environmental

samples (taken on D28, D35, D42 and D49). Spread to contact chickens was not shown in the provided studies but cannot be excluded, and appropriate care should be taken to separate vaccinated from non-vaccinated chickens, which is appropriately reflected in section 4.5 of the SPC.

Four further studies investigating the spread to turkeys and the safety in other non-target species of birds (turkeys, pigeons and pheasants) were conducted, using an overdose of around 80,000 PFU administered by the s.c. route. In two studies, it was shown that the vaccine strain is able to spread from SPF chickens to turkeys and between turkeys. However, the vaccine strain was found to be safe in turkeys. The results are adequately reflected in section 4.5 of the SPC. Two studies were conducted to evaluate the safety of Ultifend ND IBD in pigeons and pheasants, showing that the vaccine strain is safe in these species and no shedding or spreading could be detected.

It is concluded that the vaccine virus can spread from chicken to turkeys and between turkeys. Spread to contact chicken was not shown in the provided studies but cannot be excluded. Ultifend ND IBD was shown to be safe in turkeys, pigeons and pheasants.

Dissemination in the vaccinated animal

Dissemination of the vaccine strain in vaccinated animals was also investigated in one study. Seven days after vaccination, all samples taken from spleen, liver, kidney and feather tips were tested positive using a qPCR for detection of HVT and a non-quantitative gel-based PCR for detection of rHVT-ND. The strain was isolated from most of the chosen organ samples up to 49 days after vaccination.

In conclusion, the virus strain does disseminate following vaccination by the recommended route at an overdose of 80,000 PFU in animals of the target species at the minimum recommended age and therefore can be shed from vaccinated animals for up to 49 days post vaccination (shedding was demonstrated up to day 42; on day 49 all tested samples were negative).

Reversion to 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 rHVT-ND-IBD is the naturally apathogenic HVT strain FC-126 and therefore no test is necessary, the applicant has conducted a study to demonstrate that the genetic modification did not affect the safety profile of the HVT FC-126 strain and that rHVT-ND-IBD cannot become virulent through bird-to-bird passages.

The reversion to virulence of the vaccine strain was investigated in one study in accordance with the requirements of Ph. Eur. 5.2.6 and Ph. Eur. 0589 monographs, respectively.

Sequential passaging of the vaccine strain through five groups of SPF chickens was investigated; a single dose of test vaccine (8,000 PFU; MSV) was administered to 18-day-old chicken embryonated eggs in the first group by the *i.o.* route. At the following passages (2, 3, 4 and 5), 0.2 or 0.3 ml of white blood cell suspension prepared from the chickens of the preceding passage was administered to each one-day-old chicken by the intraperitoneal route. The time between passages was 7 days. Each passage group consisted of 35–38 animals. The vaccine strain was recovered at all 5 passages. No clinical signs of disease were observed at any of the passage levels.

The last passage of the MSV-containing live virus (MSV+5) was administered to 18-day-old SPF chicken embryonated eggs by the *i.o.* route. Another group of 18-day-old SPF chicken embryonated eggs was inoculated with the MSV. The results of the two groups - inoculated with the different items by the same route - were compared in order to evaluate the possible increase in virulence during the

passages.

No abnormalities were found in the birds vaccinated with either material used for the first passage or material recovered from the final passage.

It is concluded that no reversion to virulence was observed following five passages *in vivo*. The highest passage level, which may occur in the final vaccine, is MSV+7. Based on the reversion to virulence study, it can be expected that no increase in virulence will occur in passage MSV+6 and MSV+7. Thus, there is no need for further investigations.

Biological properties of the vaccine strain

No specific studies were conducted to determine the intrinsic biological properties of the vaccine strain. The results of the studies performed indicate that the biological properties of the apathogenic parental strain seem to be unaltered after insertion of the fusion (F) gene of NDV and the virion protein 2 (VP2) gene of IBDV and their regulatory elements except for the replication of these two proteins.

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

Recombination or genomic reassortment of the strains

Recombination of rHVT-ND-IBD with virulent MDV or other MDV vaccine strains in the field would only be possible if the same cell becomes infected with more than one virus at the same time (superinfection). Such events are rare due to superinfection inhibitions resulting in a very limited timeframe (between 1–4 hours) for a cell to become infected with different herpesviruses. This statement is also supported by the fact that serotype 3 HVT vaccines have been given together with serotype 2 (SB-1) and serotype 1 (Rispens) MDV vaccine strains. To date, no adverse events caused by the concurrent application of any of these vaccines or with field MD viruses (serotype 1) have been reported.

In virus strain rHVT-ND-IBD used for Ultifend ND IBD, no gene deletions have been made compared to the parental strain HVT FC-126. The fusion (F) gene of NDV and the virion protein 2 (VP2) gene of IBDV were included into HVT FC-126. The loss of the inserts would have no consequences regarding the pathogenicity of the strain.

Since HVT replicates its DNA in the cell nucleus, recombination events with viruses replicating RNA (i.e. NDV) in the cell cytoplasm are highly unlikely due to the different locations of genetic material and the different types of genetic material.

Since the genome of HVT is not segmented, genomic reassortment cannot occur.

It can be concluded that the event of recombination or genomic reassortment is considered very unlikely.

User safety

The applicant presented a user safety risk assessment, which was conducted in accordance with CVMP guideline EMEA/CVMP/IWP/54533/2006 (and EMEA/CVMP/543/03-Rev.1).

The main potential routes of accidental contact with the product were considered and it was concluded that the most likely are those of accidental self-injection during subcutaneous application and dermal exposure by accidental spilling.

In general, avian herpesviruses are not known to be a hazard to humans. HVT is not indicated in EU

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 study.

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 gentamicin present in the finished product.

Regarding the possible hazards of a vaccine stored in liquid nitrogen (risk of an ampoule exploding) and the recommended handling of the ampoules, appropriate warnings are included in sections 4.5 and 4.9 of the SPC.

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

Study of residues

No study of residues was performed. All substances included in the composition of the vaccine are listed in Table 1 of Commission Regulation (EU) 37/2010 or in the list of substances considered as not falling within the scope of Council Regulation (EC) 470/2009. Phenol red and gentamicin sulphate have no pharmacological activity at the concentrations present in the final product (the residual gentamicin is far below the lowest established MRL). 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 other veterinary immunological products and therefore proposes to include a statement in section 4.8 of the SPC that 'No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. A decision to use this vaccine before or after any other veterinary medicinal product therefore needs to be made on a case-by-case basis'.

Field studies

Four randomised, blinded field trials (combined safety and efficacy trials) were conducted to evaluate the safety of one dose of Ultifend ND IBD in commercial future layers via the subcutaneous route and in commercial broilers via the subcutaneous and *i.o.* routes. The studies were conducted in Hungary and did comply with GCP principles. The study protocols were provided upon request. In each trial, the vaccinated group was compared to a control group receiving Cevac Transmune and Cevac Vitapest L, Vaxxitek HVT+IBD and Cevac Vitapest L or Nobilis ND C2 and Cevac Gumbo L.

The studies were well designed and conducted and confirmed that the product is safe for immunisation of commercial layers and broiler chickens with one dose. However, in three field studies, the group vaccinated with Ultifend ND IBD was accidentally contaminated with a live IBDV vaccine strain and/or a live ND vaccine strain used in the control group. This had no consequences on the safety evaluation.

Clinical investigations including clinical status and mortality were carried out daily for 44 days post vaccination (until slaughter) in broilers and daily for 120 days in layers.

In a subgroup of 20 or 30 birds (30 in the layer study and 20 in the rest of the field studies), local reactions at the sites of injection were monitored and recorded daily for 14 days. After euthanasia, the injection sites were observed macroscopically. For broilers, the bodyweight gain and feed consumption

were registered in 20 birds per group until slaughter and the feed conversion ratio was compared. For layers, the bodyweight gain was checked weekly in 20 birds per group.

Necropsy was performed in broilers once a week from daily carcasses from each group and in the middle and at the end of the observation period on at least 10 birds per group (incl. organ sampling for diagnostic purposes), with special attention given to lesions compatible with MD, ND and IBD. In layers, necropsy was performed by the investigators during their visits from daily carcasses of each group, on four different time points on 10 birds per group (incl. organ sampling for diagnostic purposes) and if the daily mortality exceeded the usual percentage of daily mortality on the farm, with special attention given to lesions compatible with MD, ND and IBD. Histological examinations were performed on bursa, spleen and *n. ischiadicus* samples. In layers, ovary and oviduct samples were also examined by histology. Organ sampling included bursa, spleen, liver, kidney, caecal tonsils and *n. ischiadicus* and, in layers, also ovary and oviduct samples. PCR examination to detect potentially circulating wild type virus strains of NDV, MDV1 and IBDV was carried out.

The vaccine did not cause clinical signs of Marek’s disease in any of the vaccinated chickens. No macroscopic or microscopic lesions were observed attributable to the vaccine. No statistically significant differences regarding hatchability (≥ 80%) and mortality could be detected except in one field study, where mortality during the first four weeks was significantly lower in the vaccinated group than in the control group. No visible or palpable local reactions were observed after s.c. administration of Ultifend ND IBD. The feed conversion ratio (FCR) and European production efficiency factor (EPEF) of both groups were very similar to each other.

The data show that the product is safe when used at a commercial dose in the target species.

Environmental risk assessment

An environmental risk assessment according to the ‘Guideline for environmental risk assessment for immunological veterinary medicinal products’ (EMEA/CVMP/074/95) was provided. The applicant has also taken into account Directive 2001/18/EC (Annex II, section D) and the Notice to Applicants (‘Guidance on environmental risk assessment for veterinary medicinal products consisting of or containing genetically modified organisms’; March 2006). Based on the data provided, the ERA can stop at phase I. Ultifend ND IBD is not expected to pose a risk for the environment when used according to the SPC. Further information is provided in the next section.

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

The product Ultifend ND IBD falls within the scope of Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms. Detailed information containing the information required by Annex IIIA of Directive 2001/18/EC is provided. Documents, literature references and an environmental risk assessment in accordance with the principles of Annex II of Directive 2001/18/EC, were provided. Consequently, the proposed vaccine is compliant with Directive 2001/18/EC.

The vaccine strain was generated by insertion of the fusion (F) gene of Newcastle disease virus (NDV) and the virion protein 2 (VP2) gene of infectious bursal disease virus (IBDV) in the naturally avirulent parent turkey herpesvirus (HVT) strain FC-126. The results of the safety studies performed indicate that the biological properties of the apathogenic parental strain are unaltered after insertion of the two genes and their regulatory elements except for the replication of these two proteins. Accordingly, (*in vivo*) reversion to virulence studies did not show any tendency for genetic or phenotypic instability or

reversion.

In one study, it was shown that the vaccine virus was shed from vaccinated chicken for 42 days (mostly via feather follicle epithelium). In contrast, on day 49, none of the samples was tested positive. Spreading to contact chicken was not shown in the provided studies but cannot be completely excluded. In an additional study, spreading from vaccinated chicken to turkeys was investigated. Results of this study showed that the vaccine virus can spread from chickens to turkeys. A further safety study in turkeys revealed that the virus is safe in chickens but is shed for at least three weeks and spreads to unvaccinated turkeys. Further safety studies in pigeons and pheasants were provided. In summary, the vaccine strain was shown to be safe in chickens, turkeys, pigeons and pheasants.

The vaccine virus itself is not able to survive in the environment of the poultry house if routine cleaning and disinfection procedures are used. However, in the unlikely event of the GMO entering the environment from either the hatchery or the poultry house, the consequences would be negligible.

None of the excipients in the vaccine represents any risk, neither to the target species, nor to the environment. Components of the vaccine are commonly used in numerous IVMPs. Gentamicin may be present in the final product in very low, sub-therapeutic amounts. No toxic metabolites are known.

Overall, the applicant has provided detailed information on the potential risks for humans and for the environment.

In summary, any risk emerging from the use of the vaccine virus is expected to be negligible for humans and for the environment.

Overall conclusions on the safety documentation

The applicant has provided four pivotal laboratory studies to investigate the safety of a 10-fold overdose and one dose (approx. 7-fold overdose) to the target animal species of the minimum recommended age via the recommended routes (*i.o.* and s.c.). Batches used in these studies were pilot batches (MSV and MSV+2).

Based on these results, it was concluded that the safety for the target animals 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 four field trials (two in future layers, two in broilers).

Reproductive safety was not investigated, as the vaccine is not intended for use in breeding birds. The SPC was amended accordingly.

As this is a live vaccine, the applicant also conducted five studies to establish the potential for shedding, spread and dissemination of the vaccine strain. Shedding of the vaccine strain from vaccinated chickens was demonstrated for 42 days after the vaccination. On day 49, all samples were tested negative. It was not possible to isolate the virus from environmental samples. It was concluded that the vaccine virus can spread from vaccinated chickens to turkeys and between turkeys. Spread to contact chickens was not shown in the provided studies but cannot be excluded. Ultifend ND IBD was shown to be safe in turkeys, pigeons and pheasants.

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 was also investigated and results showed that the potential risk is very low and acceptable. The final GMO was shown to be genetically and phenotypically stable over five passages. The biological properties of the apathogenic parental strain seem to be unaltered after

insertion of the fusion (F) gene of NDV and the virion protein 2 (VP2) gene of IBDV and their regulatory elements except for the replication of these two proteins.

The product is not expected to adversely affect the immune response of the target animals and therefore no suitable tests on the immunological functions were carried out.

The data presented are considered adequate to characterise the safety profile of the vaccine and the active substance as acceptable.

A user safety assessment in line with the relevant guidance document has been presented. Based on this assessment, the product does not pose an unacceptable risk to the user when used in accordance with the SPC. The worst-case scenario for user safety is self-injection and risks associated with handling liquid nitrogen tanks and thawing frozen glass vials (i.e. injuries due to exploding glass ampoules). Appropriate warnings for the user have been included in the product literature.

Ultifend ND IBD 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.

The vaccine is considered to be safe for the target species and non-target species, the user, the consumer and the environment.

Part 4 – Efficacy

Introduction and general requirements

Ultifend ND IBD is a frozen, cell associated, live virus vaccine that contains a genetically modified HVT vector (Marek’s disease virus (MDV) serotype 3, strain FC-126) expressing the fusion (F) gene of Newcastle disease virus (NDV) and the virion protein (VP2) gene of infectious bursal disease virus (IBDV). The vaccine is filled into flame sealed ampoules, to be reconstituted before use in a sterile solvent. The vaccine is to be used in chicken embryonated eggs by *in ovo* (*i.o.*) application at a volume of 0.05 ml as well as in one-day-old chicks at a volume of 0.2 ml by subcutaneous injection (s.c.) to stimulate active immunity against MDV, NDV and IBDV.

The proposed SPC claims with regards to efficacy are:

For the active immunisation of chickens and chicken embryonated eggs:

- To reduce mortality, clinical signs and lesions caused by NDV and to reduce virus shedding;
- To reduce mortality, clinical signs and bursa lesions caused by very virulent IBDV;
- To reduce mortality, clinical signs and lesions caused by classical Marek’s disease virus (MDV).

<u>Onset of immunity:</u>

Broiler chickens
    NDV: 4 weeks, IBDV: 3 weeks, MDV: 9 days
Layer chickens
    NDV: 4 weeks, IBDV: 4 weeks, MDV: 9 days

<u>Duration of immunity:</u>

Broiler chickens
    NDV: 9 weeks, IBDV: 9 weeks, MDV: life long
Layer chicken

NDV: 18 weeks, IBDV: 9 weeks, MDV: life long

No studies on interactions were performed. Accordingly, the following sentence is included in section 4.8 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."

Challenge model:

Challenge strain of Marek's disease virus

The challenge strain (MD70) used in all laboratory efficacy studies is an MDV serotype 1, isolated in the 1970's in Hungary from 12-week-old commercial, non-vaccinated chickens with neurological disorders. Since strain MD70 was already established as a suitable strain representing vMDVs in Hungary, its virulence characteristics have not been tested according to Witter (1997) criteria. The MD70 strain can be classified as "virulent" (not "very virulent"). This strain has already been used in challenge studies performed for the centrally licensed vaccine Vectormune ND of the same manufacturer. The setup of the MD challenge model used in laboratory trials complies with Ph. Eur. monograph 0589.

Challenge strain of Newcastle disease virus

The challenge strain used in all laboratory efficacy trials (NDV Herts 33/56; batch.no: 100920) was produced by Ceva-Phylaxia Co Ltd. The strain was purchased from the Hungarian Academy of Science-Veterinarian Research Center. The strain was originally isolated from chickens in Hertfordshire, England in 1933 (Allan et al., 1978). Herts 33/56 is widely used as ND reference strain in different animal experiments all around the world and it is the reference strain of Ph. Eur. monograph 0450. The setup of the ND challenge model complies with Ph. Eur. requirements.

Challenge strain of Infectious bursal disease virus

The IBDV challenge strain MOH-94 used in the laboratory efficacy studies was isolated in Hungary in 1994 (Mató *et al*., 2001). The challenge strain was classified phylogenetically (TREECON) and based on the clinical signs as very virulent IBD virus. The classification of the strain was confirmed by amplification of the hyper-variable region of the VP2 gene (414 base pairs) by RT-PCR. The sequences were compared to relevant sequences of reference classical, very virulent and variant viruses. Three independent challenge dose calibration studies were performed to identify the lowest titre of the MOH-94 challenge virus that could satisfactorily fulfil the challenge validity requirements of Ph. Eur. monograph 0587. The challenge model finally used fulfils the requirements of Ph. Eur. monograph 0587. The small difference concerning the route of administration to apply the challenge inoculum (eye drop and oral route), where Ph. Eur. requires application by eye drop, is regarded as acceptable since challenges with different doses by eye drop and oral route are valid in terms of Ph. Eur. requirements.

Efficacy parameters and tests:

The efficacy parameters investigated in the efficacy studies are:

- Mortality, clinical signs and lesions caused by NDV as well as virus shedding (examined using validated PCR);
- Mortality, clinical signs and bursa lesions caused due to IBDV as well as measurement of bodyweight;
- Mortality, clinical signs and lesions due to classical Marek's disease virus (MDV).

Efficacy documentation

Twenty-five laboratory studies were performed to demonstrate the efficacy of Ultifend ND IBD.

Both routes of administration, i.e. s.c. (day-old chicks) and *i.o.* (18-day-old chicken embryonated eggs), were used for vaccination of the corresponding age category in these studies.

For the s.c. route, the chickens were vaccinated at one day of age under the skin of the neck with the recommended volume of 0.2 ml.

For vaccination by the *i.o.* route, adverse effects on hatchability as well as viability of the hatched chickens were examined. It must be noted that *i.o.* administration of Ultifend ND IBD is mentioned to be recommended only for broilers. Anyway, *i.o.* application for future layers can be accepted without additional studies in layers.

A volume of 0.1 ml/dose was used for *i.o.* inoculation even if a volume of 0.05 ml/dose is recommended according to the SPC. The use of a higher volume in the laboratory studies was chosen as it facilitates manual administration of the exact dose. Nevertheless, during the field studies, where *i.o.* vaccination was performed by a machine, each egg was inoculated with the recommended volume of 0.05 ml.

In all pivotal trials, a dose not higher than the minimum titre (4,000 PFU) at the most attenuated passage level that will be present in the vaccine was used (MSV+7). Batch release certificates of the batches used in the efficacy studies are provided as Annex to Part 2A. The batches used for the laboratory efficacy and field trials were representative batches of the production method.

The pivotal laboratory efficacy tests were performed using SPF chickens or SPF chicken embryonated eggs. In addition, laboratory studies were conducted in MDA positive commercial layer and broiler birds or broiler embryonated eggs.

Four combined safety-efficacy field trials were performed with MDA positive commercial broiler or layer chicks respectively embryonated eggs vaccinated with commercial doses of vaccine via the s.c. or *i.o.* route.

From one of these field studies, two complementary studies and from a second one, three complementary challenge studies were derived to demonstrate efficacy in commercial birds after vaccination in the field and NDV- or IBDV-challenge under laboratory conditions.

The laboratory efficacy tests were conducted according to previously defined protocols. They were inspected and raw data audited regarding the general requirements of good laboratory practice (GLP). The combined field safety and efficacy trials were compliant with the requirements of good clinical practice (GCP).

Overview of the laboratory efficacy studies:

Evaluation of efficacy against MDV of Ultifend ND IBD

Study title	**Dose PFU**	**Challenge day p.vacc./ train/dose**
Efficacy test in SPF chickens after *in ovo* vaccination	3,500	9 days / MD 70 (vMDV)/ 50 PFU
Efficacy test in SPF chickens after s.c. vaccination	4,000	

Onset of Immunity in broiler chickens after *in ovo* vaccination	3,500	
Onset of Immunity in broiler chickens after s.c. vaccination	4,000	
Onset of Immunity in layer chickens after s.c. vaccination	4,000	

Evaluation of efficacy against NDV of Ultifend ND IBD

Study title	**Dose PFU**	**Challenge day p.vacc.**	**Challenge strain/dose**
Efficacy test in SPF chickens after *in ovo* vaccination	3,500	day 24	Herts 33/ 5 $\log_{10}$ ELD_{50}
Efficacy test in SPF chickens after s.c. vaccination	3,500	day 21	
Onset of Immunity in broiler chickens after *in ovo* vaccination	4,000	day 31	
Onset of Immunity in broiler chickens after s.c. vaccination	3,500	day 28	
Onset of Immunity in layer chickens after s.c. vaccination	4,000	day 28	
Duration of Immunity in broiler chickens after *in ovo* vaccination	2,500	day 66	
Duration of Immunity in broiler chickens after s.c. vaccination	4,000	day 63	
Duration of Immunity in layer chickens after s.c. vaccination	4,000	day 126	

Evaluation of efficacy against IBDV of Ultifend ND IBD

Study title	**Dose PFU**	**Challenge day p. vacc/strain/ dose (log10 EID50)**	
Efficacy test in SPF chickens after *in ovo* vaccination	3,500	day 17	MOH-94 (4.0)
Efficacy test in SPF chickens after s.c. vaccination	3,500	day 14	
Onset of Immunity in broiler chickens after *in ovo* vaccination (3 weeks)	3,500	day 24	

Onset of Immunity in broiler chickens after s.c. vaccination (3 weeks)	4,000	day21	
Onset of Immunity in broiler chickens after *in ovo* vaccination (4 weeks)	4,000	day31	
Onset of Immunity in broiler chickens after s.c. vaccination (4 weeks)	4,000	day28	MOH-94
Duration of Immunity in broiler chickens after *in ovo* vaccination	4,000	9 weeks	
Duration of Immunity in broiler chickens after s.c. vaccination	3,500	9 weeks	
Onset of Immunity in layer chickens after s.c. vaccination	4,000	4 weeks 3.0	
Efficacy in layer chickens (5 weeks) after s.c. vaccination	4,000	5 weeks 2.0	
Efficacy in layer chickens (7 weeks) after s.c. vaccination	4,000	7 weeks 2.0	
Duration of Immunity (9 weeks) in layer chickens after s.c. vaccination	4,000	9 weeks 3.0	
Challenge dose calibration	-	3 and 3.5 log_{10} EID_{50}	
	-	2.5 log_{10} EID_{50}	
	-	2.0 log_{10} EID_{50}	

Dose determination

No study on the determination of the vaccine dose was performed. Minimum and maximum titres of Ultifend ND IBD are comparable to titres commonly used in Marek's disease vaccines. If safety of the maximum and efficacy of the minimum titre are demonstrated, this is considered acceptable.

Onset of immunity

MD

Two studies designed and validated according to requirements of Ph. Eur. monograph 0589, 2-4-3 Immunogenicity, were performed to determine the efficacy and onset of immunity (OOI) for MD in SPF chickens, one including birds vaccinated via the *i.o.* route and one using birds vaccinated via the s.c. route.

In summary, birds were vaccinated *i.o.* (18-day-old chicken embryonated eggs) or s.c. at one day of age with a dose of the minimum titre or lower. Challenge was performed at 9 days post vaccination as required by Ph. Eur. for both routes of vaccination with 50 PFU challenge strain MD70 (vMDV) via the intraperitoneal (i.p.) route. The claimed OOI (9 days) for the MD component corresponds to the time of challenge mentioned in Ph. Eur. for the immunogenicity test. Birds in both studies were observed for 70 days for clinical signs and necropsy was performed on dead animals and surviving animals at the end of the observation period. In case of inconclusive results, histology was conducted for clarification. Hatchability in comparison to controls was recorded for *i.o.* vaccination.

In the study “Efficacy test in SPF chickens after *i.o.* vaccination (3,500 PFU)”, 51 SPF chickens were vaccinated and 33 SPF chickens were kept as controls.

In study “Efficacy test in SPF chickens after s.c. vaccination (4,000 PFU)”, 49 SPF chickens were vaccinated and 30 SPF chickens were kept as controls.

The level of “relative percentage of protection (RPP)” after challenge was 93.9% in animals after *i.o.* vaccination and 88.9% for animals vaccinated via the s.c. route. This is in line with the efficacy pass criterion mentioned in Ph. Eur. (RPP at least 80%). The claims reduction of mortality, clinical signs and lesions caused by classical Marek’s disease virus (MDV) can be supported based on the results of both studies. The OOI at 9 days post vaccination is regarded as demonstrated for the *i.o.* as well as the s.c. vaccination.

ND

Two studies designed and validated according to requirements of Ph. Eur. monograph 0450, 2-4-5 Immunogenicity, were performed to determine the efficacy and the OOI for ND in SPF chickens, one including animals vaccinated via the *i.o.* route and one using animals vaccinated via the s.c. route.

In summary, birds were vaccinated *i.o.* (18-day-old chicken embryonated eggs) or s.c. at one day of age with a dose lower than the minimum dose (3,500 PFU). Challenge was performed at 21 days post vaccination as required by Ph. Eur. for the s.c. route and 24 days post vaccination for the *i.o.* route (at 21 days of life) with 5 $\log_{10}$ ELD_{50} challenge strain Herts 33 via the i.m. route as requested by Ph. Eur. As the claimed OOI (4 weeks) for the ND component is later than the time of challenge mentioned in Ph. Eur. for the immunogenicity test of NDV, the delay of challenge after *i.o.* use can be regarded as acceptable. Birds in both studies were observed for 14 days for clinical signs and necropsy was performed on dead animals and surviving animals at the end of the observation period. Serology was performed at the day of challenge to consider seroconversion after vaccination. Hatchability in comparison to controls was recorded for the *i.o.* vaccination.

In study “Efficacy test in SPF chickens after *i.o.* vaccination”, 20 SPF chickens were vaccinated and 10 SPF chickens were kept as controls. Challenge was performed 24 days post vaccination.

In study “Efficacy test in SPF chickens after s.c. vaccination”, 20 SPF chickens were vaccinated and 10 SPF chickens were kept as controls. Challenge was performed 21 days post vaccination.

The level of protection after challenge was 90% in birds after *i.o.* vaccination and 100% for birds vaccinated via the s.c. route. This is in line with the efficacy pass criterion mentioned in Ph. Eur. (protection at least 90%). The claims reduction of mortality, clinical signs and lesions caused by Newcastle disease virus (NDV) can be supported for both *i.o.*as well as s.c. administration.

In addition, all vaccinated birds (100%) had ND specific antibodies at the time of challenge after *i.o.* vaccination (mean ELISA titre: 6148) and 95% after s.c. vaccination. (mean ELISA titre: 5302.6).

OOI of 4 weeks for the ND component of Ultifend ND IBD at a dose of 3,500 PFU can be regarded as supported for *i.o.* vaccination as well as s.c. vaccination.

IBD

Two studies designed and validated according to the requirements of Ph. Eur. monograph 0587, 2-4-5 Immunogenicity, were performed to determine the efficacy and the OOI for IBD in SPF chickens, one including birds vaccinated via the *i.o.* route and one using birds vaccinated via the s.c. route.

In summary, birds were vaccinated *i.o.* (18-day-old chicken embryonated eggs) or s.c. at one day of age with a dose lower than the minimum dose (3,500 PFU). Challenge was performed at 14 days post vaccination as required by Ph. Eur. for the s.c. route and 17 days post vaccination for the *i.o.* route (at 14 days of life) with 4.0 log_{10} EID_{50} of the challenge strain MOH-94 by eye drop and *per os*, which is basically as requested by Ph. Eur. (per eye drop). As the claimed OOI (3 weeks for broilers and 4 weeks for layers) for the IBD component is later than the time of challenge mentioned in Ph. Eur. for the immunogenicity test of IBDV, the delay of challenge after *i.o.* use can be regarded as acceptable.

Birds in both studies were observed for 10 days after challenge for clinical signs and necropsy was performed on dead animals and surviving animals at the end of the observation period. Afterwards, histological examination of bursas as requested by and detailed in Ph. Eur. was carried out. Serology was performed at the day of challenge to consider seroconversion after vaccination. Hatchability in comparison to controls was recorded for *i.o.* vaccination.

In study "Efficacy test in SPF chickens after *i.o.* vaccination", 25 SPF chickens were vaccinated and 11 SPF chickens were kept as controls. Challenge was performed 17 days post vaccination.

In study "Efficacy test in SPF chickens after s.c. vaccination", 25 SPF chickens were vaccinated and 11 SPF chickens were kept as controls. Challenge was performed 14 days post vaccination.

The level of protection after challenge was 92% in birds after *i.o.* vaccination and 96% for birds vaccinated via the s.c. route. This is in line with the efficacy pass criterion mentioned in Ph. Eur. 0587 (protection at least 90%).

Seroconversion at challenge was seen in 68% of the vaccinated birds after *i.o.* vaccination (mean ELISA titre: 1897) but only in 16% of birds vaccinated via the s.c. route (mean ELISA titre: 496). Nevertheless, as nearly full protection could be achieved after s.c. vaccination in SPF birds, low levels of seroconversion are considered of inferior significance. The claims reduction of mortality, clinical signs and bursa lesions caused by very virulent infectious bursal disease virus (IBDV) are supported by these studies. OOI of three weeks for the IBD component of Ultifend ND IBD at a dose of 3,500 PFU can be regarded as supported for *i.o.* as well as s.c. vaccination in SPF animals.

Duration of immunity

Studies on duration of immunity (DOI) were performed in commercial birds only. This is regarded acceptable and has been accepted in other applications before.

MD

No studies are presented on the DOI, which is generally accepted for Marek vaccines as they induce life-long immunity.

ND

Three studies, basically designed according to the requirements of Ph. Eur. monograph 0450 2-4-5 Immunogenicity, were performed to determine the DOI for ND in commercial chickens, two in broilers vaccinated via the *i.o.* or s.c. route and one in future layers vaccinated via the s.c. route.

In summary, birds in studies using commercial birds were vaccinated *i.o.* (18-day-old chicken embryonated eggs) or s.c. at one day of age with a dose of minimum titre or lower. Challenge was performed at 9 weeks of age (66 days post vaccination for *i.o.* use) for broilers vaccinated by the *i.o.* or s.c. route and at 126 days (18 weeks) post vaccination for layers (vaccinated via the s.c. route).

A group of unvaccinated SPF birds was included in the studies to validate the challenge infection.

Serology was performed in 10 hatch mates at hatch to determine the MDA levels of the used animals.

Birds in all three studies were observed for 14 days for clinical signs and necropsy was performed on dead animals and surviving animals at the end of the observation period. In case of inconclusive results, histology was conducted for clarification. Serology was performed for all animals intended for challenge at the day of challenge to consider seroconversion/absence of seroconversion after vaccination. Hatchability in comparison to controls was recorded for *i.o.* vaccination. Swab sampling (oropharyngeal and cloacal) for determination of virus shedding by PCR was performed in 10/20(22) vaccinated birds and all control birds from days 1 to 5 after challenge. If animals died after challenge, sampling was omitted for these animals.

In study "Duration of Immunity in broiler chickens after *i.o.* vaccination (2,500 PFU)", mean ELISA titre at hatch: 9747), 20 layers were vaccinated and 10 layers as well as SPF chickens were kept as controls. Challenge was performed 66 days post vaccination.

In study "Duration of Immunity in broiler chickens after s.c. vaccination (4,000 PFU)", mean ELISA titre at hatch: 8906), 30 layers were vaccinated and 10 layers as well as SPF chicken were kept as controls. Challenge was performed 63 days post vaccination.

In study "Duration of Immunity in layer chickens after s.c. vaccination (4,000 PFU)", mean ELISA titre at hatch: 5052), 22 layers were vaccinated and 12 layers as well as SPF chickens were kept as controls. Challenge was performed 126 days post vaccination.

All challenges are validated by the SPF control group (0% protection). This applies to both broilers and layer chickens.

The level of protection after challenge was 100% in broilers after vaccination via the *i.o.* or s.c. route and 95% for layers vaccinated via the s.c. route. This is in line with the efficacy pass criterion mentioned in Ph. Eur. (protection at least 90%). All vaccinated broiler animals vaccinated via the *i.o.* or s.c. route had ND specific antibodies at the time of challenge as well as 95% of layers after s.c. vaccination.

The claims reduction of mortality, clinical signs and lesions caused by Newcastle disease virus (NDV) can be supported for both *i.o.*as well as s.c. administration for the DOI.

In addition, virus shedding examined by PCR in oropharyngeal and cloacal swap samples was demonstrated to be significantly lower in vaccinated animals in all studies. Altogether, the DOI of the ND component in broilers (nine weeks) and for layers (18 weeks) is regarded to be satisfactorily demonstrated.

IBD

Three studies basically designed according to the requirements of Ph. Eur. monograph 0587, 2-4-5 Immunogenicity, were performed to determine the DOI for IBD in commercial chickens, two in broilers vaccinated via the *i.o.* or s.c. route and one in layers vaccinated via the s.c. route.

In summary, commercial birds were vaccinated *i.o.* (18-day-old chicken embryonated eggs) or s.c. at one day of age with a dose of minimum titre or lower. Challenge in broilers was performed at the claimed DOI of 9 weeks post vaccination for broilers vaccinated by the *i.o.* or s.c. route with 4.0 $\log_{10}$

EID_{50} (challenge strain MOH-94 by eye drop and *per os*, which is basically as requested by Ph. Eur. (per eye drop).

Layers, vaccinated via the s.c. route, were challenged using the same challenge material and route with doses of 2.0 log_{10} or 3.0 log_{10} EID_{50} at 9 weeks after vaccination.

A group of unvaccinated SPF animals was included in one of the studies to validate the challenge infection.

Serology was performed in 10 hatch mates at hatch to determine the MDA levels of the used animals.

Animals in all three studies were observed for 10 days for clinical signs and necropsy was performed on dead animals and surviving animals at the end of the observation period. Afterwards, histological examination of bursas as requested by and detailed in Ph. Eur. was carried out.

Serology was performed for all animals intended for challenge at the day of challenge to consider seroconversion/absence of seroconversion after vaccination. Cloacal swab sampling for PCR was carried out on days 4, 7 and 11 after challenge for virus shedding (as no claim is made on IBDV virus shedding, results are not mentioned and assessed any further). The bodyweight on vaccinated birds as well as commercial controls was measured individually on the day of challenge as well as 5 and 10 days after challenge. The daily bodyweight gain was calculated for each bird between challenge and day 10 after challenge. The average daily bodyweight gain (ADWG) over 10 days after challenge was compared between vaccinated birds and controls.

Broilers

In study "Duration of Immunity in broiler chickens after *i.o.* vaccination (4,000 PFU)", mean ELISA titre at hatch: 15809), 25 broilers were vaccinated and 15 broilers were kept as controls. Challenge was performed 9 weeks post vaccination.

In study "Duration of Immunity in broiler chickens after s.c. vaccination (3,500 PFU)", mean ELISA titre at hatch: 12681), 20 broilers were vaccinated and 21 broilers were kept as controls. Challenge was performed 9 weeks post vaccination.

Future layers

In study "Duration of Immunity (9 weeks) in layer chickens after s.c. vaccination (4,000 PFU)", mean ELISA titre at hatch: 13100), 20 future layers were vaccinated and 20 were kept as controls. Challenge was performed 9 weeks post vaccination with a challenge dose of 3.0 log_{10} EID_{50}.

Broilers

At challenge 9 weeks post *i.o.* vaccination 26.7% of the control broilers remained seropositive, but all of them were unprotected upon challenge (without showing clinical signs or mortality). In contrast, 96% of the *i.o.* vaccinated broilers of group 1 had seroconverted at challenge and 84% of them were protected.

The average daily weight gain over the first 5 days after challenge as well as over the entire observation period after 10 days was not significantly higher in *i.o.* vaccinated animals than in the broiler controls.

All broiler control animals at challenge 9 weeks after s.c. vaccination of day-old broilers in a study were seronegative and the protection rate in controls was low at 4.8% (one animal did show clinical signs after challenge, all others were considered positive for IBD based on histological findings of bursa samples). Full seroconversion at challenge and full protection after challenge was found in s.c. vaccinated broilers. The average daily weight gain over the first five days after challenge as well as

over the entire observation period after 10 days was significantly higher in s.c. vaccinated birds than in the broiler controls.

Concerning reduction of bursal damage, the DOI as claimed (nine weeks) in broilers is considered demonstrated while controls were highly affected at that time. The claim of reduced bodyweight gain loss in broilers is not regarded supported in the presented DOI studies as the results provided differ and were not consistent among studies in broilers. The claim has been deleted accordingly.

Future layers

At challenge 9 weeks post vaccination, all unvaccinated layer control animals were seronegative at the time of challenge but 78.6% of them were protected after challenge, indicating decreasing susceptibility to IBD infection at that time. All vaccinated birds had seroconverted at the time of challenge and the protection rate after challenge (100%) in vaccinated layers was significantly higher than in controls. Nevertheless, there was no significant difference in clinical scores between vaccinated and control birds. The DOI as claimed (nine weeks) in future layers is regarded as demonstrated while controls were very lowly susceptible to infection at that time.

Maternally derived antibodies (MDA)

Efficacy of the vaccine in commercial chickens

Following the requirement of Ph. Eur. 5.2.7 monograph "The influence of passively acquired and maternally derived antibodies on the efficacy of a vaccine" must be adequately evaluated. "Any claims, stated or implied, regarding onset and duration of protection shall be supported by data from trials."

Validity and efficacy pass criteria according to Ph. Eur. monographs 0589, 0450 and 0587 are not applicable for use in commercial birds and are adapted by the applicant in a suitable way. Nevertheless, if criteria according to Ph. Eur. are fulfilled, this is mentioned as superior to pass criteria set by the applicant.

The studies were not performed following the guidance of reflection paper EMA/CVMP/IWP/439467/2007, where challenge is supposed to be performed if MDA levels in control animals at time of challenge are sufficiently low. The study design is rather modelled to confirm the claimed OOI. No vaccinated SPF groups are included in the studies.

MD

Three studies basically designed according to the requirements of Ph. Eur. monograph 0589, 2-4-3 Immunogenicity, were performed to determine efficacy for MD in commercial chickens, two in broilers vaccinated via the *i.o.* or s.c. route and one in future layers vaccinated via the s.c. route.

In summary, animals in studies using commercial birds were vaccinated *i.o.* (18-day-old chicken embryonated eggs) or s.c. at one day of age with a dose of minimum titre or lower. Challenge was performed at 9 days post vaccination for both routes of vaccination with 50 PFU challenge strain MD70 (vMDV) via the i.p. route.

Serology was performed in 10 hatch mates at hatch to determine the serological status of the used animals. No mean values were determined on the level of MDA. No information is given on the immunological status of the vaccinated and control chickens at the time of challenge. Accordingly, the requirements of reflection paper EMA/CVMP/IWP/439467/2007, where challenge of animals is performed at the time when unvaccinated controls are basically seronegative, were not taken into consideration. After challenge infection, commercial animals were observed for 70 days for clinical signs and necropsy was performed on dead animals and surviving animals at the end of the

observation period. In case of inconclusive results, histology was conducted for clarification. Hatchability in comparison to controls was recorded for *i.o.* vaccination. No SPF control group was included to validate the challenges.

In study "Efficacy test in broiler chickens after *i.o.* vaccination (3,500 PFU)" 45 broilers were vaccinated and 33 broilers were kept as controls; 3/10 chicks had MDA against MDV at hatch.

In study "Efficacy test in broiler chickens after s.c. vaccination (4,000 PFU)", 50 broilers were vaccinated and 32 broilers were kept as controls; 8/10 chicks had MDA against MDV at hatch.

In study "Efficacy test in layer chickens after s.c. vaccination (4,000 PFU)", 44 layers were vaccinated and 35 layers were kept as controls; 6/10 chicks had MDA against MDV at hatch.

High levels of absolute protection (APP) are achieved in all vaccinated commercial birds in all trials, 88.9% after *i.o.* vaccination in broilers, 98% after s.c. vaccination in broilers and 90.9% after s.c. vaccination in future layers, corresponding to RPP levels of approximately 88.9% after *i.o.* vaccination in broilers, 97.5% after s.c. vaccination in broilers and 90% after s.c. vaccination in future layers. Accordingly, achieved protection in vaccinated commercial birds fulfils the efficacy pass criteria of Ph. Eur. while protection in the corresponding control groups is sufficiently low to validate the challenge as required by Ph. Eur. for SPF chickens. Consequently, the achieved level of protection after challenge in vaccinated commercial animals in comparison to unvaccinated controls clearly supports the efficacy of the vaccine in the presence of MDA, even in the absence of any information on the MDA status of controls at challenge.

No data about serology that could be used as reference seem to be available despite many publications are available on this subject. The birds/eggs in the referred studies were obtained from commercial flocks; the breeders received MDV vaccination at one day of age (which is the typical age of breeder MDV vaccination). Since live MDV/HVT vaccine strains in general remain latent throughout the life of the birds, antibodies persist for life and can be transferred by the breeders to the offspring. MDA results shown in the two studies accordingly are considered as representative for the typical field situation.

In addition, there are data suggesting that the immune defences against MDV infected cells are predominantly cellular anyway (Schat et al, 2008; Diseases of poultry, 12th edition, page 483-484). Therefore, the presented data on MDA status of used commercial animals in the abovementioned studies is considered adequate.

In summary, the claims reduction of mortality, clinical signs and lesions caused by classical Marek's disease virus (MDV) could be supported in all studies. The OOI at 9 days post vaccination is regarded as confirmed for vaccination of commercial animals.

ND

Three studies basically designed according to the requirements of Ph. Eur. monograph 0450, 2-4-5 Immunogenicity, were performed to determine the OOI for ND in commercial chickens, two in broilers vaccinated via the *i.o.* or s.c. route and one in future layers vaccinated via the s.c. route.

In summary, birds in studies using commercial birds were vaccinated *i.o.* (18-day-old chicken embryonated eggs) or s.c. at one day of age with a dose of minimum titre or lower. Challenge was performed at 31 days (at 28 days of life) post vaccination for birds vaccinated by the *i.o.* route and 28 days post vaccination for birds vaccinated via the s.c. route with 5 $\log_{10}$ ELD_{50} of the challenge strain Herts 33 via the i.m. route. A group of unvaccinated SPF birds was included in the studies to validate the challenge infection. Serology was performed in 10 hatch mates at hatch to determine the MDA levels of the used birds.

Commercial birds in all 3 studies were observed for 14 days for clinical signs and necropsy was performed on dead animals and surviving animals at the end of the observation period. In case of inconclusive results, histology was conducted for clarification. Serology was performed for all birds intended for challenge at the day of challenge to consider seroconversion/absence of seroconversion after vaccination. Hatchability in comparison to controls was recorded for *i.o.* vaccination. Swab sampling (oropharyngeal and cloacal) for determination of virus shedding by PCR was performed in 10/20(22) vaccinated birds and all control animals from days 1-5 after challenge. If birds died after challenge, sampling was omitted.

In study "Onset of Immunity in broiler chickens after *i.o.* vaccination (4,000 PFU)", mean ELISA titre at hatch: 14937) 20 broilers were vaccinated and 10 broilers were kept as controls. Challenge was performed 31 days post vaccination.

In study "Onset of Immunity in broiler chickens after s.c. vaccination (3,500 PFU)", mean ELISA titre at hatch: 10475) 22 broilers were vaccinated and 12 broilers were kept as controls. Challenge was performed 28 days post vaccination.

In study "Onset of Immunity in layer chickens after s.c. vaccination (4,000 PFU)", mean ELISA titre at hatch: 5052) 22 layers were vaccinated and 12 layers were kept as controls. Challenge was performed 28 days post vaccination.

Birds used in one of the studies on OOI after *i.o.* use in broilers had high levels of antibodies resulting in 60% of unvaccinated control animals being still seropositive at the time of challenge. Accordingly, 50% of the controls were protected after challenge. The requirements of the reflection paper, where challenge of animals is supposed to be performed at the time when unvaccinated controls are basically seronegative, were not taken into consideration for this study. Accordingly, the full protection achieved in broilers vaccinated via the *i.o.* route cannot be fully attributed to vaccination in this study.

Concerning vaccination by the s.c. route, the level of protection after challenge was 81.8% in vaccinated broilers in one of the onset of immunity studies (25% of samples from unvaccinated broilers were still seropositive; protection 8.3%) and 81% in s.c. vaccinated layers (0% seropositivity in unvaccinated layer controls; 0% protection) in the other onset of immunity studies. The achieved levels of protection in s.c. vaccinated broilers and layers are considered adequate in vaccinated commercial animals in comparison to unvaccinated controls and fulfil the efficacy pass criteria as set by the applicant (not fewer than 80% protection in vaccinated animals after challenge). Seroconversion at the time of challenge was found in 81.8% of vaccinated broilers as well as layers after s.c. vaccination. The claims reduction of mortality, clinical signs and lesions caused by Newcastle Disease Virus (NDV) can be supported for both *i.o.* as well as s.c. administration. In addition, virus shedding examined via PCR in oropharyngeal and cloacal swap samples was demonstrated to be significantly lower in vaccinated animals in all studies. Altogether, the efficacy of the ND component in the presence of MDA is considered to be satisfactorily demonstrated.

IBD

Seven studies, basically designed according to the requirements of Ph. Eur. monograph 0587, 2-4-5 Immunogenicity, were performed to determine the OOI and efficacy for IBD in commercial chickens, 4 in broilers vaccinated via the *i.o.* or s.c. route (challenged at three and four weeks for each route of administration) and 3 in layers vaccinated via the s.c. route.

In summary, birds in studies using commercial animals were vaccinated *i.o.* (18-day-old chicken embryonated eggs) or s.c. at one day of age with a dose of minimum titre or lower. Challenge in broilers was performed at 21 and 28 days (24/31 days of life) post vaccination for animals vaccinated by the *i.o.* route and 21 and 28 days post vaccination for the animals vaccinated via the s.c. route with

4.0 $\log_{10}$ EID_{50} (in broilers) of the challenge strain MOH-94 by eye drop and *per os*, which is basically as required by Ph. Eur. (per eye drop).

Layer chickens, vaccinated via the s.c. route, were challenged using the same challenge material and route with doses of 2.0 $\log_{10}$ or 3.0 $\log_{10}$ EID_{50} at 4, 5 and 7 weeks after vaccination (challenges at five and seven weeks are supportive studies to evaluate age dependency on susceptibility of birds).

A group of unvaccinated SPF birds was included in studies to validate the challenge infection or reference is made to SPF groups included in other studies.

Serology was performed in 10 hatch mates at hatch to determine MDA levels of the used animals. Birds in all 7 studies were observed for 10 days for clinical signs and necropsy was performed on dead animals and surviving animals at the end of the observation period. Afterwards, histological examination of bursas as required by and detailed in Ph. Eur. was carried out.

Serology was performed for all birds intended for challenge at the day of challenge to consider seroconversion/absence of seroconversion after vaccination. Cloacal swab sampling for PCR was carried out on days 4, 7 and 11 after challenge for virus shedding (as no claim is made on IBDV virus shedding, results are not mentioned and assessed any further). The bodyweight on vaccinated as well as commercial controls was measured individually on the day of challenge as well as 5 and 10 days after challenge. The daily bodyweight gain was calculated for each bird between challenge and day 10 after challenge. The average daily bodyweight gain (ADWG) over ten days after challenge was compared between vaccinated birds and controls.

Broilers

In study "Onset of Immunity in broiler chickens after *i.o.* vaccination (3 weeks)", (3,500 PFU), mean ELISA titre at hatch: 11225), 20 broilers were vaccinated and 20 broilers as well as 10 SPF chickens were kept as controls. Challenge was performed 24 days post vaccination.

In study "Onset of Immunity in broiler chickens after s.c. vaccination (3 weeks)", (4,000 PFU), mean ELISA titre at hatch: 11666), 20 broilers were vaccinated. 15 broilers were kept as controls. Regarding a control group of SPF chickens, reference is given to one of the studies. Challenge was performed 21 days post vaccination.

In study "Onset of Immunity in broiler chickens after *i.o.* vacc. (4 weeks)", (4,000 PFU), mean ELISA titre at hatch: 15809), 20 broilers were vaccinated and 15 broilers as well as 10 SPF chickens were kept as controls. Challenge was performed 31 days post vaccination.

In study "Onset of Immunity in broiler chickens after sc. vaccination (4 weeks)", (4,000 PFU), mean ELISA titre at hatch: 14293) 20 broilers were vaccinated and 20 broilers as well as 10 SPF chickens were kept as controls. Challenge was performed 28 days post vaccination.

At challenge 3 weeks post vaccination, 90% of unvaccinated broiler control animals were still seropositive (with similar titres as vaccinated animals) in study "Onset of immunity in broiler chickens after s.c. vaccination" and 55% of the broiler controls were protected after challenge. Especially in this study, the requirements of the reflection paper, where challenge of animals is supposed to be performed at the time when unvaccinated controls are basically seronegative, were not taken into consideration. Accordingly, the 90% protection achieved in broilers vaccinated via the s.c. route cannot be clearly attributed to vaccination. Four weeks post vaccination (study "Onset of Immunity in broiler chickens after s.c. vaccination (4 weeks)", 80% of s.c. vaccinated broilers were protected (75% seroconversion) in challenge while 30% of controls were protected (20% were still seropositive with clearly lower mean titres).

At challenge 24 or 31 days after *i.o.* vaccination, 40% of unvaccinated broiler control birds were still seropositive at the time of challenge, but only 25% resp. 20% protection after challenge was observed in controls, more clearly indicating that immunity in controls is waning in these studies. Protection in *i.o.* vaccinated animals after challenge at 24 days post vaccination is comparably low with 70%, suggesting that immunity by MDA is becoming less protective and immunity by vaccination is not yet fully developed despite 85% seroconversion in vaccinated birds. At challenge 31 days after *i.o.* vaccination, 90% protection is achieved in broilers (95% seroconversion).

Neither clinical signs nor mortality due to IBD were found in unvaccinated broilers after challenge, which is not unusual in this animal category. The claim: "to reduce mortality and the clinical signs caused by IBDV" in broilers nevertheless is regarded justified, as broilers, in contrast to layers, are described in literature to often not develop clinical signs after infection with IBDV without being further affected by secondary infections. In addition, the claim for Ultifend ND IBD was well supported in SPF birds as well as in layers. It is not regarded necessary and might be confusing to the user of the vaccine to differentiate between layers and broilers with regard to the indication.

Regarding the claim on reduction of loss in bodyweight gain associated with IBDV infection, significant differences between vaccinated and non-vaccinated birds were observed over the first five days after challenge and over the whole 10 days after challenge at challenges about 4 weeks post vaccination. However, this phenomenon was not observed three weeks post vaccination over the whole observation period of 10 days.

The claim of reduced bodyweight gain loss in broilers is not considered supported in the presented studies, as provided differences in ADWG are not considered relevant and results were not consistent among studies in broilers (no significantly reduced body weight gain loss in broilers between days 1-10 post challenge in earlier challenges performed 21/24 days post vaccination). Accordingly, the claim was deleted.

Layer chickens

In study "Onset of Immunity in layer chickens after s.c. vaccination (4,000 PFU)", mean ELISA titre at hatch: 13452) 28 layers were vaccinated and 28 layers were kept as controls. Challenge was performed 4 weeks post vaccination with a challenge dose of 3.0 log_{10} EID_{50}.

In study "Efficacy in layer chickens (5 weeks) after s.c. vaccination (4,000 PFU)", mean ELISA titre at hatch: 13260) 20 layers were and 15 layers were kept as controls. Challenge was performed 5 weeks post vaccination with a challenge dose of 2.0 log_{10}.

In study "Efficacy in layer chickens (7 weeks) after s.c. vaccination (4,000 PFU)", mean ELISA titre at hatch: 13260) 20 layers were vaccinated and 15 layers were kept as controls. Challenge was performed 7 weeks post vaccination with a challenge dose of 2.0 log_{10}.

At challenge 4 weeks post vaccination, 96.4% of unvaccinated layer control animals were still seropositive (with similar titres as vaccinated animals) in study "Onset of immunity in layer chickens after s.c. vaccination" and 78.6% of the layer controls were protected after challenge. In this study, the requirements of the reflection paper, where challenge of animals is supposed to be performed at the time when unvaccinated controls are basically seronegative, were not taken into consideration. Accordingly, the 92.9% protection achieved in layers vaccinated via the s.c. route cannot be mainly attributed to vaccination.

After challenge at 5 weeks post vaccination (study "Efficacy in layer chickens (5 weeks) after s.c. vaccination") meant as supportive study to evaluate age dependency on susceptibility of birds), only 13.3% of layer control birds were protected (46.7% mortality; 80% were still seropositive at challenge) while 70% of vaccinated animals were protected (95% were seropositive with clearly higher

mean titres than controls).This study is basically regarded as suitable to demonstrate efficacy of the vaccine against IBD challenge in the presence of quite high protective percentages, but lower levels of MDA in controls at challenge.

Whereas protection after challenge at 7 weeks after vaccination increases in vaccinated layers up to 90% (100% seropositivity), the percentage of controls susceptible to challenge infection decreases (40% protection, still 46.7% seropositivity at challenge, clearly lower titres than vaccinated animals). Nevertheless, as mentioned below, all control birds, which were not protected, even died due to IBD challenge. All studies on layers met the efficacy pass criteria set by the applicant (70% protection in vaccinated birds after challenge).

The average daily clinical scores were significantly lower in vaccinated birds compared to controls after challenge 4 weeks post vaccination (study "Onset of Immunity in layer chickens after s.c. vaccination", mortality 3.5% in controls compared to 0% in vaccinated animals), as well as after challenge 5 weeks post vaccination (mortality 46% compared to 0% in vaccinated birds) and 7 weeks post vaccination (mortality 60% in controls compared to 10% in vaccinated birds). Mortality was not separately statistically evaluated but included in the statistical analysis of clinical scores. Nevertheless, it is obvious that mortality is clearly higher in controls than vaccinated birds and the claim on reduction of clinical signs and reduction of mortality due to IBDV challenge accordingly can be regarded as supported in layers.

Regarding the claim on reduction of loss in body weight gain associated with IBDV infection, significant differences between vaccinated and non-vaccinated animals were observed over the first 5 days after challenge and over the whole 10 days observation period after challenge in all studies with layers. Nevertheless, the relevance of bodyweight gain in layers is rejected. Accordingly, the claim was deleted.

In summary, the efficacy of the IBD component in the presence of MDA is regarded to be satisfactorily demonstrated. Achieved protection in challenge of 70% in broilers 24 days after *i.o.* vaccination as well as in layers five weeks after s.c. vaccination was comparably low and clearly lower than the results received in SPF birds. In layer chickens, susceptibility to IBD challenge decreases even if MDA are still present. Accordingly, a suitable time for challenge according to the cited reflection paper is not possible. Even if results of other studies in MDA positive birds could demonstrate better protection rates, a sentence on delay of OOI in the presence of (very) high levels of MDA as mentioned in the cited reflection paper EMA/CVMP/IWP/439467/2007 is included in the SPC.

Interactions

No studies on interactions were performed. Accordingly, the following sentence is included in section 4.8 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."

Field trials

Complementary efficacy tests of field trials were performed to evaluate efficacy of ULTIFEND ND IBD under field conditions regarding efficacy of the ND and IBD component. MDA levels for MDV, NDV and IBDV were measured in both field studies and confirmed to be high for NDV and IBDV. For MDV, 80% respectively 90% of the animals were seropositive.

No (complementary) field study to examine efficacy against MD under field conditions is presented for Ultifend ND IBD. The omission has been justified based on the fact that the efficacy of the vaccine (and replication of the vector) can be regarded proven by the efficacy of the inserts, which has been demonstrated in corresponding complementary studies to field studies. As well, the efficacy in presence of MDA for MDV has been adequately demonstrated in layer and broiler chickens in laboratory trials.

Detailed background information on the field studies, including efficacy parameters, is provided in the summarised study descriptions in the safety part.

Overview of the (complementary) field efficacy studies:

Study title	Dose PFU	Challenge day/strain/dose
Layer field trial-Szerencs	commercial dose	-
Complementary ND efficacy test of layer field trial		NDV Herts 33/56 50 ELD_{50}
Complementary IBD efficacy test of layer field trial		MOH-94 vv IBDV 40 EID_{50}
Layer field trial-Szendrő		-
Broiler field trial-Pusztadobos		-
Complementary ND efficacy test of broiler field trial after s.c. vaccination		NDV Herts 33/56 50 ELD_{50}
Complementary IBD efficacy test of broiler field trial after *in ovo* vaccination		MOH-94 vv IBDV 40 EID_{50}
Complementary IBD efficacy test of broiler field trial after s.c. vaccination		MOH-94 vv IBDV 40 EID_{50}
Broiler field trial-Nagyhát		-

ND

Two studies were performed to determine the efficacy for the ND component in commercial chickens, one in broilers and one in future layers vaccinated via the s.c. route. Layer and broiler chickens were vaccinated s.c. at one day of age with a commercial dose under field conditions and moved to the laboratory prior to challenge.

Basically, challenge was performed according to the requirements of Ph. Eur. monograph 0450, 2-4-5, Immunogenicity, at 55 resp. 36 days post vaccination with 5 log_{10} ELD_{50} of the challenge strain Herts 33 via the i.m. route. A group of unvaccinated SPF birds of younger age than the commercial birds was included in the studies to validate the challenge infection. Serology was performed in 20 hatch mates at hatch to determine MDA levels of the used animals.

Commercial birds in both studies were observed for 14 days for clinical signs and necropsy was performed on dead animals and surviving animals at the end of the observation period. In case of

inconclusive results, histology was conducted for clarification. Serology was performed for all birds intended for challenge on the day of challenge to consider seroconversion/absence of seroconversion after vaccination. Swab sampling (oropharyngeal and cloacal) for determination of virus shedding by PCR was performed in 10 vaccinated birds and all control animals from days 1-5 after challenge. If animals died after challenge, sampling was omitted for these animals.

Study - Complementary efficacy test of field trial study

Aim of the study was to examine the efficacy of the vaccine in layer chickens administered under routine hatchery conditions against Newcastle disease virus (NDV). This test was performed as a complementary efficacy test to a field trial.

After vaccination (day 0 of the study) in the hatchery, 22 birds (group1) were moved first to a layer chicken farm and then taken to the laboratory two days prior to challenge. 10 control birds from the same hatchery were directly transported from the hatchery to the test facilities. 10 SPF birds were placed in the test facility on study day 25 at one day of age. All three groups were kept under similar conditions. Challenge was performed 54 days after s.c. vaccination.

Considerably high levels of MDA against NDV were detected at hatch (mean ELISA titre: 18436.3); all samples were positive (100%).

Whereas 0% seroconversion at challenge and 0% protection in challenge was observed in broiler controls, full seroconversion was observed in vaccinated layers at challenge and full protection achieved after challenge in these animals. For oropharyngeal as well as cloacal swab samples examined via PCR, from examination days 2-5 after challenge, a significant reduction of virus shedding could be observed in vaccinated birds of group 1 in comparison to control group 2.

It must be noted that since some birds of group 1 were contaminated with CVP2 (different ND vaccine) approximately 20 days prior to the complementary ND laboratory challenge, the efficacy of the vaccine against ND cannot be definitely evaluated in this study.

Study - Complementary efficacy test of field trial study.

Aim of the study was to examine the efficacy of the vaccine (administered under routine hatchery conditions) against Newcastle disease challenge in broiler chickens after s.c. vaccination. This test was performed as a complementary efficacy test to a field trial.

After vaccination in the hatchery, 20 birds were moved to the laboratory two days prior to challenge. 10 control birds from the same hatchery were directly transported from the hatchery to the test facilities. 10 SPF birds were placed in the test facility as hatched (30-day-old at challenge). All three groups were kept under similar conditions. Challenge was performed 36 days after s.c. vaccination of the broilers.

Considerably high levels of MDA against NDV were detected at hatch (mean ELISA titre: 19339.6); all samples were positive (100%).

Sixty per cent of samples from unvaccinated broilers of group 2 were positive for ND specific MDA (mean ELISA titre: 1116.0) at challenge and a comparably high level of protection (60%) was observed in the animals after challenge.

Seroconversion was observed in 75% of the vaccinated birds of group 1 at challenge and 90% protection achieved after challenge. As high protection was also achieved in control birds, protection in the vaccinated group cannot be completely attributed to vaccination.

For oropharyngeal as well as cloacal swab samples examined via PCR, vaccinated birds had significantly lower copy numbers than controls at examination days 2 and 3 (Day 2: $p = 0.0052$, Day

3: p= 0.0056). On day 5 after challenge, vaccinated animals had significantly higher copy numbers than controls (Day 5: p= 0.0306).

Conclusions on field trials on ND

Both field trials cannot be completely evaluated to demonstrate efficacy of the vaccine under routine hatchery conditions in the field as some contamination occurred in study CLI-057-2018 in the vaccinated layers with CVP2 (different ND vaccine) approximately 20 days prior the complementary ND laboratory challenge. Accordingly, full protection received in the vaccinated birds (0% protection in controls) cannot be clearly attributed to vaccination. Protection in study CLI-063-2018 on broilers (90%) partly seems attributable to maternally derived immunity in the presence of high protection (60%) in the control animals. Nevertheless, as high and clearly better protection is observed in vaccinated birds compared to controls in study CLI-063-2018, the differences in protection between vaccinated birds and controls can be attributed to the vaccination. However, on day 5 after challenge, *i.o.* vaccinated birds shed significantly more virus than unvaccinated controls in contrast to days 2 and 3 (as well as all other relevant sampling times in all other studies), where virus shedding was significantly lower in vaccinated birds compared to controls.

IBD

Three studies were performed to determine the efficacy of the IBD component in commercial chickens, two in broilers vaccinated via the *i.o.* route (18-day-old chicken embryonated eggs) or at one day of age via the s.c. route and one in future layers vaccinated at one day of age via the s.c. route.

Challenge in broilers as well as layers was basically performed according to the requirements of Ph. Eur. 0587, 2-4-5, Immunogenicity, in broilers at 36 days and in layers at 43 days post vaccination with 4.0 log_{10} EID_{50} challenge strain MOH-94 by eye drop and *per os*, which is basically as required by Ph. Eur. (per eye drop).

A group of unvaccinated SPF birds is included in the studies to validate the challenge infection.

Serology was performed in 20 hatch mates at hatch to determine MDA levels of the used animals.

Birds in the studies were observed for 10 days for clinical signs and necropsy was performed on dead animals and surviving animals at the end of the observation period. Afterwards, histological examination of bursas as required by and detailed in Ph. Eur. was carried out.

Serology was performed for all birds intended for challenge at the day of challenge to consider seroconversion/absence of seroconversion after vaccination. Cloacal swab sampling for PCR was carried out on days 4, 7 and 11 after challenge for virus shedding (as no claim is made on IBDV virus shedding, results are not mentioned and assessed any further). The bodyweight on vaccinated birds as well as commercial controls was measured individually on the day of challenge as well as five and ten days after challenge. The daily bodyweight gain was calculated for each bird between challenge and day 10 after challenge. The average daily bodyweight gain (ADWG) over ten days after challenge was compared between vaccinated animals and controls.

Study - Complementary efficacy test of field trial study.

Aim of the study was to examine the efficacy of the vaccine administered under routine hatchery conditions against infectious bursal disease virus (IBDV) challenge in layer chickens 6 weeks after subcutaneous (s.c.) vaccination. This test was performed as a complementary efficacy test to a field trial.

After vaccination (day 0 of the study) in the hatchery, 25 birds were moved first to a layer chicken farm and then taken to the laboratory on the day of challenge. 25 control birds from the same hatchery were directly transported from the hatchery to the test facilities. 10 SPF birds were kept at

the test facilities after hatching. They were 36-day-old at challenge. All 3 groups were kept under similar conditions. Challenge was performed 54 days after s.c. vaccination in all SPF control birds and 23 vaccinated and control layers each, as two birds of these groups were sacrificed to examine their bursas prior to challenge.

Considerably high levels of MDA against IBDV were detected at hatch (mean ELISA titre: 20480); all samples were positive (100%).

17.4% of samples from unvaccinated layers were positive for IBD specific MDA (the mean ELISA titre of the group was 438) at challenge and only 9% of them were protected after challenge. One animal died and all but three birds showed clinical signs of IBD and severe bursa damage.

At challenge, complete seroconversion was observed in vaccinated layers and 74% of these animals were protected in challenge (mean ELISA titre: 7552). No mortality was observed in vaccinated layers and only two vaccinated birds showed clinical signs of IBD. Mean clinical scores were significantly lower in vaccinated birds compared to unvaccinated animals. Both the average daily weight gain over the first five days after challenge as well as over the entire observation period until day 10 was significantly higher in vaccinated birds compared to controls, which is not regarded relevant for layers.

Study - Complementary efficacy test to field trial study.

Aim of the study was to examine the efficacy of the vaccine (administered under routine hatchery conditions) against infectious bursal disease challenge in broiler chickens 5 weeks after *in ovo* vaccination. This test was performed as a complementary efficacy test to a field trial.

After vaccination in the hatchery (study day 0) via the *i.o.* route, 30 birds were moved to the laboratory on study day 37. 30 control birds from the same hatchery were transported from the hatchery to the test facilities on study day three after hatching. 10 SPF birds were included in the study on day nine (30-day-old at challenge). All 3 groups were kept under similar conditions. Challenge was performed 36 days after *i.o.* vaccination of the broilers in all SPF control birds and 28 vaccinated and control broilers each, as two birds of these groups were sacrificed to examine their bursas prior to challenge.

Considerably high levels of MDA against IBDV were detected at hatch (mean ELISA titre: 16384; all samples at hatch were positive [100%]).

28.6% of the samples from unvaccinated layers were positive for IBD specific MDA (the mean ELISA titre of the group was 826) at challenge and 42.9% of them were protected after challenge. Among the affected animals, two birds died (without previously showing clinical signs) and one surviving animal showed clinical signs.

78.6% seroconversion was observed at challenge in vaccinated layers and 85.7% of these animals were protected in challenge (mean ELISA titre: 4696). No mortality and clinical signs were observed in vaccinated layers after challenge. However, comparing the clinical scores, no significant difference was found between vaccinated and control birds.

The average daily weight gain over the first five days after challenge as well as over the entire observation period until day 10 was significantly higher in vaccinated birds compared to control animals.

Study - Complementary efficacy test to field trial study.

Aim of the study was to examine the efficacy of the vaccine (administered under routine hatchery conditions) against infectious bursal disease challenge in broiler chickens five weeks after s.c. vaccination. This test was performed as a complementary efficacy test to a field trial.

After vaccination in the hatchery (study day 3) via the s.c. route, 30 birds were moved to the laboratory on study day 37. Thirty control birds from the same hatchery were transported from the hatchery to the test facilities on study day three after hatching. Ten SPF birds were included in the study on day nine (30-day-old at challenge). All three groups were kept under similar conditions. Challenge was performed 36 days after *i.o.* vaccination of the broilers in all SPF control birds and 28 vaccinated and control broilers each, as two birds of these groups were sacrificed to examine their bursas prior to challenge.

Considerably high levels of MDA against IBDV were detected at hatch (mean ELISA titre: 16384) and all samples at hatch were seropositive (100%).

28.6% of the samples from unvaccinated broilers were positive for IBD specific MDA (the mean ELISA titre of the group was 826) at challenge and 42.9% of them were protected after challenge. Among the affected birds, two animals died (without previously showing clinical signs) and one surviving animal showed clinical signs.

92.2% seroconversion was observed at challenge in vaccinated layers (mean ELISA titre: 5687) and 82.1% of these animals were protected in challenge. No mortality and clinical signs were observed in vaccinated broilers after challenge. However, comparing the clinical scores no significant difference was found between vaccinated and control birds (p=0.0778, Wilcoxon rank-sum test).

The average daily weight gain over the first 5 days after challenge as well as over the entire observation period until day 10 was significantly higher in vaccinated birds compared to controls.

Conclusion on field trials for IBD

In s.c. vaccinated layer chickens, good protection rates could be achieved after challenge 43 days post vaccination (74%) while controls were highly susceptible to IBD challenge (9% protection in controls). In addition, mean clinical scores were significantly lower in vaccinated birds compared to unvaccinated birds, thus confirming the results of the laboratory studies.

In vaccinated broilers, good protection rates after challenge at 36 days post vaccination could be achieved (85.7% protection for *i.o.* vaccinated birds and 82.1% for s.c. vaccinated birds) while susceptibility of controls to IBD challenge (42.9% protection in broiler controls) was limited.

The average daily weight gain over the first five days after challenge as well as over the entire observation period until day 10 was significantly higher in vaccinated birds compared to control animals in all above-mentioned studies. As the claim on reduction of ADWG loss is not considered supported in general because this claim was neither consistently demonstrated nor regarded relevant, this finding in the field trial at a later time of challenge is of informative value only. The claim has been deleted.

Overall conclusion on efficacy

ONSET OF IMMUNITY:

MD

Two studies, designed and validated according to the requirements of Ph. Eur. monograph 0589, 2-4-3 Immunogenicity, were performed to determine efficacy and OOI for MD in SPF chickens; one including birds vaccinated via the *i.o.* route and one using birds vaccinated via the s.c. route. Challenge was performed at nine days post vaccination as required by Ph. Eur. for both routes of vaccination with 50 PFU challenge strain MD70 (vMDV) via the i.p. route. The claimed OOI (nine days) for the MD component corresponds to the time of challenge mentioned in Ph. Eur. for the immunogenicity test.

The RPP level after challenge was 93.9% in birds after vaccination via the *i.o.* route and 88.9% for birds vaccinated via the s.c. route. This is in line with the efficacy pass criteria mentioned in Ph. Eur. (RRP at least 80%).

ND

Two studies, designed and validated according to the requirements of Ph. Eur. monograph 0450, 2-4-5 Immunogenicity, were performed to determine efficacy and the OOI for ND in SPF chickens; one including birds vaccinated via the *i.o.* route and one using birds vaccinated via the s.c. route. Challenge was performed at 21 days post vaccination as required by Ph. Eur. for the s.c. route and 24 days post vaccination for the *i.o.* route (at 21 days of life) with 5 log_{10} ELD_{50} of the challenge strain Herts 33 via the i.m. route as required by Ph. Eur. As the claimed OOI (four weeks) for the ND component is later than the time of challenge mentioned in Ph. Eur. for the immunogenicity test of NDV, the delay of challenge after i.o. use can be regarded as acceptable. The level of protection was 90% after challenge in birds after vaccination via the *i.o.* route and 100% for birds vaccinated via the s.c. route. This is in line with the efficacy pass criteria mentioned in Ph. Eur. (protection at least 90%).

IBD

Two studies, designed and validated according to the requirements of Ph. Eur. monograph 0587, 2-4-5 Immunogenicity, were performed to determine efficacy and the OOI for IBD in SPF chickens; one including birds vaccinated via the *i.o.* and one using birds vaccinated via the s.c. route. Challenge was performed at 14 days post vaccination as required by Ph. Eur. for the s.c. route and 17 days post vaccination for the *i.o.* route (at 14 days of life) with 4.0 log_{10} EID_{50} of the challenge strain MOH-94 by eye drop and *per os* , which is basically as required by Ph. Eur. (per eye drop). As the claimed OOI (three weeks for broilers and four weeks for layers) for the IBD component is later than the time of challenge mentioned in Ph. Eur. for the immunogenicity test of IBDV, the delay of challenge after *i.o.* use can be regarded as acceptable.

The OOI in SPF birds is adequately and completely demonstrated for MD, NDV and IBD as claimed for both application routes (*i.o*. and s.c. use). All performed challenges were valid and adequate protection rates were received for all components.

The claims:

- to reduce mortality, clinical signs and lesions caused by NDV;
- to reduce mortality, clinical signs and bursa lesions caused by very virulent IBDV; and
- to reduce mortality, clinical signs and lesions caused by classical MDV
  could be adequately supported in presented studies.

The claim on reduction of virus shedding for NDV and the claim of reduced bodyweight gain loss in broilers was not examined in SPF birds. The claim of reduced bodyweight gain loss in broilers is deleted.

The influence of maternal antibody on the efficacy of the vaccine

According to the requirement of Ph. Eur. 5.2.7 monograph "The influence of passively acquired and maternally derived antibodies on the efficacy of a vaccine" must be adequately evaluated. "Any claims, stated or implied, regarding onset and duration of protection shall be supported by data from trials."

Validity and efficacy pass criteria according to Eur. monographs 0589, 0450 and 0587 are not applicable for use in commercial birds and are adapted by the applicant in a suitable way.

The studies were not performed following the guidance of reflection paper EMA/CVMP/IWP/439467/2007, where challenge is supposed to be performed if MDA levels in control animals at time of challenge are sufficiently low. The study design is rather modelled to confirm the

claimed OOI of the vaccine. This approach has been justified by the applicant. Nevertheless, some studies are not considered completely suitable to adequately demonstrate efficacy of the vaccine in the presence of too high MDA levels in control animals at the time of challenge. Overall data nevertheless are regarded to sufficiently allow assessing the efficacy of the vaccine in presence of MDA.

MD

Three studies, basically designed according to the requirements of Ph. Eur. monograph 0589, 2-4-3 Immunogenicity, were performed to determine efficacy for MD in commercial chickens; two in broilers vaccinated via the *i.o.* or s.c. route and one in layer chickens vaccinated via the s.c. route.

Challenge was performed at nine days post vaccination for both routes of vaccination.

High levels of APP are achieved in all vaccinated commercial birds in all trials, corresponding to RPP levels fulfilling the efficacy pass criteria of Ph. Eur. while protection in the corresponding control groups is sufficiently low to validate the challenge as required by Ph. Eur. for SPF chickens.

ND

Three studies, basically designed according to the requirements of Ph. Eur. monograph 0450, 2-4-5 Immunogenicity, were performed to determine the OOI for ND in commercial chickens; two in broilers vaccinated via the *i.o.* or s.c. route and one in layers vaccinated via the s.c. route. Challenge was performed at 31 days post vaccination (at 28 days of life) for birds vaccinated by the *i.o.* route and at 28 days post vaccination for the birds vaccinated via the s.c. route.

The study on OOI after *i.o.* use in broilers is regarded of limited value as 60% of unvaccinated control animals were still seropositive at the time of challenge and 50% of them were protected after challenge. Accordingly, the full protection seen in broilers vaccinated via the *i.o.* route cannot be mainly attributed to vaccination in this study.

Nevertheless, the achieved levels of protection in s.c. vaccinated broilers and layers are considered adequate in vaccinated commercial birds (81.8% and 81%) in comparison to unvaccinated controls (8.3% and 0%) and fulfil the efficacy pass criteria set by the applicant (not fewer than 80% protection in vaccinated animals after challenge). Seroconversion at the time of challenge was observed in 81.8% of vaccinated broilers as well as layers after s.c. vaccination. The claims reduction of mortality, clinical signs and lesions caused by NDV after s.c. (and i.o.) administration of the vaccine could be supported. In addition, virus shedding examined via PCR in oropharyngeal and cloacal swap samples could be demonstrated to be significantly lower in vaccinated birds in all presented studies. Accordingly, this claim, even if not common for ND vaccines, is accepted in the absence of data on SPF birds. In summary, it is considered that the efficacy of the ND component in the presence of MDA was satisfactorily demonstrated.

IBD

Seven studies, basically designed according to the requirements of Ph. Eur. monograph 0587, 2-4-5 Immunogenicity, were performed to determine the OOI and efficacy for IBD in commercial chickens; 4 in broilers vaccinated via the *i.o.* or s.c. route (challenged at 3 and 4 weeks for each route of administration) and 3 in layers vaccinated via the s.c. route.

Challenge in broilers was performed at 24 and 31 days post vaccination (at 21 days/28 of life) for birds vaccinated by the *i.o.* route and 21 and 28 days post vaccination for the birds vaccinated via the s.c. route. Layers, vaccinated via the s.c. route, were challenged using the same challenge material and route with lower doses at 4, 5 and 7 weeks after vaccination (challenges at five and seven weeks are supportive studies to evaluate age dependency on susceptibility of birds).

Broilers

The study on OOI (challenge at 21 days post vaccination) after s.c. use in broilers is regarded of limited value as 90% of unvaccinated control birds were still seropositive at the time of challenge and 55% of them were protected after challenge. Accordingly, the 90% protection seen in broilers vaccinated via the s.c. route cannot be mainly attributed to vaccination in this study.

Four weeks post vaccination study, 80% of s.c. vaccinated broilers were protected (75% seroconversion) after challenge while 30% of controls were protected (20% still seropositive with clearly lower mean titre).

At challenge 24 or 31 days after *i.o.* vaccination, 40% of unvaccinated broiler control birds were still seropositive at the time of challenge, but only 25% resp. 20% protection after challenge was seen in controls. This clearly indicates that immunity in controls is waning in these studies. Protection in *i.o.* vaccinated birds after challenge at 24 days post vaccination is comparably low with 70%, suggesting that immunity by vaccination is not yet fully developed despite 85% seroconversion in vaccinated birds. At challenge 31 days after *i.o.* vaccination, 90% protection is achieved in broilers (95% seroconversion).

Neither clinical signs nor mortality due to IBD was observed in unvaccinated broilers after challenge, which is not unusual in this animal category. The claim: "to reduce mortality and the clinical signs caused by IBDV" in broilers has been sufficiently justified. The claim of reduced body weight gain loss in broilers was not regarded as supported in the presented studies. The provided differences in ADWG were not considered relevant and the results were not consistent among studies in broilers (no significantly reduced body weight gain loss in broilers between days 1 to 10 post challenge in earlier challenges performed 21/24 days post vaccination and in challenge to support DOI at 9 weeks post vaccination after *i.o.* use). The claim was deleted.

Layer chickens

The study on OOI (challenge at 28 days post vaccination) after s.c. vaccination in layers, where 96.4% of unvaccinated control birds were still seropositive at the time of challenge and 78.6% of them were protected after challenge, is also considered of limited value.

After challenge 5 weeks post vaccination, only 13.3% of the layer control birds were protected (still 80% seropositive at challenge) and 70% of vaccinated birds (95% seropositive with clearly higher mean titres than controls). The study is meant as supportive study to evaluate age dependency on IBD susceptibility of birds but is basically regarded suitable to demonstrate efficacy of the vaccine against IBD challenge in the presence of lower levels of MDA at challenge.

Whereas protection after challenge at 7 weeks after vaccination (supportive study to evaluate age dependency on susceptibility of birds) increases up to 90% (100% seropositivity) in vaccinated layers, the percentage of controls susceptible to challenge infection decreases (40% protection, still 46.7% seropositivity at challenge, clearly lower titres than vaccinated birds).

The average daily clinical scores were significantly lower in vaccinated birds compared to control birds after challenge 4 weeks post vaccination as well as after challenge 5 weeks post vaccination (and 7 weeks post vaccination). Mortality was not separately statistically evaluated; but included in the statistical analysis of clinical scores. Nevertheless, it is obvious that mortality is clearly higher in control animals than in vaccinated birds. Thus, the claim on reduction of clinical signs and reduction of mortality due to IBDV challenge can be regarded as supported in layers. All studies on layers met the efficacy pass criteria set by the applicant (70% protection in vaccinated animals after challenge).

Regarding the claim on reduction of loss in body weight gain associated with IBDV infection, significant differences between vaccinated and non-vaccinated birds could be seen over the first 5 days after

challenge and over the whole 10 days observation period after challenge in all layer studies. Nevertheless, the relevance of bodyweight gain in layers is rejected. The claim is deleted accordingly.

In summary, the efficacy of the IBD component in the presence of MDA is not regarded to be at similar level as in SPF birds. Achieved protection in challenge of 70% in broilers 24 days after *i.o.* vaccination as well as in layers five weeks after s.c. vaccination was comparably low and clearly lower than the results received in SPF birds. Even if results of other studies in MDA positive birds could demonstrate better protection rates, inclusion of a sentence on delay of OOI in the presence of (very) high levels of MDA as mentioned in the cited reflection paper EMA/CVMP/IWP/439467/2007 has been included in the SPC.

The efficacy of the IBD component in the presence of MDA in *i.o.* vaccinated layers was not demonstrated. It is mentioned in the dossier but not in the SPC that this application route is not recommended for future layers. As no difference in protection after i.o. vaccination to SPF birds and broilers is expected, the *i.o*. route could be used for layers in the rare case this would be applicable.

Duration of immunity:

Studies on DOI were performed in commercial birds only. This is considered acceptable.

<u>MD</u>

No studies are presented on the DOI, which is generally accepted for Marek vaccines as they induce lifelong immunity.

<u>ND</u>

Three studies basically designed according to the requirements of Ph. Eur. monograph 0450, 2-4-5 Immunogenicity, were performed to determine the DOI for ND in commercial chickens; two in broilers vaccinated via the *i.o.* or s.c. route and one in layers vaccinated via the s.c. route. Challenge was performed at 9 weeks of age (66 days post vaccination) for broilers vaccinated by the *i.o.* or s.c. route and for layers (vaccinated via the s.c. route) at 126 days (18 weeks) post vaccination.

All challenges are fully validated by SPF as well as broiler resp. layer control groups (0% protection in all control groups).

The level of protection after challenge was 100% in broilers after vaccination via the *i.o.* or s.c. route and 95% for layers vaccinated via the s.c. route. This is in line with the efficacy pass criteria mentioned in Ph. Eur. (protection at least 90%). All vaccinated broilers vaccinated via the *i.o.* or s.c. route had ND specific antibodies at the time of challenge as well as 95.5% of layers after s.c. vaccination.

The claims reduction of mortality, clinical signs and lesions caused by Newcastle disease virus (NDV) could be supported for both *i.o.* as well as s.c. administration for the DOI.

In addition, virus shedding examined via PCR in oropharyngeal and cloacal swap samples could be demonstrated to be significantly lower in vaccinated birds in all studies. In summary, it is considered that the DOI of the ND component in broilers for 9 weeks and in layers for 18 weeks was satisfactorily demonstrated.

<u>IBD</u>

Three studies basically designed according to the requirements of Ph. Eur. monograph 0587, 2-4-5 Immunogenicity, were performed to determine the DOI for IBD in commercial chickens; 2 in broilers vaccinated via the *i.o.* or s.c. route and one in layers vaccinated via the s.c. route.

In summary, birds in studies using commercial birds were vaccinated *i.o.* (18-day-old chicken embryonated eggs) or s.c. at one day of age with a dose of minimum titre or lower. Challenge in broilers was performed at the claimed DOI of nine weeks post vaccination for broilers vaccinated by the *i.o.* or s.c. route.

Layer chickens, vaccinated via the s.c. route, were challenged using the same challenge material and route with lower doses at nine weeks after vaccination.

A group of unvaccinated SPF birds was included in the studies to validate the challenge infection.

At challenge 9 weeks post *i.o.* vaccination, 84% of the *i.o.* and all the s.c. vaccinated broilers were protected in challenge, whereas protection in broiler controls was negligible.

All unvaccinated layer control birds were seronegative at the time of challenge, but 78.6% of them were protected after challenge, indicating decreasing susceptibility to IBD infection at that time, as already seen after challenge at seven weeks post vaccination. All vaccinated birds had seroconverted at the time of challenge and the protection rate after challenge (100%) in vaccinated layers was significantly higher than in controls. There was no significant difference in clinical scores between vaccinated and control birds at that time.

The average daily weight gain over the first 5 days after challenge as well as over the entire observation period of 10 days was not significantly higher in *i.o.* vaccinated but was significantly higher in s.c. vaccinated birds compared to the broiler controls (the corresponding claim is not accepted based on lack of relevance and inconsistent results in studies on OOI and DOI). For layers, the relevance of the claim is rejected and the claim has been deleted altogether for layers and broilers.

The DOI as claimed (9 weeks) in layer and broiler chickens is regarded as demonstrated, even if it is noted that in layers, susceptibility to challenge is vading at that time.

The DOI for the IBD component in layers vaccinated via the *i.o.* route was not investigated, which is regarded acceptable. Future layers commonly are not vaccinated via *i.o.* route in practice. Anyway, in case it was performed, it could be accepted as no difference in result is expected to SPF birds and commercial broilers.

Field trials:

<u>MD</u>

No (complementary) field study to examine efficacy for MD under field conditions are presented for Ultifend ND IBD. The omission has been justified and is regarded acceptable.

<u>ND</u>

Two studies were performed to determine efficacy for the ND component in commercial chickens; one in broilers and one in layer chickens vaccinated via the s.c. route at one day of age with a commercial dose under field conditions and moved to the laboratory prior to challenge.

Challenge was basically performed according to the requirements of Ph. Eur. monograph 0450, 2-4-5 Immunogenicity at 55 resp. 36 days post vaccination with 5 log10 ELD_{50} of the challenge strain Herts 33 via the i.m. route. A group of unvaccinated SPF birds of younger age than the commercial birds was included in the studies to validate the challenge infection.

Both field trials cannot be completely evaluated to demonstrate efficacy of the vaccine under routine hatchery conditions in the field, as some contamination occurred in the study on s.c. vaccinated layers with a different ND vaccine used in the control group of the corresponding field trial approximately 20 days prior to the complementary ND laboratory challenge. Accordingly, full protection received in the vaccinated animals (0% protection in controls) cannot be clearly attributed to vaccination. Protection

(90%) in vaccinated birds in the second study on broilers seems partly based on maternally derived immunity as high protection was found in the control birds (60%). Nevertheless, as high and clearly better protection is observed in vaccinated broilers than in controls after *i.o.* vaccination, the differences in protection between vaccinated and control birds can be attributed to the vaccination in the corresponding study. However, on day five after challenge, *i.o.* vaccinated birds shed significantly more virus than unvaccinated controls in contrast to day two and three (as well as all other relevant sampling times in all other studies), where virus shedding was significantly lower in vaccinated birds than in control animals. As this is the only discordant value for NDV with regards to virus shedding and controls, as mentioned above, were considerably well protected after challenge in this study anyway, the claim on reduction of virus shedding nevertheless can be accepted.

IBD

Three (3) studies were performed to determine efficacy for the IBD component in commercial chickens; two in broilers vaccinated via the *i.o.* route (18-day-old chicken embryonated eggs) or at one day of age via the s.c. route and one in layers vaccinated at one day of age via the s.c. route.

Challenge in broilers as well as layers was basically performed according to the requirements of Ph. Eur. Monograph 0587, 2-4-5 Immunogenicity at 36 days post vaccination in broilers and at 43 days post vaccination in layers with 4.0 log_{10} EID_{50} challenge strain MOH-94 by eye drop and *per os*.

In the s.c. vaccinated layers, good protection rates after challenge could be achieved after challenge 43 days post vaccination (74%) while low protection was seen in controls in IBD challenge (9% protection in controls). In addition, mean clinical scores were significantly lower in vaccinated birds compared to unvaccinated animals, thus confirming the results of the laboratory studies.

In vaccinated broilers, good protection rates after challenge could be achieved after challenge 43 days post vaccination (85.7% protection for *i.o.* vaccinated birds and 82.1% for s.c. vaccinated birds) while high protection was seen in controls in IBD challenge (42.9% protection in broiler controls).

The average daily weight gain over the first five days after challenge as well as over the entire observation period until day 10 was significantly higher in vaccinated birds compared to control birds in all above-mentioned field studies. As the claim on reduction of average daily weight gain loss is not regarded as sufficiently supported in general and not regarded relevant, this finding in field trial at late times of challenge are of informative value only. Finally, the claim has been deleted.

It can be concluded that efficacy for IBD and ND of Ultifend ND IBD in commercial chickens, after being vaccinated in the field under hatchery conditions, could be supported for *i.o.* and s.c. vaccination altogether. The efficacy for MDV (replication of the vector) is regarded proven by the efficacy of the inserts, which, as detailed, has been demonstrated in corresponding complementary studies to field studies. As well, the efficacy in presence of MDA for MDV has been adequately demonstrated in layer and broiler chicken in laboratory trials.

Part 5 – Benefit-risk assessment

Introduction

Ultifend ND IBD is a recombinant trivalent vaccine for chicken embryonated eggs and one-day-old chicks consisting of a live recombinant herpesvirus of turkeys, strain rHVT-ND-IBD expressing the fusion protein of NDV and the capsid virion protein 2 of IBDV. The antigens are cell-associated and combined with serum and DMSO and stored frozen in liquid nitrogen. No adjuvant or preservative is added. The vaccine is diluted before use in sterile solvent. The pharmaceutical form of the final vaccine

is a suspension for injection. Ultifend ND IBD is innovative because the recombinant vaccine strain induces immunity against three relevant poultry pathogens, MDV, NDV and IBDV, which are frequently isolated in poultry stocks and they have paramount importance in the poultry production industry. Furthermore, the vaccine can be applied at an early age of the birds (one-day-old) or even in chicken embryonated eggs (18-day-old) to provide protection against early replication of virulent MDV, NDV and IBDV in case of infection.

The vaccine is intended for the active immunisation of chickens and chicken embryonated eggs to reduce mortality, clinical signs and lesions as well as to reduce virus shedding caused by Newcastle disease virus (NDV). In addition, the vaccine reduces mortality, clinical signs and bursa lesions caused by very virulent infectious bursal disease virus (IBDV). The vaccine also reduces mortality, clinical signs and lesions caused by classical Marek's disease virus (MDV). The claim on reduction of the loss of daily weight gain caused by IBDV infection has been deleted.

The proposed *in ovo* dose of 0.05 ml or subcutaneous dose of 0.2 ml, the routes of administration (*in ovo* or subcutaneously) and the vaccination scheme (one dose to 18-day-old chicken embryonated eggs or a single dose to one-day-old chicks) have been confirmed.

The application has been submitted in accordance with Article 12(3) of Directive 2001/82/EC (full application).

Benefit assessment

Direct therapeutic benefit

The benefit of Ultifend ND IBD is its efficacy in vaccinated chicken and chicken embryonated eggs to reduce mortality, clinical signs and lesions as well as to reduce virus shedding caused by NDV and to reduce mortality, clinical signs and bursa lesions caused by very virulent IBDV; furthermore, the vaccine reduces mortality, clinical signs and lesions caused by classical MDV, which was investigated in a large number of well designed, controlled laboratory challenge studies conducted in accordance with GLP. The claim for reducing the loss of daily weight gain caused by IBDV infection was not considered satisfactorily supported and has been deleted.

The proposed OOI for MDV, NDV and IBDV and the proposed DOI for NDV and IBDV (immunity of MDV is generally accepted to be lifelong) after *in ovo* or subcutaneous vaccination are supported. Some delay in the onset of IBD immunity is identified in presence of (very) high MDA levels, which is reflected in the SPC.

Studies are presented, where birds vaccinated under hatchery conditions were taken to laboratory to be challenged with virulent ND and IBD strains equivalent to challenge strains used in laboratory studies. These studies are considered supportive for IBD, whereas there are some limitations for ND based on study conditions. Nevertheless, results in field studies for NDV basically confirm results of laboratory studies.

No (complementary) field study to examine efficacy for MD under field conditions is presented for Ultifend ND IBD and this has been adequately justified.

Additional benefits

Ultifend ND IBD is easy to apply to chicken embryonated eggs by a single *in ovo* vaccination using an appropriate device/ applicator. The vaccine is also easy to apply to one-day-old chicks by a single

subcutaneous vaccination.

One single *in ovo* or subcutaneous vaccination is sufficient to stimulate immunity against three relevant poultry pathogens, MDV, NDV and IBDV.

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

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

Risk assessment

Risks for the target animal:

Administration of Ultifend ND IBD in accordance with SPC recommendations is generally well tolerated. The safety of Ultifend ND IBD in chickens was confirmed in six laboratory studies and four field trials. No adverse reactions were observed. The vaccine is based on an apathogenic vaccine strain, which is shown to be safe for chickens. Reversion to virulence after five serial passages in chickens could not be demonstrated. Spread from vaccinated chickens must be expected for at least seven weeks after the vaccination and appropriate care should be taken to separate vaccinated from non-vaccinated chickens. An appropriate warning is included in the SPC.

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 rHVT-ND-IBD behaves differently.

The vaccine is filled in glass ampoules and stored in liquid nitrogen. It must be thawed and diluted in the solvent. Special precautions are necessary for handling. The SPC gives advice how to handle the vaccine in section 4.5. Some advice on personal protective equipment is also included in section 4.9.

It is concluded that user safety for this product is acceptable when used according to the SPC recommendations.

Risk for the environment:

The vaccine virus is shed by vaccinated chickens for 49 days and spread to turkeys and between turkeys was demonstrated. No spread to chickens was observed but cannot be excluded. The genetic modification did not alter the biological properties of the apathogenic parent strain apart from expressing a surface glycoprotein from Newcastle disease virus (NDV) and a capsid protein from infectious bursal disease virus (IBDV).

Ultifend ND IBD is not expected to pose a risk for the environment when used according to the SPC recommendations. No phase II assessment is considered necessary.

Risk for the consumer:

None identified.

Risk management or mitigation measures

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

- The vaccine strain is excreted by chickens for 49 days.
- 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.

Evaluation of the benefit-risk balance

The product was shown to have a positive benefit-risk balance overall. The product was shown to be efficacious for the active immunisation of chicken embryonated eggs and one-day-old chicks to reduce mortality, clinical signs and lesions as well as to reduce virus shedding caused by NDV and to reduce mortality, clinical signs and bursa lesions caused by very virulent IBDV; furthermore, the vaccine reduces mortality, clinical signs and lesions caused by classical MDV. However, the claim "reduction of loss of daily weight gain caused by IBDV infection" was not considered satisfactorily supported and has been deleted in the SPC/PI. Some delay in the onset of IBD immunity is identified in presence of (very) high MDA levels, which is reflected in the SPC.

The objection regarding the use of additional gentamicin along with cryoprotectant 1 solution during the blending process of the bulk vaccine was satisfactorily supported by appropriate data.

Two outstanding issues on quality need to be addressed satisfactorily as post-approval recommendations to ensure that the product will be of consistent quality.

Ultifend ND IBD is well tolerated by the target animals and presents a low risk for users and the environment.

Conclusion

Based on the review of the data on quality, safety and efficacy, the Committee for Medicinal Products for Veterinary Use (CVMP) considers that the application for marketing authorisation for Ultifend ND IBD is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EC) No 726/2004 in conjunction with Directive 2001/82/EC).

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