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Veterinary Medicines Division

Committee for Veterinary Medicinal Products (CVMP)

CVMP assessment report for Divence IBR Marker Live (EMEA/V/C/006260/0000)

Vaccine common name: Bovine herpesvirus type 1 (live) vaccine

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

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Introduction

The applicant Laboratorios Hipra, S.A. submitted on 25 April 2023 an application for a marketing authorisation to the European Medicines Agency (The Agency) for Divence IBR Marker Live, through the centralised procedure under Article 42(2) a of Regulation (EU) 2019/6 (mandatory scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 8 December 2022 as Divence IBR Marker Live has been developed by means of a biotechnological process, i.e. using recombinant DNA technology (Article 42(2)(a)(i)).

The eligibility to the centralised procedure was agreed upon by the CVMP on 8 December 2022 as no other marketing authorisation has been granted for the veterinary medicinal product within the Union.

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

Active immunisation of cattle from 10 weeks of age to reduce virus shedding, hyperthermia and clinical signs caused by BoHV-1.

Vaccinated animals can be differentiated from field virus infected animals due to the marker deletion (gE-) by means of commercial diagnostic kits.

Onset of immunity: 3 weeks after completion of the basic vaccination scheme.

Duration of immunity: 6 months after completion of the basic vaccination scheme. 1 year after re-vaccination scheme.

The active substance of Divence IBR Marker Live is a live recombinant bovine herpesvirus type 1 (BoHV-1) with *gE*- and *tk*- genes deleted and it is intended for the active immunisation of cattle against infectious bovine rhinotracheitis. The marker deletion (*gE*-) allows to differentiate vaccinated animals from field-virus-infected animals by means of commercial diagnostic kits. The target species is cattle.

The basic vaccination scheme consists of two intramuscular injections (2 ml each), the first dose being administered to calves from 10 weeks of age, and the second dose three weeks later. Re-vaccination is recommended at an interval not longer than 6 months after completion of the basic vaccination scheme by the administration of a single intramuscular dose. Afterwards, subsequent re-vaccinations are recommended at an interval not longer than 12 months.

The vaccine contains a GMO and is a fall-out product of Divence Penta vaccine, which was authorised under the centralised procedure. Thus, the components (antigens, composition of excipients and adjuvant) are identical for both vaccines and it is only the number of active ingredients which is decreased. Both vaccines are reconstituted with the same solvent.

Divence IBR Marker Live lyophilisate and solvent for emulsion for injection contains $10^{6.3}$ - $10^{7.6}$ $CCID_{50}$/dose of live recombinant infectious bovine rhinotracheitis virus, strain CEDDEL, gE- tk- double-gene deleted and is presented in packs containing one vial of lyophilisate (5, 20, 40, 50 doses) and one vial of solvent (10, 40, 80, 100 ml).

The rapporteur appointed is Jacqueline Poot and the co-rapporteur is Cristina Muñoz Madero.

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

On 19 June 2024, the CVMP adopted an opinion and CVMP assessment report.

On 9 August 2024, the European Commission adopted a Commission Decision granting the marketing authorisation for Divence IBR Marker Live.

Scientific advice

The applicant received scientific advice from the CVMP in June 2019. The scientific advice pertained to the quality part of the dossier (including batch potency test).

The advice given was generally followed.

Part 1 - Administrative particulars

Summary of the Pharmacovigilance System Master File

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

Manufacturing authorisations and inspection status

Active substance

Manufacture, storage and/or distribution of the active substance live *gE- tk-* double-gene deleted bovine herpesvirus type 1 (BoHV-1) strain CEDDEL is performed at Laboratorios Hipra S.A., Amer, Spain.

A GMP declaration for the active substance manufacturing site was provided from the qualified person (QP) at the EU batch release site. The declaration was based on an on-site audit by the manufacturing site responsible for batch release which has taken into consideration the Good manufacturing practice (GMP) certificate available for the active substance site issued by the competent authority of Spain (AEMPS) following inspection.

Finished product

Manufacture and primary packaging of the finished product takes place at Laboratorios Hipra S.A., Amer, Spain.

The site has a manufacturing authorisation issued on 28-11-2022 by AEMPS.

GMP certification, which confirms the date of the last inspection and shows that the site is authorised for the activities indicated above, has been provided.

Batch release, manufacture of solvent, quality control testing (biological; chemical/physical; microbiological), primary packaging, secondary packaging and storage and/or distribution take place at Laboratorios Hipra S.A. Amer, Spain.

The site has a manufacturing authorisation issued on 28-11-2022 by AEMPS.

GMP certification, which confirms the date of the last inspection and shows that the site is authorised for the activities indicated above, has been provided.

Overall conclusions on administrative particulars

The summary of the pharmacovigilance system master file is considered to be in line with legal requirements. The GMP status of the active substance and of the finished product manufacturing sites has been satisfactorily established and is in line with legal requirements.

Part 2 - Quality

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

Qualitative and quantitative composition

The finished product is presented as a lyophilisate containing live gE- tk- double gene-deleted bovine herpesvirus type 1 (BoHV-1) strain CEDDEL ($10^{6.3-7.6}$ $CCID_{50}$), as active substance at the potency/titre per dose indicated.

Other ingredients of the lyophilisate are dipotassium phosphate, gelatin, glycine, potassium dihydrogen phosphate, sorbitol and sucrose.

The solvent contains the adjuvant Montanide IMS. Other ingredients of the solvent are disodium phosphate dodecahydrate, potassium chloride, potassium dihydrogen phosphate, sodium chloride and water for injections.

The product is available as lyophilisate in 10 ml vials containing 5, 20, 40 or 50 doses combined in carboard boxes with 10, 50 or 100 ml vials containing 10, 40, 80 or 100 ml of solvent as described in section 5.4 of the SPC.

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

Container and closure system

The vaccine is packed in type I glass vials of 10 ml, closed with a type I rubber stopper and aluminium cap. The solvent is packed in colourless PET vials of 10, 50 or 100 ml, closed with type I rubber stoppers and aluminium cap.

The containers and closures are in compliance with the pharmacopoeial requirements, and their sterilisation is adequate.

Product development

The active substance of the vaccine is live attenuated bovine herpesvirus type 1 (BoHV1), also called infectious bovine rhinotracheitis virus (IBRV) in the dossier. The product was developed as a fall-out of the larger combination product Divence Penta, containing live attenuated bovine respiratory syncytial virus (BRSV), strain Lym-56; live gE- tk- double gene-deleted bovine herpesvirus type 1 (BoHV-1), strain CEDDEL; inactivated bovine parainfluenza 3 virus (PI-3), strain SF4; E2 recombinant protein from bovine diarrhoea virus type 1 (BVDV-1) and E2 recombinant protein from bovine diarrhoea virus type 2 (BVDV-2). The composition of the two products is identical but for the presence of the additional antigens in Divence Penta.

An explanation and justification for the composition and presentation of the vaccine has been provided. The lyophilised form was chosen in order to achieve stability of the active substance. Different freeze-drying components were tested in various compositions to achieve an optimal excipient.

The adjuvant, Montanide IMS, is an immunostimulatory compound. It was chosen from a battery of adjuvants tested in calves together with the antigens in the Divence Penta vaccine. The selected composition was found to have the best capacity to induce humoral and cellular immune responses.

A justification is given regarding the relevance of the chosen vaccine strain within the EU.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 2 of the SPC.

The justification provided for the choice of potency tests is acceptable.

From the calculation of the worst-case scenario for antibiotic remnants in the finished product, it can be concluded that there is no risk to the consumer.

The formulation of batches used during clinical studies was the same as that intended for marketing.

Description of the manufacturing method

The manufacturing process consists of four main steps: manufacturing of the active substance, the freeze-drying excipient, the finished product and the solvent.

BoHV-1 antigen is produced on suitable cells in bioreactors. The different steps are well described. The production and composition of the freeze-drying excipient is also well described.

For the finished product the antigen is transferred to the reactor, then freeze-drying excipient is added. The bulk is mixed, and filling and freeze drying is performed. Capped vials are kept at 2 °C – 8 °C for a maximum of 18 months.

The components for the solvent (WFI, disodium phosphate dodecahydrate, potassium dihydrogen phosphate, sodium chloride, potassium chloride) are introduced into a tank. The adjuvant is added once the rest of the substances have been dissolved. The pH is checked and adjusted if necessary. The solution is sterile filtered into a sterile tank. Samples for bioburden testing are taken and the bulk is filter-sterilised again into the filling machine. If necessary, the bulk can be stored.

The process is considered to be a standard manufacturing process.

Production and control of starting materials

Starting materials listed in pharmacopoeias

The applicant provided a list including the name, the function and the applicable monograph of each starting material listed in a Pharmacopoeia. All of them are monographs of the European Pharmacopoeia.

Example certificates of analysis (CoA) have been provided for all substances listed and all substances conform to the relevant Ph. Eur. or USP monograph requirements. Where applicable, certificates of suitability and certificates of irradiation have been provided. The nature of the starting

materials, controls and treatments applied guarantee sterility of the vaccine and absence of extraneous agents (EAs).

Starting materials not listed in a pharmacopoeia

Starting materials of biological origin

BoHV-1 strain CEDDEL (*gE- tk-*)
The original virus strain (FM) was isolated from an IBR outbreak. The double deletion of *gE* and *tk* to obtain the CEDDEL strain was performed at the Institute of Biotechnology and Biomedicine (IBB) of the Universitat Autònoma de Barcelona (Spain) in cooperation with Laboratorios Hipra. The MSV was tested for titre, identity, sterility, mycoplasma and extraneous agents testing. The MSV and WSV preparation, control and storage are adequately presented. The MSV and WSV are manufactured and handled in a seed lot system in line with Ph. Eur. 0062. The WSV is produced from the MSV by passages in cell cultures again meeting the requirements of Ph. Eur. 0062.

Description and validation of all methods is provided. Management of extraneous agents was performed in accordance with Ph. Eur. 5.2.5.
A transmissible spongiform encephalopathy (TSE) and extraneous agents risk assessment for BoHV-1 CEDDEL in the final product is provided. It is concluded that the material poses no risk for transmission of TSE or extraneous agents.

GBK cells
The GBK cell line is controlled by a cell seed system in line with Ph. Eur. 5.2.4 on cell cultures for the production of veterinary vaccines. The history of the cell line in terms of origin, number of passages, media used, storage conditions and preparation are adequately described.
The MCS was tested for general microscopy, karyotype, identification of species, sterility, mycoplasma and endogenous retrovirus. The presence of extraneous agents was assessed in accordance with Ph. Eur. 5.2.5. Test methods and their validations are provided. The risk of tumorigenicity has been assessed and can be considered acceptable.

The WCS is tested for general microscopy, viability, sterility, mycoplasma and extraneous agents.

A TSE and extraneous agents risk assessment for GBK cells is provided. This includes materials used in the obtainment/storage of the MCS. It can be concluded that the material poses no risk for transmission of TSE or EA.

Tryptose phosphate broth (TPB)
TPB is a buffered dextrose broth used as an ingredient of the culture medium in different production phases. All components are processed using heat at different temperatures (minimum 80 °C) and different pH conditions.
An extraneous agents risk assessment for TPB was performed in accordance with Ph. Eur. 5.2.5. In conclusion, the risk of contamination with extraneous agents is considered negligible. The material is not considered a TSE risk since it contains porcine materials (non-TSE species) and bovine milk fit for human consumption.

Trypsin
Trypsin is an enzyme derived from porcine pancreas and is used to detach cells. The trypsin is supplied irradiated.
An extraneous agents risk assessment for trypsin was performed, concluding that the risk is considered negligible, since it is terminally irradiated. The material is not considered a TSE risk since it contains only porcine materials.

Cytodex 3 surface microcarriers
Cytodex 3 surface microcarriers are support matrices allowing the growth of GBK cells in a bioreactor. A CoA is provided.

Gelatin
An extraneous agents risk assessment for gelatin was performed. In conclusion, the risk of contamination with extraneous agents is considered negligible. The material is not considered a TSE risk since it contains only porcine materials.

Starting materials of non-biological origin

Certificates of analysis are provided for the starting materials of non-biological origin and all of them are conforming to in-house specifications. Appropriate documentation was provided.

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

During the production of the vaccine, several media are used. Detailed information on the qualitative and quantitative composition, methods of preparation, sterilisation and storage of media and solutions are provided for the in-house prepared media and solutions.

Control tests during the manufacturing process

The tests performed during the manufacturing process are detailed below for each production phase, manufacturing of individual antigens, finished product and solvent. The methods listed were all appropriately validated and for Ph. Eur. methods suitability was shown. The in-process tests are sufficient to control all the critical steps in the manufacturing.

gE$^-$ *tk*$^-$ BoHV-1 strain CEDDEL
Sterility is tested in accordance with Ph. Eur. 2.6.1. The suitability of the method is in accordance with the monograph. The virus titre is also determined.

Freeze-drying excipient
Appearance, sterility by membrane filtration, pH and density are tested on each batch.

Solvent
Bioburden is tested prior to filter sterilisation. During filling, the volume is continuously checked by weight.

Control tests on the finished product

The proposed finished product tests are generally considered adequately described and validated and appropriate to control essential properties of the product.

1) General characteristics of the finished product

Appearance is tested on each batch of lyophilised fraction. It should be a white-to-yellow lyophilisate. Solubility is tested on each batch of lyophilised fraction. Any observations are noted. Appearance is tested on each batch of solvent bulk and filled product. It should be a white translucent emulsion. The pH is tested on each bulk batch of solvent.

2) Identification of the active substance

The antigen is identified in the potency test.

3) Batch titre or potency

BoHV-1 virus titre and identity are determined in the lyophilised product. The method was appropriately validated and is performed in accordance with Ph. Eur. 0696 requirements.

Scientific advice was requested for the titration assays. The applicant's proposal was considered in principle acceptable if appropriately validated. Appropriate data were provided and therefore, the potency test is considered acceptable and the scientific advise provided followed.

4) Identification and assay of adjuvants

The identity and concentration of Montanide IMS in the solvent is determined on each bulk batch. The method was appropriately validated.

5) Identification and assay of excipient components

No tests are performed. The absence of testing for excipients is considered justified for the lyophilisate, while for the solvent the buffering capacity is considered adequately controlled by the pH determination.

6) Sterility and purity tests

Each batch of lyophilised product and solvent is tested for bacterial and fungal sterility in accordance with Ph. Eur. 2.6.1. Absence of mycoplasma is tested on each batch of lyophilised product, by culture method, in accordance with Ph. Eur. 2.6.7. No tests for extraneous agents are performed since this is not considered necessary based on the risk-based approach following Ph. Eur. 5.2.5.

7) Residual humidity

Residual moisture is tested on each batch of lyophilised fraction by the Karl-Fisher method in accordance with Ph. Eur. requirements.

8) Filling volume

Filling volume is checked on each batch of filled solvent. The contents of a vial are transferred to a graduated cylinder and volume is measured.

Batch-to-batch consistency

In order to support the consistency of the manufacturing process, data on 3 consecutive batches of the larger combination vaccine Divence Penta are provided. These batches were manufactured in accordance with the method described in part 2.B. of the Divence Penta dossier. The batches met the requirements for all tests.

Since Divence IBR Marker live has a new larger presentation of 50 doses and this is considered a worst-case scenario, additional data on three consecutive 50-dose batches are provided in part 2.F. These batches met the requirements for all tests.

Data on six batches of solvent are provided. These batches met the requirements for all tests.

It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible and consistent manner.

Stability

Stability of the bulk antigens

The proposed storage period for BoHV-1 antigen is 24 months at – 70 ºC and it has been demonstrated with the satisfactory results of three batches manufactured according to the method described in the quality part of the dossier.

Stability of pre-inoculum and inoculum

The stability of the pre-inoculum and inoculum of BoHV-1 was demonstrated by data. Data show that the claimed shelf lives are acceptable.

Stability of the finished product

Three batches of the 5-dose presentation of Divence Penta (bigger combination vaccine) were put on a long-term stability study. Batches are to be stored at 2 °C – 8 °C for 33 months. All finished product tests were planned to be performed at regular intervals.

The batches appear stable and remain well within the requirements. Stability of the 50-dose presentation of Divence IBR Marker Live was investigated. Data up to 18 months storage are provided The batches appear stable and remain well within the requirements. The data are considered supportive of a shelf life of 18 months.

Stability of the finished product lyophilisate after freezing for 6 months at ≤-20 °C was investigated. Two production batches were used, one 5-dose batch of Divence Penta and one 50-dose presentation of Divence IBR Marker Live. The batches met all requirements, and no remarkable change was observed for any of the parameters.
Data up to T=6 are provided for the 50-dose batch, no remarkable changes were observed.
Similarly, stability of the finished product lyophilisate after freezing for 12 months at ≤-20 °C was investigated. The study was set up as described above, using 2 batches of 5-dose presentation of Divence Penta and one batch of 50-dose presentation of Divence IBR Marker Live. Data up to T=27 months are provided for the 5-dose batches; these batches met all requirements and no remarkable change was observed for any of the parameters. Data up to the time of defrosting (T=0) are provided for the 50-dose batch, no remarkable change was observed.
Together, the data are considered to support the frozen storage for up to 12 months followed by a shelf life of 18 months at +2 °C to +8 °C.

Three consecutive production batches of 10 ml and of 100 ml solvent were put on long-term stability study in support of the proposed shelf life of 3 years. An accelerated study was also performed. In the long-term stability study, batches were stored at 2 °C – 8 °C. Samples were taken from each batch at regular intervals. The appearance, pH, Montanide concentration and identification were checked at each time point. For the 100 ml batches, data are provided up to 18 months of storage. All parameters were within limits at all time points. For the accelerated stability study, batches were stored at 25 °C ± 2 °C for 6 months and samples were taken from each batch at T=0, 3 and 6 months. The results for three 10 ml batches and three 100 ml batches give no indication of a particular trend for any of the parameters. All parameters were within the limits at all time points.

Together the data are considered adequate to support a shelf life for the solvent of 3 years.

Overall conclusions on quality

The quality part of the dossier complies with the Annex II to Regulation (EU) 2019/6. General and, where relevant, specific Ph. Eur. monographs have been followed and the data are adequate in support of a consistent and well-controlled manufacturing process.

The composition of the product is described in sufficient detail. The development of the product has been adequately described and justified. Reasonable justification is given regarding the relevance of the chosen vaccine strain within the EU. All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards. There are no novel excipients used in the finished product formulation.

The manufacturing process consists of four main steps: manufacturing of the active substance, the freeze-drying excipient, the finished product and the solvent. The manufacturing process has generally been described in adequate detail.

Starting materials have been listed and shown to comply with pharmacopoeial or in-house requirements. The extraneous agents risk assessment and testing strategy is considered adequate.

Control tests performed during the manufacturing process have generally been adequately described and appropriately validated. The range of control tests is generally considered to provide adequate control of the consistency of the manufacturing process and critical points.

Finished product control tests have been adequately described and appropriately validated. The range of tests is generally considered to provide adequate control of the quality of the final product with respect to its critical attributes.

Data on stability of the active substance as well as the finished product and solvent have been provided. The results of testing give no clear indication of a reduction in potency or change in composition of the lyophilisate or the solvent. The data are considered adequate to support a shelf life of 18 months for the lyophilisate and 3 years for the solvent.

Part 3 – Safety documentation (safety and residues tests)

General requirements

The active substance of Divence IBR Marker Live is a live gE- tk- double-gene deleted bovine herpesvirus type 1 (BoHV-1), strain CEDDEL. The applicant states that the antigen is already part of a centrally authorised vaccine. The lyophilisate fraction includes the antigen together with a well-known freeze-drying excipient intended to provide cryoprotection as well as a stability to the antigen. The solvent contains PBS and the adjuvant (Montanide IMS).

The vaccine is intended for the active immunisation of cattle from 10 weeks of age. The recommended vaccination programme includes a basic vaccination scheme, which consists on the administration of two intramuscular injections (2 ml each), the first dose administered to calves from 10 weeks of age, and the second dose three weeks later. Re-vaccination is recommended at an interval not longer than 6 months after completion of the basic vaccination scheme by the administration of a single intramuscular dose. Afterwards, subsequent re-vaccinations are recommended at an interval not longer than 12 months.

A full safety file in accordance with Article 8(1)(b) has been provided.

Safety documentation

Ten safety studies were conducted to investigate the safety of the product; this included 8 pre-clinical studies investigating the safety of the administration of a 10-fold overdose and repeated dose, reproductive performance and 2 clinical trials. Studies applicable to live vaccines and GMO products were conducted to investigate the dissemination of the vaccine strain, the spread from vaccinated animals to non-vaccinated contacts and reversion to virulence. The vaccine strain was administered by the intramuscular route as recommended, or intranasally. Pre-clinical studies were reported to be Good laboratory practice (GLP) compliant and carried out in target animals at or below the minimum age recommended for vaccination, using pilot batches of the vaccine. Commercial batches were used in the clinical trials, performed under Good clinical practice (GCP).

The requirements for safety testing of Ph. Eur. monograph 5.2.6 "Evaluation of safety of veterinary vaccines and immunosera" and the specific monograph 0696 "Infectious bovine rhinotracheitis vaccine (live)" have been taken into account to demonstrate the safety of this vaccine. VICH guidelines (GL 41 and GL 44) have also been taken into account.

Divence IBR Marker Live is a fall-out vaccine of Divence Penta vaccine, which has received a positive CVMP opinion under Centralised Procedure. Thus, the components (antigens, composition of excipients and adjuvant) are identical in each case and it is only the number of active ingredients which is decreased. Consequently, the studies presented in part 3 of the dossier are exactly the same as the ones presented in the Divence Penta file.

The IBR strain CEDDEL is a genetically modified organism (GMO). Specific documentation is provided in part 3.E of the registration dossier under Directive 2001/18/EC.

Pre-clinical studies

Safety of the administration of one dose

No studies on the safety of one dose were performed, this is considered to be covered by the study of the safety of an overdose. Adverse events observed after the application of an overdose of the vaccine are listed in section 3.6 of the SPC.

Safety of one administration of an overdose

A randomised, blinded, controlled study was performed in calves of 10 weeks of age that were free of antibodies to BVDV, BoHV-1 and BRSV and had no or low levels of antibodies to PI3. The study was performed to study the safety of an overdose and of a repeated dose. The test group (n=8) received a 10-fold dose of Divence Penta in a volume of 20 ml divided over 4 injection sites. This is acceptable considering the vaccine contains both live and inactivated antigens and since the injection volume per site exceeds the standard volume of 2 ml. At 14-day intervals, three further single doses of Divence Penta were applied at alternating sites. The control group received injections with PBS at the same time, in the same volume and locations.

Calves were observed for clinical signs daily for the duration of the study. Rectal temperature was measured after each vaccination at different time points. In case of rectal temperatures over 39.5 ºC at day 7 post-vaccination (p.v.), temperature measurement continued daily until the temperature decreased to below 39.5 ºC. Injection site reactions were observed daily for 14 days following 1st, 2nd and 3rd administrations and 21 days following the 4th administration.

No generalised clinical signs were observed in any of the animals after vaccination. The body temperature of control animals remained at baseline level throughout the study. In the vaccinated group, average temperatures increased with a peak (avg. 40.7 ºC, max. 41.1 ºC) at day 1 and a gradual decrease to baseline level on day 9 for all animals. After the second vaccination on Day 14, average temperatures increases in the vaccinates were close to 0, with a maximum of 40.3 ºC on day 14 + 4hrs. After the third vaccination on Day 28, average temperature increases in the vaccinates were again close to 0, with a maximum of 40.3 ºC on Day 28 + 4hrs. After the fourth vaccination on Day 42, rectal temperatures in the vaccinated group increased (in all animals) on Day 43 with a maximum of 40.3 ºC. At day 44 temperatures had returned to baseline levels.

No local reactions were observed in the control animals. Local reactions were observed in 5 out of 8 vaccinates, scores up to 3 (max. 10 cm diameter) were recorded. Lesions disappeared completely by day 8. After the second vaccination, local reactions were observed in all vaccinates with a maximum size of 13 cm and a maximum duration of 5 days. After the third vaccination local reactions were observed in all vaccinates, with a maximum size of 7 cm and a maximum duration of 6 days. After the fourth vaccination local reactions were observed in 5 out of 8 vaccinates, with a maximum size of 6 cm and a maximum duration of 6 days.

The adverse events are considered to be acceptable for the type of vaccine and did not appear to affect the overall health of the calves. An adequate warning has been included in the SPC indicating the temperature increases and maximum observed size of local reactions.

Safety of the repeated administration of one dose

Repeated administration of one dose of Divence Penta has been investigated in the study summarised above for safety of an overdose. A tenfold maximum dose was tested, as appropriate for the live components of the vaccine. The study schedule included vaccinations at Day 0 (10-fold overdose), 14, 28 and 42 (all one dose). Some adverse events were reported after the repeated administrations. Slight increases in rectal temperature occurred (max. 1.3 ºC) for 1 day. Injection site reactions with a swelling up to 13 cm and a duration up to 6 days were observed. No indication of an increase in adverse events with repeated vaccinations was found.

Examination of reproductive performance

A study was performed with the aim to study the safety of a basic vaccination followed by one booster vaccination 6 months later, in pregnant cows. The study was appropriately designed, in accordance with Ph. Eur. 5.2.6 and monograph 1952 (BVDV vaccine, inactivated), and performed in compliance with GLP. A total of 28 pregnant FH and FH-crossbreed cows, free from BVDV-1, BDVD-2, IBR, BRSV, PI3 antibodies or with very low PI3 antibodies and not vaccinated against bovine parainfluenza virus were included in this study. Divence Penta was used in the study and this is acceptable since the larger combination vaccine can be regarded as a worst-case scenario for safety. Nine cows in the third trimester of gestation received two vaccine doses with a 21 day interval; in addition, 9 animals in the second trimester of gestation also received 2 vaccine doses with a 21 days interval and 10 animals in the first trimester of gestation received 3 vaccine doses, two doses with a 21 days interval and one dose 6 months after the basic vaccination schedule. Due to the grouping based on gestation, the study was not blinded. Animals were observed for local reactions [14 days post-vaccination (p.v.)] and clinical signs (daily), rectal temperature (day -1 to day 7) and adverse effects on pregnancy and offspring (up to three days of age).

No systemic adverse reactions were observed in any of the animals during the study. Local adverse

reactions were observed in 5 cows after the first vaccination, with a maximum size of 14 cm diameter and a maximum duration of 16 days. After the second vaccination 9 cows presented local reactions, with a maximum swelling size of 11 cm diameter and a maximum duration of 14 days. No animals presented local reactions after the third vaccination.

Rectal temperatures increased on the day after the first vaccination, to a maximum of 40.9 ºC and returning to baseline levels by day 4. After the second vaccination only slight increases were observed in few animals (up to 39.8 ºC) on the day after vaccination, returning to normal two days later. After the third vaccination, a slight increase in temperature occurred in two cows at T=0 + 4hrs.

One animal in the second group (2nd trimester) had dystocia, with the calf presenting in an abnormal position. The calf was born dead due to hypoxia and showed no clinical abnormalities. A calf born to a cow in the same group was found in the morning with a severe injury to the ribcage, likely due to crushing, but otherwise normal. This calf was euthanised. In the third group (boostered), one cow presented with dystocia, the calf presenting in an abnormal position. The calf was born dead due to hypoxia. The remaining 25 cows gave birth to normal healthy calves.

The study results gave no indication of negative effects of vaccination on the outcome of pregnancy. The components of the vaccine are not expected to negatively affect the development of the reproductive system.

Data from the field study support the safety for lactating cattle. Abortigenicity and passage through the placenta studies for BoHV-1 as stated by the specific monograph Ph. Eur. 0696 (IBRV) were provided and the vaccine strain was found to be safe.

Examination of immunological functions

Taking into consideration the nature and composition of the vaccine there is no reason for suspecting an impairment of the immune system under the claimed conditions of use of the vaccine. There are no data suggesting a negative influence on the immune response of the vaccinated animal for the live BoHV-1 component that is included in a centrally authorised vaccine. No studies were performed and the absence of specific data is considered justified.

Special requirements for live vaccines

Special requirements for live vaccines are applicable. It is noted the vaccine strain is a component of centrally-authorised vaccines.

Spread of the vaccine strain

A study was performed to assess spread of the vaccine strain from vaccinated to unvaccinated target animals. A group of six 3-month-old calves was vaccinated intramuscularly (i.m.) with a vaccine containing the same BoHV-1 strain CEDDEL in a dose equivalent to a tenfold dose of the strain in Divence IBR Marker Live and three weeks later with a 0.5-fold maximum dose. The calves were housed together with 3 untreated calves. The possible spread of the vaccine antigen was monitored by means of the serological response against BoHV in the unvaccinated animals. Serology of all animals was performed at the day of vaccination, the day of re-vaccination and 21 days after re-vaccination. At Day 42 all vaccinated animals showed strong seroconversion, in-contact animals remained seronegative. The use of calves slightly older than the minimum age is considered acceptable as is the use of this vaccine instead of Divence IBR Marker Live vaccine. The

results support absence of spreading of the vaccine strain.

Dissemination in the vaccinated animal

A study was performed in 6 susceptible 2-month-old calves free from antibodies against IBR and vaccinated by intramuscular route with the BoHV-1 MSV at a tenfold maximum dose. Two were sacrificed at 2, 4 and 6 days post-vaccination and samples were obtained and tested for virus isolation and virus presence by PCR. The presence of the virus was tested in all the samples by isolation/titration in GBK cell cultures ($CCID_{50}$) and by specific differential PCR. The vaccine virus was not detected in any of the tested samples of nasal, ocular, saliva, vaginal and balanal swabs, urine, faeces, whole blood, serum, white blood cells, ocular conjunctiva, nasal mucosa, trachea, lungs, trigeminal ganglion, testis, seminal vesicle, prostate, ovaries, uterine mucosa and vaginal mucosa origin. The use of MSV instead of the Divence IBR Marker Live vaccine is acceptable, since the other vaccine components are unlikely to affect behaviour of the BoHV-1 strain. It is noted the trigeminal ganglion samples (preferred location for BoHV to go into latency) were PCR negative. It can be concluded that the vaccine virus does not disseminate to any significant degree.

Increase in virulence of attenuated vaccines

The reversion to virulence of the BoHV-1 vaccine strain was investigated in three studies.

In the first study, 8 calves of 3 months of age were included, 2 served as sentinels and 6 were vaccinated i.m. at day 0, 21 and 42 with a tenfold overdose of BoHV-1 CEDDEL strain (10^7 $TCID_{50}$) 3 of these vaccinated calves were treated with prednisolone. Throughout the study, the sentinel animals remained seronegative, indicating absence of transmission/spread of the vaccine strain. No BoHV-1 was isolated from any of the salivary, nasal or ocular samples of any of the vaccinated animals, indicating absence of shedding. In the second study, 8 calves of 1.5 to 2.5 months old were included, 2 calves served as negative controls and 6 were vaccinated twice with a 3 week interval (BoHV-1 CEDDEL strain (10^6 $TCID_{50}$) i.m.). No virus was detected in conjunctival or nasal samples taken after the two vaccinations. The results of the two studies are considered to support the conclusion that no shedding of virus occurs after intramuscular administration of the BoHV-1 vaccine strain.

In previous studies, 12 sero-negative calves (2 for each passage) of 3 months of age were vaccinated by the intranasal route with MSV. The use of intranasal application instead of intramuscular vaccination is acceptable since it appears to be the only method to get excretion of virus and thus the possibility of passage. Serum antibodies were detected by commercial tests. Virus-positive nasal samples were pooled and used to inoculate the next group. In the 4th passage one calf was BoHV-1 positive in the nasal swab sample taken before inoculation. Since the passage was invalid, one in vitro passage was performed using the remainder or the virus inoculum and two new calves were inoculated with this material. This is considered acceptable, also in light of the 3Rs. All calves were evaluated for clinical signs, local reactions and rectal temperature daily for 21 days. No clinical signs were observed that could be attributable to vaccination; there was thus no increase in clinical signs. No clinically significant increases in temperature were observed for any of the calves. In the last passage group the temperatures were somewhat higher than normal in both calves, but this had already occurred before inoculation. There was no indication of an increase in rectal temperatures with later virus passages. The virus recovered from the swabs decreased in titre with subsequent passages. It is noted that attenuation of the strain was achieved by deletion of two genes, which reduces the chances of reversion to virulence to practically zero. The study provides adequate evidence of the absence of reversion to virulence.

Biological properties of the vaccine strain

No specific studies have been conducted to determine the intrinsic biological properties of the vaccine strain. BoHV-1 strain CEDDEL was obtained by genetic engineering; two virulence genes (thymidine kinase and glycoprotein E) were deleted. The strain was shown to be safe, was shown not to spread to in-contact calves and has no potential for reversion to virulence. On the basis of the data presented the safety profile of the strain can be considered acceptable: in addition, it is noted the vaccine strain is a component of centrally authorised vaccines for the same target species.

Recombination or genomic reassortment of the strains

Regarding the genomic reassortment or recombination/redistribution of the strain with other strains of BoHV-1 virus, no specific trials have been performed.

In the BoHV-1 strain CEDDEL genes have been deleted. Any potential recombination is not expected to increase the virulence to more than the virulence of circulating wild-type strains. Together with the intrinsic characteristics of the recombination events (necessity of closely related parental viruses for successful homologous recombination) and epidemiological reasons (prevalence of different herpesviruses in different geographical zones, host predilection) it can be concluded that the risk arising from the potential recombination between CEDDEL strain and other herpesviruses is likely to be negligible.

User safety

A user risk assessment performed according to the revised guideline on User Safety for Immunological Veterinary Medicinal Products (EMEA/CVMP/IWP/54533/2006) is provided.

BoHV-1 is not considered a zoonotic agent. Regarding the components of the lyophilisate and the solvent, no local or systemic harmful effects have ever been reported. No toxicity studies were considered necessary. The adjuvant "Montanide IMS" contains a mineral oil component that is known to cause severe pain and swelling particularly if injected into a joint or finger.

The vaccine is a lyophilisate and a solvent for emulsion to be administered by a veterinary surgeon or under his/her supervision. Accidental self-injection is considered the most likely route of exposure, although the probability is very low. The probability of exposure as a consequence of accidental breakage of the container is considered low and any potential exposure is considered to be very short. Deliberate ingestion is considered to be very unlikely.

Except for the mineral oil, no hazard has been identified for the user and potential exposure to the vaccine is considered very limited; the risk is considered negligible. Only professionals or trained personnel under supervision is allowed to administer the product. Therefore, no measures other than the standard warning sentence for mineral oil-containing products, which is included in the SPC, are considered necessary to reduce the risk of exposure to the vaccine.

MRLs

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

The excipients, including adjuvants, listed in section 2 of the SPC are either allowed substances for which Table 1 of the Annex to Commission Regulation (EU) No 37/2010 indicates that no MRLs are

required or are considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this product.

The antimicrobial substances used in the manufacturing process are present at low residual levels in the finished product, which is not considered to constitute a risk to the consumer.

Withdrawal period

The withdrawal period is set at zero days.

Interactions

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

Clinical studies

Two multi-centre, randomised, double-blinded and placebo-controlled clinical trials investigating safety and efficacy have been performed. The first study was carried out in calves and a second study in heifers and cows. Divence Penta was applied in these studies which is acceptable as it is considered a worst-case scenario with respect to efficacy. The studies are summarised in the tables below.

Efficacy and safety assessment under field conditions of DIVENCE PENTA vaccine in calves	
Objectives	To evaluate clinical safety and efficacy of the vaccine
Study design	Randomised, blinded, placebo controlled, multicentre study designed to assess superiority of the vaccine over control.
Study sites	7 farms (feedlots) in Spain, with historical records of respiratory disease and entering batches of 50 calves or more.
Compliance with regulatory guidelines	GCP
Animals	A total of 1,017 calves, 10-12 weeks of age. The animals were obtained from several regions (Czech republic, Belgium, Germany, France) and were of various breeds (Friesian, Blanc-bleu, Montbeliarde and Montbeliarde cross-breed). On most farms only male calves were kept, but on one farm only blanc-bleu females and on one farm male and female blanc-bleu calves were kept. 506 calves were vaccinated, 511 were treated with placebo, on each farm vaccinated and placebo animals were housed together.
Eligibility criteria	The animals were clinically healthy and not previously vaccinated

	for BRSV, BVD, IBR and/or PI-3.
Test product	One group received Divence Penta whereas the other group received placebo (PBS)
Control product/ Placebo	
Vaccination scheme	Vaccination with 1 dose of 2 ml on Day 0 and Day 21
Safety parameters	Overall safety: recording of adverse events Post-vaccinal safety: 30 calves per group, in 3 different batches from 3 farms were followed closely during the first two days after each vaccination for systemic reactions (scoring), rectal temperature and local reactions at the injection site (scoring).
Statistical method	For all statistical tests a nominal significance level of 5 % ($p<0.05$) was applied. A descriptive analysis was performed for each variable. For quantitative variables appropriate tests were used and for qualitative variables appropriate tests for comparison between treatments were used. All analyses were performed including farm as a random factor into the appropriate statistical model.
Results	
Safety parameters	At total of 1017 animals received at least the first vaccination and form the safety dataset: 506 vaccinates, 511 controls. Post-vaccinal safety: 60 calves from 3 farms were closely monitored (10 calves/group/farm). No systemic reactions were reported. Rectal temperature in the vaccinates increased slightly on D1 and was returning to normal on D2 (average vaccinates D1: 39.5°C, controls: 38.6°C). After the second vaccination, again a slight increase in temperature was observed in the vaccinates, only on D1 (average vaccinates D1: 39.4°C, controls: 38.9°C). After the first vaccination, local reactions (swelling) were observed in 2 control animals on D1 (and one animal on D2). In the vaccinates, swelling up to 3 cm was observed in 2 and 3-5 cm in 3 calves on D1, decreasing rapidly (within 4 days). After the 2nd dose, no reactions were observed in controls, in vaccinates one calf had a swelling of 3-5 cm lasting 1 day. No induration was observed in any calf.
Adverse reactions	Two adverse reactions were observed. One calf experienced an anaphylactic type reaction within 15 minutes after the first application of the vaccine. The calf died and field necropsy revealed acute pulmonary emphysema. One calf after receiving the 2nd dose of vaccine and within 15 minutes presented with loss of balance and prostration. Within 10 minutes the calf started to recover (without treatment), stood up and was totally recovered. (anaphylactic shock in 2 out of 1003 administrations = 0.2%).
Discussion/conclusions further to assessment	
The study was appropriately designed and executed to an acceptable standard (GCP). The animals were of the youngest age for vaccination (10-12 weeks of age). The use of a standard/commercial	

dose is acceptable; the normal vaccination schedule (two applications, three weeks apart) was applied. The general follow-up of animals performed mainly by the farmers and the close follow-up of 30 calves in each group around the days of vaccination revealed no significant safety issues [no clinical signs, no clinically relevant increases in body temperature and no large reactions at the injection site (swelling of max. 5 cm and max. 4 days)]. Two anaphylactic-type reactions were observed in this study; a warning is included in the SPC section 3.6.

Efficacy and safety assessment under field conditions of DIVENCE PENTA vaccine in cattle	
Objectives	To evaluate clinical safety and efficacy of the vaccine
Study design	Randomised, blinded, placebo controlled, multicentre study designed to assess superiority of the vaccine over control.
Study sites	3 dairy farms in Spain and 1 in Hungary.
Compliance with regulatory guidelines	GCP
Animals	A total of 1,255 female HF cattle from 10 weeks of age onward were included. Stratified by (age) category: vaccinated 295 heifers and 336 cows, controls: 296 heifers, 328 cows. At inclusion, around 48.5 % was pregnant, in all stages of pregnancy.
Eligibility criteria	The animals were clinically healthy and not previously vaccinated for BRSV, BVD, IBR and/or PI-3.
Interventions: Vaccine	One group received DIVENCE PENTA vaccine whereas the other group received the placebo (PBS). The route of administration was the recommended one (intramuscular)
Control product/ Placebo	
Vaccination scheme	Vaccination with 1 dose of 2ml on Day 0 and Day 21. 3rd dose at 6 months after 2nd dose Booster at 12 months after 3rd dose (not yet reported for safety) Follow-up for 24 months total.
Safety parameters	Overall safety: recording of adverse events Post-vaccinal safety: 24 heifers and 24 cows per group, in 3 farms were randomly selected at inclusion and followed closely during the first two days after each vaccination for systemic reactions (scoring), rectal temperature and local reactions at the injection site (scoring). Milk yield was compared between groups for 14 days after each dose in two farms with an automatic daily milk production recording system (40 cows/group, with the highest milk production at the time of vaccination).
Statistical method	Descriptive analysis was performed for each variable.
Results	
Safety parameters	At total of 1,255 animals received at least the first vaccination and form the overall safety dataset: 631 vaccinates, 624 controls. Post-vaccinal safety: 8 heifers and 8 cows in each group, in each of 3

	farms were closely monitored. No systemic reactions were reported. Rectal temperature in the vaccinates increased slightly on D1 and was returning to normal on D2 (average vaccinates D1: 39.3°C, controls: 39.1°C). The increase was somewhat higher in heifers (maximum increase 2.16°C from baseline in a vaccinated heifer). After the second and third vaccination, the temperature pattern was very similar to the first administration. After the first vaccination, local reactions (swelling, 3-5 cm) were observed in 12 vaccinated animals on D1 (and 3 animals on D2); none were observed in controls. After the 2nd dose, no reactions were observed in controls or in vaccinates. After the 3rd dose, reactions (up to 3 cm) were observed in 5 controls on the day after vaccination (and in 2 controls on the second day). In the vaccinates, 3 animals had reactions (<3cm) on the first day and one on the second day. No induration was observed in any animal. Milk production was monitored in 20 animals/group/farm on 2 farms. No clinically relevant differences were observed between the groups with respect to average daily milk production for 14 days following the three vaccinations.
Adverse events	No adverse reactions were observed.
Discussion The study was appropriately designed and performed to an acceptable standard. From the results it can be concluded that the vaccine is generally safe in adult cattle since it did not give rise to general clinical signs and no clinically relevant increases in rectal temperature were observed. The local reactions observed were relatively small (<3 cm) and disappeared within a few days. There was no apparent effect on milk yield after vaccination. The evaluation of reproductive safety was performed by analysing pregnancy losses that occurred within two days after (each) vaccination. No losses were observed in the vaccinated group. The overall outcome of pregnancies was highly similar for the vaccinated and control groups, which give a further indication of the safety of the vaccine for the pregnant animals.	

Environmental risk assessment

An environmental risk assessment was performed in accordance the "Guideline for environmental risk assessment for immunological veterinary medicinal products" (EMEA/CVMP/074/95).

Considerations for the environmental risk assessment

The vaccine contains a live attenuated virus. The live gE- tk- BoHV strain CEDDEL, which is also used as the active substance in Hiprabovis IBR Marker Live (authorised 27 January 2011), was shown to be highly attenuated and safe and not to revert to virulence or spread to in-contact animals when applied via the intramuscular route. In conclusion, the probability of the active substance having a negative impact on the environment is considered negligible.

Apart from the active substance, the rest of vaccine components, i.e. the excipients, including the adjuvant, are well-known ingredients used in numerous vaccines currently authorised. None of the ingredients can be considered as hazardous for the environment. Moreover, the vaccine is administered individually by the intramuscular route, thus, the risk of the product being released

into the environment is considered negligible.

As the use of Divence IBR Marker Live does not result in an environmental risk, no specific mitigation measures are considered necessary in addition to general management recommendations and precautions included in the product information regarding the handling and disposal of unused veterinary medicinal product or waste materials derived from the use of thereof.

Considering the approach outlined in Annex I to the "Guideline for environmental risk assessment for immunological veterinary medicinal products" (EMEA/CVMP/074/95), the risk for the environment when using Divence IBR Marker Live can be considered to be effectively zero, based on a low likelihood of hazard occurrence and experience in the use of similar vaccines. Consequently, the environmental risk assessment can stop in phase I and no phase II environmental risk assessment has been considered necessary.

Divence IBR Marker Live is expected to pose a negligible risk to the environment when used as recommended.

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

The antigen live gE- tk- double-gene deleted bovine herpes virus-1 strain CEDDEL is a genetically modified organism (GMO). This strain was constructed by recombinant techniques, by deleting two genes in the virus genome: the coding sequences for the glycoprotein E (gE) and for the enzyme thymidine kinase (tk). The risk assessment for the gE- tk- BoHV-1 strain CEDDEL mandated by Directive 2001/18/EC was previously assessed by the CVMP during the centralised procedure for Hiprabovis IBR Marker Live in 2011.

Written consent for the deliberate release into the environment of the gE- tk- BoHV-1 strain CEDDEL was issued by the Spanish competent authority in 2007, a copy of which has been provided in the dossier.

A complete technical file containing all information required under Annex III and IV to Directive 2001/18/EC has been provided.

Briefly, the modification method used was *in vivo* homologous recombination on cells in culture co-transfected with non-enveloped viral DNA and a shuttle vector. After rounds of plaque-purification, the strain CEDDEL was isolated. Due to the nature of the gene deletion, i.e. by homologous recombination, no foreign DNA sequences remain in the recombinant virus strain. Stability of the construct after 5 passages on GBK cells was confirmed.

The possibility of recombination of the vaccine strain with field virus strains is considered to be low, but not impossible. This would lead to characteristics in line with normal BoHV, with a more complete genome. Selection of the vaccine virus leading to the expression of unexpected or undesirable traits is thus highly unlikely, considering the vaccine strain does not contain foreign sequences. The vaccine strain has limited transmission capacity.

In conclusion, the BoHV-1 strain CEDDEL has been first released into the environment in 2007, in the frame of field studies for the Hiprabovis IBR Marker Live vaccine. After authorisation of this vaccine in 2011, it was placed on the market and thus the vaccine strain is currently present in the field in the EU. A risk assessment for this GMO was previously submitted and accepted by the CVMP and is again presented in this dossier. In Divence IBR Marker Live, the BoHV-1 strain CEDDEL is applied via the same route (i.m.) at a similar dose and to the same target species as for

the Hiprabovis IBR Marker Live vaccine. The previous risk assessment provided by the applicant and the Committee's previous conclusion can thus be accepted. Any risk emerging from the use of the attenuated vaccine virus is expected to be negligible for humans and for the environment.

Overall conclusions on the safety documentation

The vaccine is intended for the active immunisation of cattle from 10 weeks of age. The recommended vaccination programme includes a basic vaccination scheme, which consists of the administration of two intramuscular injections (2 ml each), the first dose administered to calves from 10 weeks of age, and the second dose three weeks later. Re-vaccination is recommended 6 months after completion of the basic vaccination scheme by the administration of a single intramuscular dose. Afterwards, subsequent re-vaccinations are recommended at an interval not longer than 12 months.

A full safety file in accordance with Article 8(1)(b) has been provided. For the safety studies, the larger combination vaccine Divence Penta was used, which is considered acceptable as it represents a worst-case scenario.

No studies on the safety of one dose were performed; this is considered to be covered by the study of the safety of an overdose. A randomised, blinded, controlled study was performed in seronegative calves of two months of age. The calves received a 10-fold overdose of the larger combination vaccine Divence Penta followed by three single maximum doses at 2 week intervals. Calves remained clinically healthy for the duration of the study. Rectal temperatures increased after vaccination with a maximum of 41.1 ºC and a gradual decrease to baseline over one week. Local reactions were observed with a maximum diameter of 13 cm and a maximum duration of 6 days. The adverse events are considered to be acceptable for the type of vaccine and did not appear to affect the overall health of the calves. An adequate warning is included in the SPC indicating the maximum temperature increases and the maximum observed size of local reactions. The risk of increasing (local) adverse events with subsequent vaccinations is acceptably small.

Reproductive safety of a basic vaccination with the larger combination vaccine Divence Penta followed by one booster vaccination 6 months later using Divence Penta, was investigated in pregnant seronegative cows. Cows were vaccinated twice with a vaccine at maximum potency, with a 3-week interval while in the second or third trimester of pregnancy. Animals in the first trimester received the basic vaccination followed by a booster 6 months later. No systemic adverse reactions were observed in any of the animals. Local reactions of up to 14 cm and up to 14 days duration were observed, as were rectal temperature increases to a maximum of 40.9 ºC. All cows carried to term. The results are considered to support reproductive safety.

A study on abortigenicity and passage through the placenta performed in accordance with the specific monograph Ph. Eur. 0696 was provided. The result showed no abortions and no transplacental infection due to the injection of a 10-fold dose of the vaccine strain. The BoHV-1 vaccine strain can be considered safe.

Data from the clinical study are considered to support safety during lactation as well as reproductive safety.

No studies on immunological functions were performed; this is considered justified.

Special requirements for live vaccines are applicable. It is noted the virus strain is a component of a centrally authorised vaccine.

Spread of the BoHV-1 live vaccine strain was investigated. Three-month-old calves were

vaccinated i.m. with a 10-fold maximum dose and 3 weeks later with a standard dose. The calves were housed together with untreated calves for 42 days. In-contact calves remained seronegative. The results support absence of spreading of the BoHV-1 vaccine strain.

Considering the dissemination of the vaccine strain in the target animals, a study was performed in 6 seronegative calves that received a tenfold maximum dose of the BoHV-1 CEDDEL MSV i.m. The vaccine virus was not detected in any of the tested samples. It is noted the trigeminal ganglion samples (preferred location for BoHV to go into latency) were PCR negative. It can be concluded that the BoHV-1 vaccine virus does not disseminate to any significant degree.

Reversion to virulence was investigated in accordance with the Ph. Eur. requirements. Absence of shedding was reported for two studies. A third study was performed that was appropriately designed and performed under GLP. The intranasal application of MSV instead of intramuscular is considered acceptable since it appears to be the only method to get excretion of virus and thus the possibility of passage. Albeit a lower than 10-fold maximum titre was applied, the first and subsequent passages were successful. No evidence of reversion to virulence was found in this study.

No specific studies have been conducted to determine the intrinsic biological properties of the vaccine strains; this is considered acceptable based on the data provided.

Regarding the genomic reassortment or recombination/redistribution of the strains with other strains of BoHV-1 virus, no specific trials have been performed. The chances of recombination occurring are considered very small. Any potential recombination is not expected to increase the virulence to more than the virulence of circulating wild-type strains.

A user risk assessment performed according to the revised guideline on User Safety for Immunological Veterinary Medicinal Products (EMEA/CVMP/IWP/54533/2006) was performed. A potential risk to the user was identified, posed by the mineral oil contained in the adjuvant. An appropriate standard warning is therefore included in section 3.5 of the SPC, which is considered to sufficiently address the risk.

Regarding the MRLs, the active substance being a principle of biological origin intended to produce active immunity is not within the scope of Regulation (EC) No 470/2009.

The excipients, including adjuvants, listed in section 2 of the SPC are either allowed substances for which Table 1 of the Annex to Commission Regulation (EU) No 37/2010 indicates that no MRLs are required or are considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this product. The antimicrobial substances used in the manufacturing process are present at low residual levels in the finished product which is not considered to constitute a risk to the consumer. The withdrawal period is set at zero days.

No specific studies have been carried out to investigate the possible interactions of Divence IBR Marker Live vaccine with other veterinary medicinal products. An appropriate warning has been included in the SPC.

Two multi-centre, randomised, double blinded and placebo-controlled clinical trials investigating safety and efficacy have been performed. In the first trial 1017 calves were included, in the second trial 1255 heifers and cows. Both studies were appropriately designed and performed to GCP standards. Follow up of the calves revealed no significant safety issues but for the occurrence of two anaphylactic type reactions. A warning is included in section 3.6 of the SPC as follows: *"In cases of anaphylactic-type reactions, an appropriate symptomatic treatment should be administered."* Local reactions and rectal temperature increases were comparable to what was found in the pre-clinical studies. From the results of the study in heifers and cows, it can be

concluded that the vaccine is generally safe in adult cattle since it did not give rise to general clinical signs and no clinically relevant increases in rectal temperature were observed. Local reactions were similar to what was observed in pre-clinical studies. There was no apparent effect on milk yield after vaccination and reproductive safety is supported by the results of the study.

An Environmental risk assessment was performed, in accordance with Commission Delegated Regulation (EU) 2021/805 of 8 March 2021 amending Annex II to Regulation (EU) 2019/6 of the European Parliament and of the Council and the guideline for Environmental risk assessment for immunological veterinary medicinal products (EMEA/CVMP/074/95). In conclusion, Divence IBR Marker live is expected to pose a negligible risk to the environment when used as recommended.

The antigen live gE- tk- double-gene deleted Bovine Herpes Virus-1, strain CEDDEL is considered a genetically modified organism (GMO). Any risk emerging from the use of the attenuated vaccine virus is expected to be negligible for humans and for the environment.

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

General requirements

The vaccine is intended for the active immunisation of cattle from 10 weeks of age to reduce virus shedding, hyperthermia and clinical signs caused by IBRV.

The recommended vaccination programme includes a basic vaccination scheme, which consists of the administration of two intramuscular injections (2 ml each). The first dose may be administered to calves from 10 weeks of age, and the second dose three weeks later. Re-vaccination is recommended at an interval not longer than 6 months after completion of the basic vaccination scheme by the administration of a single intramuscular dose. Afterwards, subsequent re-vaccinations are recommended at an interval not longer than 12 months.

The efficacy of Divence IBR Marker Live has been demonstrated by the results of the laboratory tests and the field studies performed with Divence Penta and provided in this part of the dossier. Studies were carried out in accordance with the general principles and requirements of the Commission Delegated Regulation (EU) 2021/805, as well as with the current version of the general monograph 5.2.7 of the European Pharmacopoeia, "Evaluation of efficacy of veterinary vaccines and immunosera". In addition, the requirements described in the following current specific Ph. Eur. monographs have also been followed: no. 1177 "Bovine Respiratory Syncytial virus vaccine (live)", No 0696 "Infectious Bovine Rhinotracheitis vaccine (live)", no. 1952 "Bovine Viral Diarrhoea vaccine (inactivated)" and no. 1176 "Bovine Parainfluenza virus vaccine (live)".

Challenge model

The challenge model used for BoHV-1 was based on published data and the suitability was previously confirmed by the results obtained in the control group of the efficacy trials conducted for the Hiprabovis IBR Marker Live vaccine.

Efficacy parameters and tests

Parameters assessed in the pre-clinical studies include virus shedding (titre and duration), general and respiratory clinical signs, rectal temperature and serological response. The parameters used are

in accordance with the specific Ph. Eur. monograph (0696) and the proposed claims. Methods to determine antibody and virus titres were appropriately validated.

Efficacy documentation

Seven studies were conducted to investigate the efficacy of the product and included 5 pre-clinical studies and 2 clinical trials. Laboratory studies were well documented and carried out in target animals of around the minimum age recommended for vaccination, using production and pilot batches containing a minimum dose. Commercial batches were used in the clinical trials.

Study title
Immunological response induced in young calves after administration of Divence Penta vaccine and other fall-out vaccines containing a smaller combination of antigens.
Efficacy of Divence Penta vaccine Infectious Bovine Rhinotracheitis (IBR) disease in young calves
Influence of maternally derived antibodies (MDA) on Divence Penta vaccine's efficacy against Infectious Bovine Rhinotracheitis (IBR) disease in young calves
Study on the duration of immunity (DOI) of Divence Penta vaccine against Infectious Bovine Rhinotracheitis (IBR) disease in calves
Study on the duration of immunity (DOI) of the booster administration of Divence Penta vaccine against Infectious Bovine Rhinotracheitis (IBR) disease in young calves
Efficacy and safety assessment under field conditions of Divence Penta vaccine in calves
Efficacy and safety assessment under field conditions of Divence Penta vaccine in cattle

Pre-clinical studies

Studies in support of efficacy of Divence IBR Marker Live were performed using the larger combination product Divence Penta. The two vaccines are identical but for the presence of BRSV, PI3 and BVDV 1 and 2 antigens in Divence Penta.

A study has been performed to assess absence of interference between the active substances by evaluating the immunological (serological) response in calves as induced by Divence Penta and fall-out vaccines. The results indicate no relevant differences in antibody responses in calves vaccinated with the larger combination or the fall-out vaccines. With respect to efficacy against BoHV-1, vaccination with Divence Penta may indeed be considered a worst-case scenario. It can be concluded that the data generated with Divence Penta can be used in support of the efficacy of the fall-out Divence IBR Marker Live.

Dose determination

No specific dose determination studies were performed.

Onset of immunity

Onset of immunity to BoHV-1 was studied in seronegative calves of 12-14 weeks of age. The study was randomised, controlled and blinded. Vaccination was performed with two doses of vaccine, three weeks apart, containing a minimum titre of BoHV-1. The control group received PBS. After challenge at three weeks post vaccination, a significant reduction of virus shedding was observed compared to controls and duration of shedding was significantly shorter in vaccinates, both parameters being in compliance with the Ph. Eur. 0696 requirement. Clinical signs and increases in rectal temperature were clearly and significantly decreased in vaccinates compared to controls. The study was performed to an acceptable standard and can be considered valid in accordance with Ph. Eur. 0696. The results support the claimed onset of immunity at 3

weeks after completion of the basic vaccination scheme.

Duration of immunity

A randomised, blinded, controlled DOI study for protection against BoHV-1 was performed. MDA- calves of 10-13 weeks of age were vaccinated twice with a 3-week interval with Divence Penta batch containing a minimum titre of BoHV-1. Controls received PBS. Challenge was given at 6 months post vaccination and calves were monitored for 3 weeks. The mean titre and duration of virus excretion were significantly higher in the control group compared to the vaccinates. Average rectal temperatures were significantly higher in controls. After challenge, clinical scores increased notably in the control group but only minimally in the vaccinates. Overall, the difference in scores was significant. The study was appropriately designed, in accordance with Ph. Eur. 0696. The study was valid. The vaccine complied with the test for immunogenicity and the results are supportive of a duration of immunity of 6 months after completion of the basic vaccination scheme against BoHV-1.

A randomised, blinded, controlled study to assess the DOI against BoHV-1 after re-vaccination was performed. MDA- calves of 12-13 weeks of age were vaccinated twice with a 3-week interval with Divence Penta batch containing a minimum titre of BoHV-1 and were re-vaccinated six months later. Controls received PBS. Challenge was given at 1 year after the re-vaccination and calves were monitored for 3 weeks. The mean titre and duration of virus excretion were significantly higher in the control group compared to the vaccinates. Average rectal temperatures were significantly higher in controls. Clinical scores were significantly higher in the control group; only mild clinical scores were observed in the vaccinates. The study was appropriately designed and valid, in accordance with Ph. Eur. 0696. The vaccine complied with the test for immunogenicity and the results are supportive of a duration of immunity against BoHV-1 of 1 year after the first re-vaccination.

Maternally derived antibodies (MDA)

For BoHV-1 a randomised, blinded, controlled study in MDA+ and MDA- calves of the youngest age for vaccination was performed. MDA+ and MDA- calves were vaccinated with Divence Penta batch containing the minimum titre of BoHV-1. The remaining MDA+ control animals were treated with PBS. When MDA levels had dropped to undetectable (45 days post vaccination), all calves were challenged intranasally and monitored for 3 weeks. Virus shedding (titre and duration) was significantly higher in controls compared to vaccinates. Rectal temperatures increased in the controls but not the vaccinates; the difference was not significant. The average clinical score was significantly lower in both vaccinated groups compared to controls. The study was appropriately designed, in accordance with the requirements of Ph. Eur. 0696, and can be considered valid. The vaccine met the requirements of the monograph, both for MDA+ and MDA- animals. It can be concluded there was no effect of MDA on protection against IBR in calves at the youngest age for vaccination.

Interactions

No specific studies have been carried out to investigate possible interactions of Divence IBR Marker Live or Divence Penta vaccines with other veterinary medicinal products. For this reason, the following recommendation is included in the relevant section of the summary of product characteristics and package leaflet: "No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. A decision to use this vaccine before

or after any other veterinary medicinal product therefore needs to be made on a case by case basis." This warning sentence is acceptable.

It is claimed that Divence IBR Marker Live can be used in subsequent re-vaccinations in animals previously vaccinated with Divence Penta vaccine. Whereas no specific (challenge) data to this extent has been provided, it can be accepted that the results of a study investigating serological responses to Divence Penta and the fall-out vaccines support the absence of significant interaction between the vaccine components and as such it can be expected that re-vaccination with a fall-out vaccine will induce the same immunological response (to the relevant antigen) as re-vaccination with Divence Penta.

Clinical trials

Two field trials were performed in order to assess safety and efficacy under field conditions of use. One study was performed in calves on farms in Spain whereas another study was performed in heifers and cows on farms in Spain and Hungary.

The general outline of the studies has been summarised in section 3.C.

Briefly, the first study included 506 calves vaccinated with Divence Penta and 511 control calves. The following efficacy parameters were applied:

Primary: incidence of new cases of respiratory disease (RD) during an outbreak.
Secondary: overall incidence of new cases of RD, number of concomitant treatments due to RD, severity of respiratory clinical signs, mortality due to RD, serological response, lung lesions at slaughter, productive performance.
Results: three outbreaks of RD were reported, at 23, 26 and 36 days after the start of vaccination respectively. The causative agent could only be diagnosed in one outbreak; this concerned BRSV as a single (detected) pathogen.

The mean number of concomitant treatments was comparable between the groups.

The mean severity of respiratory clinical signs as recorded during the whole study follow-up was 0.80 in controls and 0.61 in vaccinates; this difference was statistically significant (p=0.004). The mean severity of RD during an outbreak could only be calculated from the outbreak and was 2.11 in controls and 1.70 in vaccinates (p=0.055). Mortality due to RD was similar in control (2.4%) and vaccinated (2.8%) groups.

Vaccination induced antibody responses that were significantly greater compared to control animals against all antigens included in the vaccine. Lung lesions scores were evaluated at slaughter in animals. No statistically significant differences were observed and scores were generally absent to low. With respect to production parameters, there were no differences between the groups.

The trials were appropriately designed and performed to an acceptable standard (GCP). The results of the study in calves provide no additional support with regard to protection against BoHV-1. However, this can be accepted in principle since an effect was shown in accordance with the specific claims and no productivity claims are made.

The other study was performed in heifers and cows. The study was a multicentre, randomised, controlled and blinded trial performed in 4 dairy farms, a total of 1,255 animals were included from the age of 10 weeks onwards. The vaccinated group received four doses of the vaccine (primary vaccination D0 and D21, re-vaccination 6 months and booster vaccination one year later), whereas the controls received a placebo. The follow-up period was 24 months. For the overall efficacy

population, pregnancy loss (including early embryonic death, abortion and stillbirth) was highly similar for the control and vaccinated groups (resp. 13.8% and 13.1%). When the data were analysed separately for cows and heifers, the same result (highly similar for control and vaccinated groups) was obtained.

Overall conclusion on efficacy

The challenge model developed to test the efficacy of the vaccine against BoHV-1 was appropriately validated. The parameters chosen can be considered appropriate and tests used were validated and fit for purpose. Seven studies were conducted to investigate the efficacy of the product and included 5 pre-clinical studies and 2 clinical trials. Laboratory studies were well documented and carried out in target animals. Where these studies concerned calves, it is noted that animals were often older than the minimum age recommended for vaccination; this has been appropriately justified. Production and pilot batches of the larger combination product Divence Penta, at minimum potency for BoHV-1, were used in the pre-clinical studies. Commercial batches, containing a standard potency BoHV-1, were used in the clinical trials.

Adequate evidence of onset of immunity at 3 weeks post vaccination was obtained in a study performed in accordance with the specific Ph. Eur. monograph 0696. The study was valid and the vaccine was shown to meet the requirements.

Duration of immunity was studied in two separate challenge studies in calves. Studies were performed in accordance with the specific Ph. Eur. monograph, the studies were valid and the vaccine was shown to meet the requirements. Protection was shown to last 6 months after the primary vaccination schedule. Re-vaccination at 6 months after the primary vaccination was shown to provide protection lasting for 1 year.

The influence of MDA on the onset of protection was studied. The level of MDA compared to the level generally observed in the field and in calves born to vaccinated dams has been justified. No differences in protection level against BoHV-1 between MDA+ and MDA- calves were observed. It can be concluded there was no effect of MDA on the protection against IBR in calves at the youngest age for vaccination.

No specific studies have been carried out to investigate the possible interactions of Divence IBR Marker Live or Divence Penta vaccines with other veterinary medicinal products. A warning sentence to this effect has been included in the SPC. The SPC states that Divence IBR Marker Live can be used in subsequent re-vaccinations in animals previously vaccinated with Divence Penta vaccine. Whereas no specific (challenge) data to this effect have been provided, it can be accepted that the results of a study investigating serological responses to Divence Penta and the fall-out vaccines support the absence of significant interaction between the vaccine components and as such it can be expected that re-vaccination with a fall-out vaccine will induce the same immunological response (to the relevant antigen) as re-vaccination with Divence Penta.

Two multicentre field trials were performed in order to assess safety and efficacy of Divence Penta under field conditions of use. A study in calves included 506 vaccinated and 511 control animals. The trial was appropriately designed and performed to an acceptable standard (GCP). The results of the efficacy analysis provide no additional support with regard to protection against BoHV-1. However, this can be accepted in principle since for the active substance in the vaccine an effect was shown in pre-clinical studies, fully in accordance with the claims, and no productivity claims are made.
Data from the clinical study in heifers and cows support the safety and do not contradict the results of efficacy studies but provide no further evidence of efficacy.

Part 5 – Benefit-risk assessment

Introduction

Divence IBR Marker Live is a lyophilisate and solvent for emulsion for injection containing $10^{6.3}$ - $10^{7.6}$ $CCID_{50}$/dose live recombinant Infectious bovine rhinotracheitis virus, strain CEDDEL, *gE- tk-* double-gene deleted.

The product is intended for use in cattle and the applicant initially applied for the active immunisation of cattle from 10 weeks of age to reduce virus shedding, hyperthermia and clinical signs caused by BoHV-1.

Vaccinated animals can be differentiated from field virus infected animals due to the marker deletion (gE-) by means of commercial diagnostic kits.

Onset of immunity: 3 weeks after completion of the basic vaccination scheme.

Duration of immunity: 6 months after completion of the basic vaccination scheme. 1 year after re-vaccination scheme.

The vaccination scheme initially proposed by the applicant consisted of two intramuscular injections (2 ml each), being the first dose administered to calves from 10 weeks of age, and the second dose three weeks later. Re-vaccination is recommended 6 months after completion of the basic vaccination scheme by the administration of a single intramuscular dose. Afterwards, subsequent re-vaccinations are recommended at an interval not longer than 12 months.

The proposed withdrawal period is zero days.

The effective dose of $10^{6.3-7.6}$ $CCID_{50}$ BoHV-1 strain CEDDEL, applied twice via intramuscular injections with a three week interval, has been confirmed.

The application has been submitted in accordance with Article 8 of Regulation (EU) 2019/6 (full application)

Benefit assessment

Direct benefit

The benefit of Divence IBR Marker Live is its efficacy in reduction of virus shedding, hyperthermia and clinical signs due to BoHV-1, which was established in a number of well-designed pre-clinical studies conducted to an acceptable standard.

The onset of immunity is 3 weeks. The duration of protection is 6 months after completion of the basic vaccination scheme and 1 year after re-vaccination.

Efficacy was shown not to be affected by the presence of MDA.

The indication accepted by the CVMP is as follows:

Active immunisation of cattle from 10 weeks of age to reduce virus shedding, hyperthermia and clinical signs of IBR (infectious bovine rhinotracheitis).

Onset of immunity: 3 weeks after completion of the basic vaccination scheme.

Duration of immunity: 6 months after completion of the basic vaccination scheme. 1 year after completion of the re-vaccination scheme.

Additional benefits

The product is a differentiating infected from vaccinated animals (DIVA) vaccine; the *gE* deletion permits the use of this strain as marker vaccine in IBR eradication programmes.

Risk assessment

Quality

Information on development, manufacture and control of the active substance and finished product has generally been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use. The type of containers and the method of administration are commonly used in veterinary vaccines.

Safety

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

Risks for the target animal

Administration of Divence IBR Marker Live in accordance with SPC recommendations is generally well tolerated. The main reported adverse reactions include local swelling and increases in rectal temperature.

The safety of Divence IBR Marker Live in pregnant cattle was confirmed in a pre-clinical study. Adverse reactions were local swelling and increases in rectal temperature. The vaccine was found to be safe for use in lactating animals.

Risk for the user

The product contains mineral oil; this is known to cause severe pain and swelling particularly if injected into a joint or finger. The applicant has included in section 3.5 of the SPC a standard warning sentence concerning mineral oil. No further hazards were identified and the overall risk to the user is considered to be negligible.

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

Risk for the environment

Divence IBR Marker Live is not expected to pose a risk for the environment when used according to the SPC recommendations. Standard advice on waste disposal is included in the SPC.

Risk for the consumer:

Divence IBR Marker Live is not expected to pose a risk for the consumer when used according to the SPC recommendations.

Risk management or mitigation measures

Appropriate information has been included in the SPC to inform on the potential risks of this product relevant to the target animal. A user risk warning relating to mineral oil is included.

The veterinary medicinal product is subject to a veterinary prescription.

Evaluation of the benefit-risk balance

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

The product has been shown to be efficacious for the active immunisation of cattle from 10 weeks of age to reduce virus shedding, hyperthermia and clinical signs of IBR (infectious bovine rhinotracheitis).

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

The product information has been reviewed and is considered to be satisfactory and in line with the assessment.

Conclusion

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

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