EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Veterinary Medicines and Product Data Management

# Scientific discussion

This module reflects the initial scientific discussion for the approval of Hiprabovis IBR Marker Live (as published in January 2011). For information on changes after this date please refer to module 8.

## 1. Summary of the dossier

Hiprabovis IBR Marker Live is a lyophilisate and solvent for suspension for injection, intended for the active immunisation of cattle from 3 months of age against Bovine Herpes Virus type 1 (BoHV-1) to reduce the clinical signs of Infectious Bovine Rhinotracheitis (IBR) and field virus excretion.

The active substance of Hiprabovis IBR Marker Live is a live double-gene deleted (deleted glycoprotein E (gE-) and deleted thymidine kinase (tk-)) Bovine Herpes Virus type 1, strain.

Hiprabovis IBR Marker Live was eligible for the submission of a dossier for granting of a Community marketing authorisation via the centralised procedure under Article 3.1 of Regulation (EC) No. 726/2004 as the antigen is obtained through recombinant DNA technology. The company Laboratorios Hipra S.A. submitted an application to the EMEA on 3 March 2009 for the granting of a Community marketing authorisation. The Rapporteur and Co-Rapporteur's assessment reports were circulated to all CVMP Members on respectively 28 May 2009 and 12 June 2009. The consolidated list of questions was adopted on 15 July 2009 an the CVMP Opinion was adopted on 13 October 2010.

The benefits of Hiprabovis IBR Marker Live is the stimulation of active immunity against bovine herpesvirus type 1 in cattle from 3 months of age reducing clinical signs of IBR and virus shedding. The onset of immunity is 21 days after completion of the basic vaccination scheme and the duration of immunity is 6 months after completion of the basic vaccination scheme.

The most common side effects are a slight increase in body temperature up to 1° C within 4 days following vaccination. Occasionally, an increase in rectal temperature up to 1.63º C in adult cows and up to 2.18º C in calves may be observed. This transient rise in temperature is spontaneously resolved within 48 hours without treatment and it is not related to a febrile process. A transient inflammation at the inoculation site is common in cattle within 72 hours post-vaccination. This slight swelling lasts for less than 24 hours in most cases.

Infectious Bovine Rhinotracheitis is a respiratory disease affecting cattle of major economic importance. Eradication programmes are in place in a number of countries, but this is hampered by the

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latency of the virus. This vaccine is a marker vaccine and can enable a differential testing of vaccinated and naturally infected cattle which is a useful tool in eradication campaigns.

A detailed description of the pharmacovigilance system was provided and was considered satisfactory and in compliance with relevant requirements. Appropriate certification was also provided for the manufacturing site; an inspection was not considered necessary.

# 2. Quality assessment

The applicant had applied to the CVMP Scientific Advice Working Party (SAWP) in January 2007 requesting advice on questions covering mainly quality issues, including testing of seedlots, process validation, finished product testing and safety testing.

# Composition

The composition for a dose of 2 ml is provided in the following table.

| | NAME | COMPOSITION PER DOSE | FUNCTION | REFERENCE STANDARDS |
|---|---|---|---|---|
| **ACTIVE INGREDIENTS** | Live $gE^-$ $tk^-$ double-gene deleted IBRV, strain CEDDEL | $10^{6.3}$ - $10^{7.3}$ $TCID_{50}$ | Induces a specific and active immune response against Infectious Bovine Rhinotracheitis (IBR) | Ph. Eur., monograph no. 0696 |
| **COMPONENTS OF THE EXCIPIENT (freeze dried)** | **COMPOSITION PER VIAL (10 ml)** | | | |
| | Povidone | | Cryoprotector | Ph. Eur., monograph no. 0685 |
| | Gelatin | | Cryoprotector | Ph. Eur., monograph no. 0330 |
| | Disodium phosphate dodecahydrate | | Buffer | Ph. Eur., monograph no. 0118 |
| | Potassium dihydrogen phosphate | | Buffer | Ph. Eur., monograph no. 0920 |
| | Sucrose | | Cryoprotector | Ph. Eur., monograph no. 0204 |
| | Sodium chloride | | Osmotic agent | Ph. Eur., monograph no. 0193 |
| | Potassium chloride | | Osmotic agent | Ph. Eur., monograph no. 0185 |
| | Monosodium glutamate | | Protein stabiliser | USP 30 NF 25 |
| | Water for injections | | Dilution medium | Ph. Eur., monograph no. 0169 |

| | | | | |
|---|---|---|---|---|
| **COMPONENTS OF THE SOLVENT** | **COMPOSITION PER DOSE (2 ml)** | | | |
| | Disodium phosphate dodecahydrate | | Buffer | Ph. Eur., monograph no. 0118 |
| | Potassium dihydrogen phosphate | | Buffer | Ph. Eur., monograph no. 0920 |
| | Sodium chloride | | Osmotic agent | Ph. Eur., monograph no. 0193 |

| | | | | |
|---|---|---|---|---|
| | Potassium chloride | | Osmotic agent | Ph. Eur., monograph no. 0185 |
| | Water for injections | | Dilution medium | Ph. Eur., monograph no. 0169 |

### *Container*

The immediate container is a Type I colourless glass bottle with rubber closure and aluminium cap.

The method of preparation, sterilisation and closure were described.

Freeze-dried fraction:

Bottles are colourless neutral glass, Type I (10 ml) in accordance with Ph. Eur. 3.2.1.

Solvent:

Bottles are colourless neutral glass, Type I for the 10-ml (5 doses) and Type II for the 50-ml (25 doses) in accordance with Ph. Eur. 3.2.1.

The containers and closures comply with the relevant Ph. Eur. requirements. The sterilisation specifications were in excess of Ph.Eur. requirements and were acceptable.

### *Development Pharmaceutics*

The vaccine was developed according to the model of a live freeze-dried vaccine for resuspension in solvent to be administered by the intramuscular route. The applicant selected this pharmaceutical form due to the generally high and long-lasting immunity conferred by live vaccines. In addition, freeze-dried is the most common presentation for live vaccines due to the generally high stability of the live virus throughout a long period of time.

The choice of the method of attenuation was based on the need to accurately establish attenuation and to enable differentiation between vaccinated and naturally infected animals. Attenuation was by the construction of a double deletion corresponding to the genes of the glycoprotein E (gE) and the enzyme thymidine kinase (tk). Both deletions are known to reduce virulence in the vaccine strain. Moreover, the gE deletion permits the use of this strain as marker vaccine in IBR eradication programmes.

### *Components of Hiprabovis IBR Marker Live*

## Active substances

**IBRV gE$^-$ tk$^-$, strain** CEDDEL
The IBRV strain CEDDEL was obtained from a virulent strain isolated from an outbreak of IBR. Two deletions were constructed on this field strain in the genes coding for the glycoprotein E (gE) and the enzyme thymidine kinase (tk). Deletions in both genes are known to reduce virulence of IBRV. Moreover, the gE deletion permits the use of this strain as marker vaccine in IBR eradication programmes.

The Geogia Bovine Kideny (GBK) cells were selected for virus propagation. The freeze-dried presentation was chosen because live microorganisms keep their properties stable for long periods of time when they are stored freeze-dried. The solvent enclosed for the reconstitution of the freeze-dried virus is composed of a phosphate buffer solution. No other specific functions are attributed to the solvent fraction except for acting as a vehicle for the antigen administration and protect it from pH oscillations. No adjuvants or other active substances are included in the composition of the solvent.

## Excipients and other non-active ingredients

The freeze-drying excipient is composed of buffers, osmotic agents, stabilisers and cryoprotective agents which the applicant considered as known for maintaining the stability of IBRV. The applicant justified the presence of each excipient.

## Solvent

The solvent is sterile phosphate buffered solution (PBS) which lacks any immunostimulant effect. All of the ingredients used for the manufacture of the solvent are also included in the composition of the freeze-drying excipient described above, which allows the resuspension of the freeze-dried vaccine without modifying the virus properties.

## Conclusions

The development pharmaceutics were adequately described and the choice of each aspect of the product reasonably well explained and justified, therefore this section was considered satisfactory.

### *Composition of the batches used in the clinical trials*

The batches used in the clinical trials were pilot batches and their Manufacturer's Batch Protocols were provided.

### *Method of manufacture*

The production process is based on the seed-lot-system described in the Ph. Eur. general monograph 0062, which is considered the most appropriate for the manufacture of the vaccine. The manufacture and filling of the finished vaccine are carried out according to Good Manufacture Practices (GMP), thereby establishing a process that is reproducible and appropriate for the manufacturing of the vaccine. Flow charts detailing the steps taken during the preparation of the finished product were submitted. Details of the production steps involved in the growth of the IBRV vaccine strain, as well as the production of the freeze-drying solution were provided.

### *Description of the method of preparation*

**Production of the IBRV strain CEDDEL**
The standard batch size of the viral harvest is a range between 175 litres and 360 litres. The production of virus is done through a series of scale up steps including a pre-inoculum inoculum and viral harvest stage. Culture is in GBK cells using Glasgow Minimum Essential Media (MEM) at each stage.

Data were provided to confirm that the virus is stable during frozen storage, therefore no adverse effect on the finished product is anticipated. In addition, there are adequate in-process and finished product tests in place to ensure that the stability of the finished product will be ensured in-line with the stability data provided. The applicant has also confirmed that the maximum number of passages is Master Seed Virus (MSV)+5. This was considered satisfactory.

**Production of GBK cells**
The production of sufficient GBK cells for viral propagation is done by sequential passages.

**Preparation of Freeze-drying Excipient**
The composition (per litre) of the freeze-drying excipient was described.

**Preparation of Freeze-dried Fraction**
A standard batch is described as between 18,000 and 60,000 vials. Each vial contains a total of 4 ml, comprising 3 ml IBRV CEDDEL strain virus harvest and 1 ml freeze-drying excipient.

**Preparation of Solvent**
A standard batch is described as 600 litres and 1200 litres. The composition (per litre) of the solvent was described.

## *Validation studies*

The applicant provided an extensive list of test methods and a number of validation reports for the techniques used. The sensitivity, specificity and the robustness of the techniques described were investigated in each study and were considered acceptable.

## *Control of starting materials*

## *Listed in a Pharmacopoeia*

| Name |
|---|
| Glycerol |
| Gelatin |
| Monosodium glutamate |
| Potassium chloride |
| Potassium dihydrogen phosphate |
| Povidone |
| Sodium chloride |
| Sodium hydroxide |
| Sucrose |
| Water for injections |
| Ultrapure sterile water |
| Disodium phosphate dodecahydrate |
| Gentamicine sulphate |
| Sodium ampicillin |
| Nystatin |
| Sodium hydrogen carbonate |
| Trypsin |
| Disodium EDTA |
| Foetal Bovine Serum |
| Adult Bovine serum |

The Certificate of Analysis for the above materials were provided and were compliant with the relevant guidelines and Eur. Ph. monographs.

Foetal Bovine Serum (FBS)
The FBS is sourced from approved abattoirs in Australia or US. A Certificate of Analysis together with the company's specifications were provided and a EDQM Certificate of Suitability for the serum.

Adult bovine serum
The adult bovine blood used to produce this serum sources from clinically healthy cattle from Australia. The control tests are carried out on each batch of serum by the supplier and Hipra and were described. They were considered acceptable. A Certificate of Analysis was presented, together with company specifications and a EDQM Certificate of Suitability for the serum.

## *Not listed in a Pharmacopoeia*

**Starting materials of biological origin**

| Name |
|---|
| Live gE- tk- IBRV, strain CEDDEL |
| GBK cell line |
| Tryptose Phosphate Broth |
| Cytodex$^{TM}$3 surface microcarriers |

***Active substance*: Live marker gE- tk- Infectious Bovine Rhinotracheitis Virus (IBRV), strain CEDDEL**

The IBRV strain CEDDEL is a double-deleted strain obtained from the original strain, clinical isolate from an IBR outbreak in Spain in 1988. The constitution of the double-deleted IBRV strain was carried out by the Institute of Biotechnology and Biomedicine (IBB) of the University of Barcelona in Spain by recombinant DNA technology. Detailed methodology for the production of the strain and characteristics are detailed further on.

Master Seed Virus (MSV)

The Master Seed Virus was obtained from the Original Virus, (obtained from the original CEDDEL strain), by passages in GBK cells using the same culture medium used in the manufacturing method established for the vaccine and it was distributed in vials of different volumes, frozen and stored at -80°C.

The following testing were conducted on the MSV: titre, identity, bacterial/fungal sterility, detection of mycoplasmas, detection of cytophatic and heamadsorbent viruses, detection of pestiviruses, detection of rabies virus, detection of BRSV, absence of FMD/Bluetongue and Bovine Leucosis Viruses and detection of Mycobacterium tuberculosis and paratuberculosis.

Detailed methodology and validation reports for the above were provided.

Working Seed Virus (WSV)

WSV was obtained from the MSV, by passages in the GBK cells and distributed in vials of different volumes.

The following tests were performed on the WSV: Titre $TCID_{50}$/ml, Bacterial and fungal sterility

The tests conducted in the MSV complied with requirements of the relevant Ph. Eur. Monograph. The freedom from IBR wild type, can be deduced from the identity test conducted on the MSV, where the lack of gE and tk genes is shown.

The applicant provided information regarding the extraneous agents testing of the MSV, based on the requirements of the GRIMV guideline. Additional testing of the agents specified in the guideline as described above was conducted. Detailed methodology was provided for most of the methods used.

The CVMP considered it appropriate to request the applicant to develop a Brucella specific test, through, within a precise timetable (eg 6-9 months post authorisation).

**Genetic engineering**

**a) Source materials**

a.1.) Glycoprotein E gene

The gE is one of the envelope glycoproteins of Bovine Herpes Virus type-1 (BoHV-1) that have been related to a variety of functions involved in different aspects of the virus/host interaction. The gE deletion mutants are virtually avirulent in vivo and have been used as a live vaccine that clinically protected calves against challenge with a wild type strain.

The steps for the production of a gE-deleted virus strain were described adequately.

a.2.) Thymidine kinase gene

The tk was selected because is related both with viral neurothropism and latency and reactivation in infected animals and therefore, an additional deletion comprising this gene would improve the safety of the viral strain. The BoHV-1 tk gene was identified and mapped in 1987 and the precise location and sequence established in 1989-1990.

The preparation of the production strain was described in detail.

**c) Description of the production strain**

c.1.) Biological properties of the various elements found in the final construct

The BoHV-1 FM gE$^-$ tk$^-$ does not express an active form of glycoprotein E, nor thymidine kinase enzyme, as a consequence of a deletion in the coding region of the gE gene and tk gene.

c.2.) Demonstration that the construction is actually identical to that desired

The precise deletion of the gE and tk genes was investigated both by restriction endonuclease digestion of viral DNA and Southern blot analysis. In both cases, the results indicated that the strain is the product of the intended recombination between the BoHV-1 genome and the constructed plasmids. The absence of additional changes in the restriction patterns of the recombinant virus agree with the absence of undesired rearrangements in the genome of the BoHV-1 FM gE$^-$ tk$^-$ virus.

**e) Genetic stability**

DNA from the recombinant strain BoHV-1 FM gE$^-$ tk$^-$ obtained from the first cell passage after virus purification and obtained after 5 passages (maximum passage level used for production) from the MSV stock in GBK cells were digested with several restriction enzymes and analyzed in an agarose gel eletrophoresis. No changes in relation to the restriction patterns are found in the lanes corresponding to the digested DNA from the preparations with the initial virus and the full scale production.

The size of the deletions indicates that the restoration of the gene function is very unlikely and the genetic stability of the strain constructed is shown by restriction patterns obtained from the first virus obtained and 5 times passages virus, showing both identical patterns.

**Georgia Bovine Kidney (GBK) cell line**

This cell line is composed of kidney cells of bovine origin, it is not deposited in any collection, but its use in propagating different viruses of bovine origin is widely documented in the international bibliography. Successive passages were done to obtain an homogeneous cell line, including a cloning procedure, in the same culture medium used for current vaccine production.

Master Cells Seed (MCS)

The stock of MCS was obtained from the Original Cell stock. The following testing was conducted on the MCS: Microscopic observation, Bacterial and fungal sterility, Detection of mycoplasmas, Detection of mycoplasmas (Hoechst stain), Detection of mycoplasmas by means of PCR, Detection of adventitious virus, Viability, Chromosome analysis and identification of the cell line in comparison with passage 20. Detailed methodology for the above tests was provided. The MCS is stored in liquid nitrogen with freezing medium composed of FBS and dimethylsulfoxide. The stock of MSC is considered valid as long as the controls on the WCS comply with the specifications. Confirmation was provided that identification of the species of the MCS and cells at the highest passage was carried out by RT-PCR.

Working Cells Seed (WCS)

The stock of WCS was obtained from the MCS. The following testing has been conducted on the WCS: Microscopic observation, Bacterial and fungal sterility, Detection of mycoplasmas, Detection of mycoplasmas (Hoechst stain), Detection of mycoplasmas by means of PCR, Detection of adventitious virus, Viability. The methods used were the same provided for the MCS testing. The WCS is stored in liquid nitrogen with freezing medium composed of FBS and dimethylsulfoxide. The stock of WCS is considered valid as long as the following passage complies with the specifications.

The applicant has provided additional information regarding the extraneous agents testing of the MSC, based on the requirements in the GRIMV guideline. The CVMP considered appropriate to request the applicant to develop a Brucella specific test, through, within a precise timetable (eg 6-9 months pist authorisation).

***Excipients:***

**Tryptose Phosphate Broth**

TPB is used as an ingredient of the culture medium used in the various production phases of the antigen of the vaccine, acting as a source of phosphates. It contains materials of animal origin derived from porcine tissue and from bovine milk.

**Cytodex$^{TM}$3 surface microcarriers**

CytodexTM 3 surface microcarriers are support matrices allowing for the growth of the GBK cells in a fermentor. They provide convenient surfaces for the growth of animal cells and can be used in suspension culture systems.

The applicant provided a risk assessment which takes into consideration the type and source of materials used during the manufacture of the microcarriers. In addition, the sterilisation treatments involved at various stages during the production of the microcarriers is taken into account. Based on the risk assessment the conclusion is that the risk of extraneous agents being introduced through use of the microcarriers is effectively negligible.

**Starting materials of non-biological origin**

**Glasgow MEM (BHK-21)**

Glasgow MEM (BHK-21) is the principal ingredient of the culture media used to propagate the GBK cell line and for propagating the IBR virus. Documentation on its composition, as well as the corresponding certificate of analysis were submitted.

**In House preparation of media**

Thr composition and preparation procedures for the following were presented: Glasgow infection medium , Growth medium for cells , Trypsin solution

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

The following materials of animal origin are used for the preparation of the Hiprabovis IBR Marker Live vaccine:

| Material | Origin of material by species |
|---|---|
| IBR marker virus gE- tk-, CEDDEL strain | Bovine |
| GBK cell line | Bovine |
| Foetal bovine serum | Bovine |
| Adult bovine serum | Bovine |
| Tryptose Phosphate Broth | Bovine milk<br>Porcine pancreas<br>Porcine stomach |
| Gelatine | |
| Cytodex$^{TM}$3 surface microcarriers | Porcine skin<br>Bovine milk powder |

IBR marker virus gE$^{-}$ tk$^{-}$, CEDDEL strain

Origin of strain:

- Clinical isolate from a calf infected with IBR, from an outbreak in Spain in 1988. Original strain propagated in GBK and identified as IBRV by IF
- Construction of double-deleted strain, strain CEDDEL , by recombinant DNA technology

The first cases of BSE in Spain were diagnosed in 2000 and therefore the risk that the tissue of the IBRV was infected is minimal, the age of the animal and tissue from which the isolation was made is unknown but it is assumed that the tissue was part of the respiratory system. All tissues from the

respiratory system are classified as low infectivity tissue, the possibility of propagating any TSE infection by the processes used for deletion, cloning and isolation of the CEDDEL strain is practically null. The following materials of ruminant origin were used for the construction process: GBK cell line (risk assessment shown later on), FBS (EDQM certificate available, see risk assessment later on) and Tryptose phosphate broth (contains bovine milk from healthy animals deemed for human consumption). The following tests have been performed in the MSV to confirm the absence of prion proteins: Western Blotting technique and bioassay test (inoculation in sensitive laboratory animals) and were presented.

GBK cell line

Origin of strain:

- Supplied in 1990 by the Livestock Laboratory of Asturias (Spain) at an unknown passage
- Origin from bovine foetal kidney cells, unknown when the cell line was established (prior to 1975), probably from the region of Georgia in US

It was considered that the isolation of the cell line was performed when the risk of contamination with TSE was negligible (prior to 1980), the animal origin is a bovine foetus and therefore the age of the animal do not pose any risk of infectivity, bovine renal tissue is classified as low infectivity; embryonic tissues are categorised as undetected infectivity, the first cases of BSE in the USA were diagnosed in 2005 and therefore the risk of tissues infectivity is minimal, the following materials of ruminant origin were used to obtain the Original cells stock, MCS and WCS: FBS (EDQM certificate available, see risk assessment later on) and Tryptose phosphate broth (contains bovine milk from healthy animals deemed for human consumption). The following tests have been performed in the MSV to confirm the absence of prion proteins: Western Blotting technique and bioassay test (inoculation in sensitive laboratory animals). Results were presented.

The TSE was considered low for the following materials as they originated from countries of GBR III level or milk sourced:

Foetal bovine serum, Adult bovine serum, Tryptose phosphate broth, Cytodex$^{TM}$3 surface microcarriers

The applicant considered that the risk of spreading TSE among the manufacturing process is null, given that the ingredients of ruminant origin used in the manufacturing process of the pre-inoculum, inoculum and viral harvest are the IBR strain, GBK cells, FBS, ABS and Trytose phosphate broth, which have been previously assessed and considered to have a very low risk of being contaminated with TSE agents. The risk that infectivity the MCS could still be present in the final product and transmit infection is virtually zero, given that the original antigen and cells were obtained from animal tissues with low infectivity risk and the specific controls done.

Finally, the applicant provided a benefit/risk evaluation of using Hiprabovis IBR marker live vaccine in relation to the prevention of TSE, considering that the route of administration is intramuscular, the amount of vaccine received per animal would be proportionally very low and the huge dilution factor that the original materials undergo before obtaining the antigen MS stock and therefore the amount of seed material present in the final product is extremely low. The final conclusion is that the risk can be considered as minimal.

Overall, the applicant presented a thorough assessment of the risk of transmission of TSE of the different starting materials and manufacturing phases of the vaccine. The statement about the first cases of BSE in Spain being diagnosed in 2000 was confirmed. In addition, laboratory testing have been done on MSV and MCS to confirm that they are negative for the presence of prion proteins. Detailed reports have been provided.

On the basis of the above the starting materials of animal origin used in the production of the final product comply with the current regulatory texts related to the TSE Note for Guidance (EMEA/410/01-Rev.2) and Commission Directive 1999/104/EEC.

## Control tests during production

Production includes scale up process involving culture through three stages: Pre-inoculum, inoculum and viral harvest. At each stage the following in process control test conducted are the following:

- Bacterial and fungal sterility to verify the bacterial and fungal sterility
- Titre $TCID_{50}$/ml to check that the viral culture contains the $TCID_{50}$ / ml required

The above were considered satisfactory.

### *Control tests on the finished product*

The applicant had requested scientific advice from the CVMP SAWP-V concerning appropriate control test to be conducted on the finished product. The tests presented below were largely in line with the advice provided to the applicant by the CVMP SAWP-V.

### *General characteristics of the finished product*

- Appearance
- Solubility
- Volume control
- pH of the solvent

### *Identification and assay of active ingredients*

- Identity:
- Titre $TCID_{50}$/dose**: Timing/Frequency:** on each batch of vaccine (freeze-dried fraction reconstituted with its corresponding solvent). **Limits of acceptance:** Between $10^{6.3}$ and $10^{7.3}$ $TCID_{50}$ / dose.

### *Safety tests*

**Safety: Timing/Frequency:** On each batch of vaccine (freeze-dried fraction reconstituted with its corresponding solvent). **Description:** Administration of 10 vaccine doses to 2-3 month old calves. Observation for 21 days **Limits of acceptance:** No notable local or general reactions are observed for 21 days post-vaccination.

Tests parameters comply with the requirements of the specific monograph 01/2008:0696.

### *Residual humidity*

Residual moistness: The applicant established a finished product residual humidity specification of between 1-3% based on data. This range was considered acceptable.

### *Sterility and purity tests*

- Sterility

- Absence of mycoplasmas
- Absence of extraneous viruses

## *Batch to batch consistency*

The results of the control tests carried out on 3 consecutive batches of IBRV, strain CEDDEL were provided and were satisfactory. The results showed that the specifications of the different tests were met with a good level of consistency.

## *Stability*

## *Stability of the finished product*

**Stability of the freeze dried component**

The applicant presented two studies to show the stability of the freeze dried component of the vaccine: a study on the real-time stability of the live vaccine and a study of the real-time stability of the live vaccine after one year frozen at -20 ± 5°C. The stability data covered three consecutive batches of all available presentations, as outlined in the relevant guidelines.

The real time study investigated stability up to 27 months. These results confirmed that the product remained within specification for the duration of the study. This minimum release titre takes into consideration the expected fall in titre and a safety margin and is intended to ensure that the revised minimum efficacious titre is present at the end of the proposed shelf-life (24 months). A final stability report confirmed that the vaccine titre does not decrease significantly during 27 months storage.

As a result of the above a shelf life of 24 months was acceptable.

**Stability of the solvent**

Data were provided from three batches of solvent filled in both 10ml and 50ml vials, tested at 0, 3, 6, 9, 12, 18 and 24 months using the proposed finished product tests (appearance, pH and sterility). These data confirmed that the solvent remains within specification for all tests during 24 months storage at +2 to +8 ◦C. Therefore the proposed shelf life of 24 months for solvent is acceptable.

## *Stability of the reconstituted product*

A study was provided to support the stability of the reconstituted product.
The vaccine titre showed an acceptable maximum decrease in titre at 6 hours post-reconstitution for one batch and an acceptable maximum decrease in titre at 4 hours post-reconstitution for another batch. The vaccine remained sterile after 6 hours post-reconstitution. The decrease in titre seen was within the variability of the test, therefore it was accepted that the minimum titre is maintained for 6 hours following reconstitution.

## *Data related to the environmental risk assessment for products containing or consisting of genetically modified organisms (GMO)*

The applicant had requested scientific advice from the CVMP SAWP-V concerning requirements to demonstrate safety for humans, other non-target species and the environment from release of the GMO. The applicant was advised of the requirements and principles for an Environmental Risk Assessment under Directive 2001/18/EC. The applicant has followed this advice when compiling the dossier for Hiprabovis IBR Market Live.

## 1. Introduction

Hiprabovis IBR Marker Live is a live marker vaccine intended for use in cattle to reduce the respiratory distress caused by the Infectious Bovine Rhinotracheitis Virus (IBRV) and contribute to the eradication programmes for this disease. The vaccine contains a live attenuated double-deleted strain of IBRV (Strain CEDDEL) that has been constructed using genetic modification techniques to delete regions of the virus genome that code for glycoprotein E (gE) and for the enzyme thymidine kinase (tk).

Bibliographic data were provided.

## 2. Copy of the written consent for the deliberate release of the GMO as required by Part B of Directive 2001/18/EC

A copy of the written consent to the deliberate release into the environment of the GMO for research and development purposes according to Part B of Directive 2001/18/EC, issued by the competent authorities in Spain (dated 07/12/04) , together with the corresponding translation into English were provided.

## 3. Technical information required by Annex IIA of Directive 2001/18/EC, including the results of investigations performed for the purposes of research and development.

Information concerning the notifier (Hipra) and the responsible scientists was submitted.

**Information relating to the GMO**

### A) Characteristics of the recipient or (when appropriate) parental organism.

The organism to which this application refers is an IBRV in whose genome a double deletion has been constructed in the regions coding the (gE) and (tk) enzyme. This section describes the characteristics of the initial working strain (BoHV-1), which was the recipient of both deletions in its genomic material.

#### 1. Scientific name

The name of the recipient organism of the double deletion is IBRV.

#### 2. Taxonomy

IBRV belongs to the *Herpesviridae* family, *Alphaherpesvirinae* subfamily, Varicellovirus genus, bovine Herpesvirus group, serotype 1 (BoHV-1). This group and serotype also includes Infectious Pustular Vulvovaginitis Virus (IPV) and Infectious Balanoposthitis Virus (Straub, 1990).

#### 3. Other names (usual name, strain, name, etc.)

The isolate was obtained by Laboratorios Hipra, S.A. from an outbreak of IBR in Spain in 1988. This isolate was identified as IBRV using immunoflourescence and referenced as BoHV-1

#### 4. Phenotypic and genotypic markers

The inclusion of a virus in the Herpesviridae family is based on the structure of the virion, which is composed of:

a) a nucleus which contains a linear dsDNA molecule.

b) an icosahedral capsid of 100 nm in diameter.

c) a complex of material of proteineic nature, amorphous and often asymmetrical, which coats the capsid and is called *tegument.*

d) a membrane called *envelope* in which viral glycoproteins are embedded and from which they project towards the outside.

Average virion size, estimated from negative staining, varies between 120 and 300 nm. In addition to these structural characteristics, the Herpes viruses present four specific properties:

1. All Herpesviruses possess enzymes and other factors related to the synthesis of nucleic acids (e.g. polymerase, helicase, primase, origin of replication binding proteins, etc.) as well as a variable number of enzymes related to the metabolism of DNA (e.g. thymidine kinase, thymidilate synthase, dUTPase, ribonucleotide reductase, etc.). The Herpesviruses specify at least one protease and a variable number of protein kinases.

2. The synthesis of the viral DNA and the assembly of the capsids occur in the cell nucleus. The acquisition of an envelope during the transit of the capsids through the nuclear membrane is also characteristic of the Herpesviruses.

3. The release of the viral progeny is invariably accompanied by the destruction of the infected cell.

4. All the Herpesviruses examined to date are capable of remaining latent in their natural host. In the carrier cells of the latent virus, the viral DNA acquires the closed circular DNA form and only expresses a small group of viral genes. These are the BoHV-2 (or Bovine Mammillitis Herpesvirus), BoHV-4 (or Movar Herpesvirus) and BoHV-5 (or Bovine Encephalitis Herpesvirus). Since 1981, the Herpesvirus Study Group has proposed the subdivision of members of the *Herpesviridae* family into three subfamilies: *alphaherpesviridae*, *betaherpesviridae* and *gammaherpesviridae*, depending on the biological properties of each virus. The biological characteristics of BoHV-1 have led it to be classified in the *Alphaherpesviridae* subfamily (Roizman et al. 1992). The members of the *Alphaherpesviridae* subfamily present the following characteristics:

1. Broad host specificity.
2. Relatively short viral cycle.
3. Rapid spread in cell culture.
4. Effective destruction of the infected cells.
5. Capacity to establish latency in sensorial ganglions.

Whilst BoHV-1 rarely infects other animals other than its usual host there have been isolated cases of infections in goats, pigs and some wild animals (Mohanty et al. 1972; Derbyshire and Caplan, 1976; Stauber et al. 1977). BoHV-1 is not pathogenic in humans.

## 5. The degree of relation between the donor and recipient or between parental organisms

Since the final resulting organism is obtained by the deletion of coding DNA sequences for glycoprotein E and the tk enzyme from the parental stain, there will be identity with the recipient organism in all other regions of its genome.

## 6. Description of identification and detection techniques

Details of the available range of techniques for the identification and detection of Infectious Bovine Rhinotracheitis Virus were listed and described.

## 7. Sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques

Serological techniques, which enable the detection of antibodies aimed specifically against the virus (SN, IF, IPMA or ELISA), and techniques which permit the specific detection of the same virus (PCR) were available.

## 8. Description of the geographical distribution and of the natural habitat of the organism, including information on symbionts and hosts

The natural habitat of the parental organism, IBRV, is cattle. Despite being prevalent in the five continents, IBR is considered a pathologic entity exclusive to bovines, since, although strains of Herpesvirus closely linked from the antigenic point of view to Bovine Herpesvirus serotype 1 (BoHV-1) have been isolated in other domestic ruminants (goats) and wild ones (deer, buffalo), they are different strains.

Ferrets are highly susceptible to the disease on the American continent, but not in Europe (Porter et al., 1975). Rabbits ((Lupton et al., 1980; Rock and Reed, 1982), new-born skunks and hamsters (Straub, 1978), are also susceptible under experimental conditions.

Whilst bovine Herpesviruses have been present in Europe for over a century, the real reservoir of the microorganism are wild African ruminants, which establish a more symbiotic association where the virus reaches a state of latency from which it can emerge and replicate following a situation of stress.

Infectious Bovine Rhinotracheitis virus is the cause of respiratory, genital and/or nervous infections principally in cattle. Natural infections caused by IBRV have been reported in goats (Mohanty et al., 1972), swine (Saxegaard and Onstand, 1967; Derbyshire and Caplan, 1976), buffalo (St. George and Philpott, 1972), gnu (Karstad et al., 1974), mink and ferrets (Porter et al., 1975). Experimental infections have been carried out in deer (Chow and Davis, 1964), pigs (Woods et al., 1968; Nelson et al., 1972) and ferrets (Smith, 1978). Serological studies indicate that infection by IBRV may occur in wild African ruminants.

## 9. Organisms with which transfer of genetic material is known to occur under natural conditions

The parental organism, IBRV, belongs to the family of Herpesviruses, which are DNA viruses where replication occurs in the cell nucleus.

Genomic recombination may occur spontaneously in nature and which may occur both between virulent field strains and between field strains and vaccine strains. Different studies carried out on Aujeszky's Disease virus, also classified as Alphaherpesvirus, (Henderson et al., 1990; Cowen et al., 1990) have demonstrated that genomic recombination is possible both *in vivo* and *in vitro*.

However, for this to occur, it would be essential for a cell to be infected simultaneously by two different strains, regardless as to whether these viruses are attenuated or virulent. In this case, recombination occurs in the early phase of the infective process, before the replication of DNA.

During vaccination it is not possible to know whether an animal is latently infected with a virulent virus. Methods to identify latent infection are not practical as they require sacrifice of the animal. Therefore, there will always be the risk of vaccinating an animal which is infected latently, regardless of the vaccine used.

Recombinants obtained by the recombination of vaccine strains with virulent strains do not appear to be more virulent than field strains nor than the parental strain which gave rise to the recombinant, since the latter always maintains the functional copies of all the known virulence genes (Henderson et

al., 1991). Therefore, the possibility that *in vivo* recombination will create more virulent strains is practically non-existent.

## 10. Verification of the genetic stability of the organisms and factors affecting it

The recipient organism, IBRV, is a DNA virus. In general, DNA viruses have greater genetic stability than RNA viruses, since DNA viruses have mechanisms for repairing errors which occur during the genome replication.

The FM strain of IBRV has a characteristic restriction pattern (Mayfield et al., 1983) which is a "fingerprint" of this virus subtype, which permits both its characterisation and the detection of possible alterations in the structure of its genome. This fact indicates that it is a virus with a very stable genetic structure. In the same way, throughout the different studies carried out with the FM strain of IBRV, alterations have never been detected in its restriction patterns other than those expected due to the two deletions carried out in its genome.

## 11. Pathological, ecological and physiological traits:

### a) Classification of hazard according to existing Community rules concerning the protection of human health and the environment

IBRV does not appear in the list in Annex II of Royal Decree 664/1997 of 12th May, concerning the protection of workers against risks associated with exposure to biological agents in the workplace. It is not included in this list since IBRV is not pathogenic in humans.

IBRV is included in the list of diseases which should be reported to the O.I.E., which lists the organisms considered to be of socio-economic importance and which are relevant to the international trade of animals and animal products.

### b) Generation time in natural ecosystems, reproductive cycle

Knowledge of the infective cycle of the Alphaherpesviridae comes largely from studies carried out on Herpes simplex virus (HSV-1), which is considered a prototype virus of this subfamily. A detailed description of the infection cycle within the cell was provided.

### c) Information on survival, including seasonality and the ability to form survival structures

IBRV is distributed all over the world. The presence of outbreaks of the disease depends on different factors such as the climate, situations of stress (transport), density of young susceptible animals, general health status, the presence of antibodies against IBR in the population, etc. A determining factor in the appearance of IBR outbreaks is the introduction of infected animals into farms.

The survival structures characteristic of viruses are the virus particles or virions. The virions of the herpesviruses are, in general, fairly stable and resistant to adverse conditions such as freezing temperatures. Studies carried out with IBR attenuated vaccine strains show that the viral preparations are very stable at temperatures below –65 ºC. Stability of the virus at –20 ºC is shorter (less than 1 year). The virus is inactivated slowly at +4 ºC and survives for around 10 days at +37 ºC (Straub, 1990).

IBRV is also able to survive in the environment as a persistent infection and the ability to remain as a latent infection in animals. The viral genome can remain latent in the nucleus of some infected cells (principally neurons of the trigeminal ganglion) and remain inactive indefinitely. However, in conditions of stress, the latent virus can be reactivated, giving rise to secretion of virus, and sometimes clinical signs.

**d) Pathogenicity: infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organisms. Possible activation of latent viruses (proviruses). Ability to colonise other organisms**

The applicant provided a detailed description of the development of a natural infection by a virulent IBRV. The respiratory symptoms were described and consequences of systemic infection are summarised. In particular, a systemic invasion of the animal by IBRV leads to the colonisation of different organs and tissues. The virus can be transported by peripheral leucocytes to the placenta and foetus; the foetus is highly susceptible to infection by IBRV and an acute infection is usually fatal. Infection during the last term of pregnancy can produce abortions, mummifications, still-born calves or very weak calves which present lesions typical of IBR together with lesions in the stomach and intestines.

Variation in the severity of the clinical symptoms observed following a natural infection by IBRV is believed to be due to differences in pathogenicity of the strain, the age of the infected animals and environmental factors. The clinical disease is slight in dairy cows and more serious in fattening calves. The severity of the clinical form is also greater on farms with overcrowding of animals and in those where animals from different origins are housed.

Dairy animals recover within 10-14 days. In fattening calves, the course of the disease is longer and the clinical symptoms are more severe, with some deaths occurring in the initial fever period and many others as a consequence of secondary bronchopneumonia.

The susceptibility of other animal species to IBRV infection is discussed and it is concluded that IBRV is species-specific and that in Europe at least there do not appear to be potential for colonising other organisms.

IBRV, like all the Herpesviruses, is able to establish latency in the neuronal cells of the trigeminal ganglion.

**e) Antibiotic resistance and potential use of these antibiotics in humans and domestic organisms for prophylaxis and therapy**

IBRV does not present specific resistance to antibiotics. Its life cycle takes place in eukaryotic cells and its sensitivity to different antibiotics is due to the sensitivity presented by the infected cells. The virus does not contain any gene that confers resistance to antibiotics of interest in terms of health.

**f) Involvement in environmental processes: primary production, nutrient turnover, decomposition of organic matter, respiration, etc.**

IBRV as such does not play a specific role in any primary production process, or in any cycle related to the decomposition of organic material. As with all viruses in their virion form, it does not show any type of metabolic activity. Only once it has infected its target cell does it express enzymatic activities which are characteristic of it. None of the enzymes produced contribute to any decomposition process of organic material.

The influence IBRV in the primary production process stems from its ability to infect cattle. In this way, the virus presents a negative influence on the final performance of infected individuals, which is reflected in a lower conversion rate. For this reason, there is interest in developing effective vaccines against it.

**12. Nature of the indigenous vectors: a) sequence, b) frequency of mobilisation, c) specificity, d) presence of genes which confer resistance**

The presence of mobile genetic elements such as proviruses, transposons or plasmids in IBRV has not been reported.

## 13. History of previous genetic modifications

Published literature on the range of genetic modifications conducted on IBRV was reviewed. Thymidine kinase (tk-) mutant herpesviruses were shown to be less virulent than tk+ strains, presented a lower likelihood of reactivating latent forms, protected inoculated laboratory animals against experimental infections carried out with virulent tk+ strains and reduced the probability of superinfection by virulent tk+ strains. Vaccinated calves subjected to an immunosuppressive treatment with dexamethasone, showed that such strains can establish latency and be reactivated (Kit et al., 1985, Whetstone et al., 1992)

Flores et al., 1993, assessed the efficacy of a gC-/tk- mutant obtained by Kit et al., in 1991. The results obtained demonstrated that the use of this GMO strain in conjunction with an anti gC specific ELISA could constitute an effective tool in IBR eradication programmes.

IBRV containing a deletion in gE- (Kaashoek et al., 1994) provided protection against experimental infection and that the gE- virus was not isolated after treatment with dexamethasone. These results led to the conclusion that the gE- strains of IBRV are attenuated, immunogenic and present the advantage of being able to be used as markers.

In a later study (Van Engelenburg et al., 1994) the virulence and immunogenicity of three deleted IBRV strains, gE-, tk- and gE-/tk- (double deletion), were compared. After inoculation by intranasal route of the three mutants, the tk- strain showed certain residual virulence, while the gE- and gE-/tk- strains were avirulent. The three mutants protected calves against an experimental infection. In a later study, none of the three mutants was recovered after subjecting the inoculated calves to a treatment with dexamethasone (Kaashoek et al., 1996).

A later study (Kaashoek et al., 1998) investigated the virulence and immunogenicity of various IBRV mutants (gC-, gG-, gI-, gE- and gI-/gE-). All mutants, except gC-, were less virulent in comparison with the parental organism; the avirulence of the gI- and gI-/gE- mutants was complete. The level of reduction in excretion of virulent virus after an experimental infection was related to the virulence of the vaccine strain used: the greater the virulence, the less the excretion of virulent virus. In this study the reduction in viral excretion was lowest in the animals vaccinated with the gI- and gI-/gE- mutants. When vaccinated animals were given an immunosuppressive treatment with dexamethasone, the gC- and gG- mutants were reactivated. The results obtained in this study indicate that the gC- mutant is too virulent and the gI- and gI-/gE- mutants are the least immunogenic, so gE- mutants are the best candidates for the preparation of live marker vaccines against Infectious Bovine Rhinotracheitis.

## B) Characteristics of the vector

### 1. Nature and source of the vector

The application described an IBRV which presents two deletions in its genome; one in the reading frame of the gE gene and the other in the reading frame of the tk enzyme gene. The process designed for the construction of both deletions required the prior construction of a "shuttle vector".

In view of all the information provided throughout this responses document, together with the documentation included in the original dossier, there was sufficient evidence supplied in support of the Environmental Risk Assessment (ERA), which enables confirmation that the release of the GMO will not present any hazard to humans, other non-target species or the environment.

Consequently, according to the intrinsic properties of IBRV strains, such as host specificity and low capability of surviving in the environment, and the specific properties of the gene-deleted CEDDEL strain (no virus dissemination in the treated animal's body, no transmission from vaccinated to unvaccinated animals, genetic stability, absence of foreign genomic sequences and absence of

reversion to virulence), the overall risk can be established as minimum and a full sequence analysis seems not considered necessary.

Construction of the shuttle vectors

A detailed description of the construction of shuttle vectors was presented.

**C) Characteristics of the modified organism**

**1. Information about the genetic modification**

**a) Methods used for the modification**

The modification method used was *in vivo* homologous recombination on cells in culture co-transfected with non-enveloped viral DNA and a shuttle vector.

**b) Methods used to construct and introduce the insert(s) into the recipient or to delete a sequence**

The deletions were introduced by *in vivo* homologous recombination. Homologous recombination was obtained by co-transfection of the shuttle vectors with the non-enveloped viral DNA and selection of the recombinant viruses. Transfection is infection by a non-enveloped nucleic acid, whose entry in the cell does not depend on specific receptors. Co-transfection is used to describe the entry into the cell of non-enveloped viral DNA jointly with the DNA of the shuttle which is the method used.

**2. Information on the final GMO**

**a) Description of the genetic traits or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed**

The final GMO is designated IBRV, strain CEDDEL. This strain is characterised by presenting two deletions in the regions of its genome which encode glycoprotein E (gE) and the thymidine kinase (tk) enzyme. The modified virus thus loses its capacity to express the functional forms of these proteins. Therefore, the main characteristics of the GMO virus are its inability to express glycoprotein E, and the lack of thymidine kinase activity. The lack of expression of both proteins does not alter the ability of the virus to spread *in vitro* on cell cultures (in terms of final viral titre), nor the immunogenic capacity of the virus. Therefore, the non-expression of glycoprotein E in calves inoculated with CEDDEL induces the development of an immune response intended to provide protection against subsequent infections with virulent IBRV, but whilst failing to develop anti-gE antibodies.

Glycoprotein E (gE) is known to be important for the dissemination of the virus by direct cell-to-cell transmission (Rebordosa et al., 1996). Therefore, the non-expression of this glycoprotein considerably reduces the capacity of the virus to spread in the infected animal.

The replication of the CEDDEL strain appears restricted to the inoculation site. The applicant considers that there is no evidence that the strain causes a generalised infection nor is the virus detected in internal organs or secretions of vaccinated calves. Consequently, the non-expression of gE reduces the pathogenicity of the virus without reducing its antigenic properties (van Engelenburg et al., 1994; Kaashoek et al., 1994; Bosch et al., 1996; Strube et al., 1996).

Since IBR gE- strains are incapable of expressing gE, animals inoculated with these IBR strains will not produce anti-gE antibodies, which would normally be induced following natural infection with wild-type virus. The use of an ELISA to detect anti-gE antibodies makes it possible to differentiate animals which are seropositive due to vaccination (gE- animals) from those animals which are seropositive due to infection (gE+ animals).

It should be noted that virus encoded thymidine kinase activity is important for BoHV-1 virulence, for replication in nerve cells which lack inherent thymidine kinase activity, and therefore a functional tk gene is important to allow IBRV to establish infection in nerve cells and potentially to enable

recrudescence, typically following stress. Deletion in the tk gene therefore reduces the ability of IBRV to establish an infection in nerve cells.

**b) Structure and amount of any vector and/or donor nucleic acid remaining in the final composition of the modified organism**

The applicant refered to section II.C.1.d, and supported that the final recombinant virus does not possess in its sequence any segment of DNA which is not its own.

**c) Stability of the organism in terms of genetic traits**

The CEDDEL strain of IBRV has been subjected to genetic stability studies through successive passages both in cell lines as well as in calves.

A comparison of the restriction patterns of the recombinant gE- tk- strain after the first cell passage after viral purification and after 5 cell passages from the first stock of Master Seed Virus were presented and showed no differences.

The IBR gE- tk- GMO virus has been subjected to genetic stability studies using successive passages, both in cell lines as well as in calves. In these studies no change has been detected in the biological characteristics of the virus both with regard to its behaviour in cell cultures (growth kinetics, viral titre reached, morphology and plate size, cytopathic effect, etc.) and its effects on infected calves (pathogenicity, tropism, viral titre in different tissues, etc.). The studies show that the deletions remain stable throughout a number of passages.

**d) Rate and level of expression of the new genetic material. Method and sensitivity of measurement**

Given that the deletions eliminate the expression of glycoprotein E and the regions of the thymidine kinase enzyme related to the catalytic activity, the presence of glycoprotein E or tk enzymatic activity is not expected in the GMO gE- tk- strain of IBRV. Studies conducted both with Western blot and ELISA, using specific polyclonal antibodies of glycoprotein E, shows that the CEDDEL strain does not express this glycoprotein. In addition, none of the animals inoculated experimentally with this strain developed detectable levels of antibodies against glycoprotein E.

**h) History of previous releases or uses of the GMO**

IBRV, strain CEDDEL has been used in containment conditions to carry out studies to assess its efficacy and its safety as a vaccine for cattle. In addition, IBRV CEDDEL has been used in a field trial. There have been no previous releases of this strain into the environment.

**3. Considerations for human health and animal health, as well as plant health**

**i) Toxic or allergenic effects of the non-viable GMOs and/or their metabolic products**

The applicant referred to the studies submitted in support of Safety Part. The results of these studies confirmed the safety of the vaccine virus and also demonstrated that the vaccine virus is not detected in organs or body fluids and secretions of vaccinated animals, so its transmission capacity to non-vaccinated animals can be considered as zero. The virus does not have the capacity to revert to virulence after being subjected to different serial passages in calves and in cell cultures.

The conclusions of the safety studies are discussed in detail in Part III, however there do not appear to be any specific safety concerns.

**ii) Comparison of the modified organism to the donor, recipient or (where appropriate) parental organism regarding pathogenicity**

The parental organism to which the two genomic deletions were added was isolated from a clinical case of Bovine Rhinotracheitis diagnosed in the province of Ciudad Real (Spain), in 1988. This strain was identified as IBRV and referenced as BoHV-1. It is therefore a virulent strain.

The defined deletions, gE- and tk-, have been selected based on current knowledge that deletions in each of these genes has been shown to reduce the pathogenicity of IBRV. Therefore, it is reasonable to conclude that the GMO containing both deletions will be less pathogenic than the parental strain.

**iii) Capacity for colonisation**

A study has been carried out to establish the dissemination capacity of IBRV strain CEDDEL in vaccinated animals. The results obtained demonstrated that the GMO was not detected in organs, body fluids or secretions of inoculated animals, and therefore, its transmission capacity to non-vaccinated animals can be considered as zero.

**iv) If the organism is pathogenic to humans who are immunocompetent: Diseases caused and mechanisms of pathogenicity, including invasiveness and virulence; communicability; infective dose; host range, possibility of alteration; possibility of survival outside of human host; presence of vectors or means of dissemination; biological stability; antibiotic-resistance patterns; allergenicity and availability of appropriate therapies.**

Bovine Herpesviruses type 1 (BoHV-1) strains are not pathogenic in humans.

**v) Other product hazards**

None known

***3. Information relating to the conditions of release and the recipient environment***

**A) Information about the release**

**1. Description of the proposed deliberate release, including its purpose**

The previous deliberate release carried out in a field trial which involved intramuscular inoculation of the GMO in cattle at farms in Spain, housed in premises with adequate isolation measures was described in detail.

***4. Information relating to the interactions between the GMO and the environment***

**A) Characteristics affecting survival, multiplication and dissemination**

**1. Biological features which affect survival, multiplication and dispersal**

The survival and multiplication of IBRV is affected by a series of physical and chemical agents. Dispersal of IBRV is principally by the aerosol route. Nasal exudates and the droplets expelled in coughs are the main sources of infection. In genital-related diseases caused by bovine Herpesvirus serotype 1 (BoHV-1), this is transmitted by venereal route, through genital secretions, semen, placenta and tissues and foetal fluids being the main routes of infection.

**2. Known or predicted environmental conditions which may affect survival, multiplication and dissemination (wind, water, soil, temperature, pH, etc.)**

Environmental conditions which may affect survival and multiplication of IBRV are temperature, pH and environmental humidity, which affect stability of the virus are discussed. Since aerosols are the main means of dissemination, so the action of the wind can be considered relevant as an environmental factor having an effect on dissemination. The introduction of infected animals on IBR-free farms may lead to the development of an outbreak of the disease.

**3. Sensitivity to specific agents**

The sensitivity of IBR to a range of specific agents, both physical and chemical, which affect the survival and multiplication of IBRV are reviewed.

1) Physical agents

*1.1) Temperature.*
IBRV survives in the environment for 30 days in winter. On cattle farms, it can survive for 6-13 days in winter, and for 5-9 days in spring. The virus is inactivated in seconds at temperatures over 63 ºC.

Various thermal sensitivity studies have demonstrated that the virus is very stable at temperatures below -65 ºC but this stability decreases if the storage temperature is around -20 ºC. The virus is slowly inactivated at 4 ºC and survives some 10 days at 37 ºC. Consequently IBRV can remain stable in semen storage containers, and may contaminate IBRV-free samples stored in the same container.

*1.2) Ultraviolet radiation*
Ultraviolet radiation can inactivate the virus when exposed for between 8-12 hours.

*1.3) pH*

The virus is stable at pH values between 6.0 and 9.0, values considered normal in the upper respiratory tracts and in the genital tract. The virus is very sensitive to low pH values. For example, 40 mM Citrate pH 3.0 buffer inactivates the virus in less than 15 minutes.

*1.4) Environmental humidity*
Environmental humidity plays an important role in the survival of the virus in the environment. Survival of the virus is optimal in 90% relative humidity and low temperatures (Elazhary and Derbyshire, 1979). As the environmental temperature increases, the optimal rate of relative humidity decreases.

2) Chemical agents

*2.1) Virucides*
Formaldehyde, most commonly used at 1%, 2% or 5% of the commercial solution of 40% pure formaldehyde, is very effective in inactivating the virus.

Beta-propiolactone is often used to inactive the virus at a final concentration of 10% in less than 180 minutes at 37 ºC.

**B) Interactions with the environment**

**1. Predicted habitat of the GMOs**
The GMO life cycle is in cattle. The animals included in the clinical trials conducted were isolated from other animals and so spread was not expected to occur. The habitat of the GMO was therefore the target animals on modern conventional farms.

**2. Studies of the behaviour and characteristics of the GMOs and their ecological impact carried out in simulated natural environments, such as microcosms, growth rooms, greenhouses, animal houses etc. may also be of relevance to medicinal products**

Studies have been carried out on the dissemination of the virus under controlled laboratory conditions to determine the horizontal transmission capacity of the virus by calf-to-calf contact, and the shedding of the vaccine virus from vaccinated animals. During these experiments, calves inoculated intramuscularly with the GMO were housed in contact with uninoculated calves. A range of different samples of organs and body secretions were taken from the vaccinated animals to assess the presence of the vaccine virus.

**3. Genetic transfer capacity:**

**a) Post-release transfer of genetic material from GMOs into organisms in affected ecosystems**

**b) Post-release transfer of genetic material from indigenous organisms to the GMOs**

It is considered that the only potential genetic transmission which could be foreseen is genetic exchange by homologous recombination between the recombinant virus and virulent strains of IBRV. For this to take place, both viruses would have to co-infect the same cell, in the same animal, which limits the chances of such an event occurring. Should such a recombination occur between the GMO and a wild-type IBRV strain, the deleted sequences of the genes of glycoprotein E (gE) and the thymidine kinase (tk) enzyme could only be replaced by the corresponding genetic sequences donated by the virulent strain. The expected result of such a recombination would the creation of a recombinant IBRV strain with the same complement of genes as the wild-type virus.

**4. Likelihood of post-release selection leading to the expression of unexpected and/or undesirable traits in the modified organism**

The probability of the GMO showing the expression of unexpected traits is considered unlikely, given that it does not incorporate genomic sequences donated by another organism. A possible recombination event between the GMO and a virulent IBRV strain would lead to the creation of a recombinant IBRV strain with the same complement of genes as the wild-type virus. Therefore, there are no reasons to believe that once released the GMO will behave in a different way to that seen in laboratory and field trials. It seems reasonable to accept that the likelihood is extremely low that post-release selection will lead to the expression of unexpected and/or undesirable traits in the modified organism is negligible.

**5. Measures employed to ensure and verify genetic stability; description of genetic traits which may prevent or minimise dispersal of genetic material; methods to verify genetic stability**

Genetic stability has been investigated by serial passages of the GMO both in calves and cell cultures. These studies have not shown any changes in biological characteristics: growth kinetics, cytopathic effect, pathogenicity, tropism, viral titre in different tissues. The limited transmission capacity shown by the GMO is considered an additional control against the risk of dispersal of genetic material.

**6. Known routes of biological dispersal or potential modes of interaction with the disseminating agent, including inhalation, ingestion, surface contact, etc.**

IBRV is predominantly transmitted by the aerosol route and enters the host principally through the upper respiratory tract. The GMO, strain CEDDEL, has a very limited transmission capacity (see section II.C.2.).

**7. Description of ecosystems to which the GMO could be disseminated**

The dissemination capacity of the GMO gE- tk- IBRV, strain CEDDEL, has proved to be zero. Dissemination into the ecosystem was considered low.

**8. Potential for excessive population increase in the environment**

Laboratory and field studies indicated that horizontal transmission is practically non-existent: cattle inoculated by intramuscular route do not excrete the deleted IBRV, so the possibility of excessive excretion of the virus is highly unlikely.

**9. Competitive advantage of the GMOs in relation to the unmodified recipient or parental organism(s)**

The GMO is defective in the expression of two proteins which are present in all the wild-type IBRV strains, which a priori means a competitive disadvantage for the deleted virus. Therefore, it is not expected that the GMO will spread better in cattle populations than natural strains of the virus already

adapted to the environmental conditions of each area. The GMO will be transmitted, in a worst case, the same as natural indigenous strains and data indicate that transmission will be less.

Consequently, no competitive advantage of the CEDDEL strain compared to wild-type IBRV (or parental BoHV-1FM strain) is expected. The vaccinated animal acts almost as a dead end host, therefore the virus would be eliminated from the population. Consequently no competitive advantage is expected.

**10. Identification and description of the target organisms if applicable**

The target organisms are fattening calves and adult cows.

**11. Anticipated mechanism and result of interaction between the released GMOs and the target organisms, if applicable**

The GMO is intended to be used as a vaccine for cattle. In these animals, the GMO will have a certain degree of replication and will induce the development of an immune response which will finally eliminate the virus. The intention is that the vaccinated animals will have a degree of protection against IBRV.

**12. Identification and description of non-target organisms which may be adversely affected by the release of the GMO, and the anticipated mechanism of any identified adverse interaction**

None.

**13. Likelihood of post-release shifts in biological interactions or in the host range**

None expected. Reference to data indicate that the gene deletions are stable and that the GMO does not behave in a manner that would lead to a release in biological interactions or host range.

**14. Known or predicted effects on non-target organisms in the environment, impact on population levels of competitors, hosts, symbionts and pathogens**

None known.

**15. Known or predicted involvement in biogeochemical processes**

None known.

**16. Other potential interactions with the environment**

None expected.

**5. Information on monitoring, control, waste treatment and emergency response plans**

**A) Monitoring techniques**

**1. Methods for tracing the GMOs and for monitoring their effects**

During the prior release the vaccinated animals were observed for any possible effects of inoculation. Blood samples were also taken to assess the production and persistence of anti-IBR antibodies and the productive parameters of the vaccinated animals were recorded. All the results obtained were compared with those obtained in a non-vaccinated control group (inoculated with a blank vaccine).

**2. Specificity (to identify the GMOs and to distinguish them from the donor, recipient or, where appropriate, the parental organism), sensitivity and reliability of the monitoring techniques**

There are a number of methods to detect the CEDDEL strain of the BoHV-1 which are sensitive and specific, both in cell culture and in biological samples.

**3. Techniques for detecting the transfer of the donated genetic material to other organisms**

Since the CEDDEL strain does not contain any genetic material donated by another microorganism (only deletions in its own genetic material, the transfer of donated genetic material to other microorganisms is not possible.

**4. Duration and frequency of the monitoring**

During the prior release (during the field trial) the vaccinated animals were observed monitoring was carried out according to a pre-established schedule. The duration of monitoring lasted for the whole study period of the clinical trial. The frequency was according to a pre-established study plan.

**B) Control of the Release**

The procedures that were in place during the field trial to control the release were reviewed and were acceptable.

**C) Waste treatment**

The waste treatment is in accordance with local requirements.

**D) Emergency response plans**

The methods and procedures in place for the control of an unexpected spread, decontamination, waste management, and plans to protect humans and environment are reviewed. If an unexpected dissemination causing adverse effects had occurred the animals would have been culled and farms fumigated. No signs of unexpected spread were reported.

**4. Environmental Risk Assessment in accordance with the principles in Annex II to Directive 2001/18/EC**

**A. Objective**

The objective of this Environmental risk assessment is to identify and assess potential adverse effects, whether direct or indirect, immediate or delayed, on human health and the environment that the deliberate release or placing on the market of the IBRV strain CEDDEL, may have.

**B. General principles**

In accordance with the precautionary principle, this environmental risk assessment was carried out in compliance with the following general principles:

1. The characteristics of the genetically modified organism and its use which may have the potential to cause adverse effects have been compared with those presented by a non-modified organism from which it is derived and its use in similar situations.
2. In a scientifically safe and transparent manner, based on available scientific and technical data.
3. On a case-by-case basis.
4. Current information available on the genetically modified organism and its effects on human health or the environment.

## C. Methodology

### C.1. Characteristics of the genetically modified organism and releases

Details of the parental organism (BoHV-1) were provided, together with the method used to construct the IBRV gE- tk- double deletion GMO (IBRV, strain CEDDEL). Information on the nature of the deleted genes, their role in pathogenesis and the consequences of these deletions on the characteristics of the GMO were submitted.

The nature of the release of the GMO are given as inoculation of cattle of breeds normally used for meat production and industrial milk production. The nature of the receiving environment was discussed as well as the potential interaction between the GMO and this environment.

### C.2. Risk assessment phases

**1. Identification of characteristics which may cause adverse effects**

The IBRV, strain CEDDEL, was designed to have reduced pathogenicity (compared to wild-type virus) for the target species, whilst providing a level of protection against infection with virulent strains of IBRV. Laboratory safety studies provided, indicated that the GMO administered to calves by the intramuscular route does not disseminate in inoculated animals, is not transmitted to non-vaccinated animals and does not revert to virulence after being subjected to five serial passages in calves.

Wild-type IBRV is not known to affect humans or any other animal species other than cattle. No possible toxic effects have been identified in plants either. The genetic modifications used to create the GMO (gE- and tk-) do not alter these characteristics of the virus.

The IBRV strain CEDDEL does not contain any genes that code for resistance to antibiotics.

IBRV does not play a specific role in any primary production process, or in any cycle related to the decomposition of organic material. As with all viruses in their virion form, it does not show any type of metabolic activity.

The potential adverse effects from the deliberate release of a GMO were reviewed:

1. The spread of the GMO: the CEDDEL strain of the IBRV does not multiply in the organism of animals inoculated by intramuscular route. Therefore, no release of the GMO into the environment is expected, provided the GMO is administered correctly. In addition, IBRV strains are very sensitive to variations in temperature and environmental humidity. They present an average survival of between 6-13 days in winter and 5-9 days in spring. In addition, ultraviolet radiation (direct sunlight) inactivates the virus in some 8-12 hours. Therefore, a low survival of the IBRV strain CEDDEL is expected in the event of direct accidental release in the environment.

2. The transfer of the genetic material: the IBRV strain CEDDEL does not contain any fragment of foreign genetic material, so the possibility of transfer to other organisms is nil. The only genetic transmission which could occur is the genetic exchange by homologous recombination between the recombinant virus and virulent strains of the IBRV. For this to happen, both viruses would have to co-infect the same cell, in the same animal, which is not likely to occur under field conditions. Should there be a recombination event between the GMO and a virulent IBRV strain then this would lead to the creation of a recombinant IBRV strain with the same complement of genes as the wild-type virus.

3. Phenotypic and genetic instability: The CEDDEL strain of IBRV has been subjected to genetic stability studies through successive passages both in cell lines as well as in calves. A comparison of the restriction patterns of the recombinant gE- tk- strain after the first cell passage after viral purification and after cell passages from the first stock of Master Seed Virus were presented showing no differences. In addition, no change in biological characteristics (growth kinetics, cytopathic effect, pathogenicity, tropism, viral titre in different tissues, etc) were detected. The limited transmission

capacity shown by the GMO is also a form of additional control against the risk of dispersion of the genetic material.

4. Interactions with other organisms: no interactions between the IBRV, strain CEDDEL, and field IBRV are anticipated, nor is a reversion of the deleted genes expected. The applicant supports this by reference to laboratory studies in which the viral vaccine was subjected to different passages in calves and in cell cultures. The results obtained in this study showed the absence of reversion to virulence of the IBRV, strain CEDDEL. Different published works also show that both deletions are stable (Kit et al., 1985; Kaashoek et al., 1994).

5. Changes in management including, where applicable, in agricultural practices:

None expected.

**2. Evaluation of the potential consequences of each adverse effect, if it occurs**

The potential consequences and any potential adverse events were investigated. In brief, the lack of dissemination, low transmission rate, and poor survival capability in the environment mean that the GMO will not persist should it become released into the environment in an uncontrolled way. In addition, the defined gene deletions in the GMO a priori means a competitive disadvantage for the deleted virus compared to the wild-type virus.

**3. Evaluation of the likelihood of the occurrence of each identified adverse effect**

Inoculation of the animals is expected to be carried out by specialised staff and therefore the possibility of accidental release is minimal.

The potential consequences and any potential adverse events were investigated. In the hypothetical event of prolonged survival, no adverse effects are expected in the flora and fauna in the environment since IBRV only affects cattle.

**4. Estimation of the risk posed by each identified characteristic of the genetically modified organism or organisms**

The risk to human health or the environment posed by each of the characteristics identified is practically zero, since the likelihood that these will occur is very low and, if they did occur, no adverse consequence is expected for humans or the environment.

**5. Application of management strategies for risks from the deliberate release or marketing of the genetically modified organism or organisms**

None of the possible risks associated with the release of the GMO requires special management.

Any unused veterinary medicinal product or waste materials derived from such veterinary medicinal products should be disposed of in accordance with local requirements.

**6. Determination of the overall risk of the genetically modified organism or organisms**

According to all the information given above, the overall risk posed by the genetically modified organism can be established as negligible.

## D. Conclusions on the potential environmental impact from the deliberate release or the placing on the market of genetically modified organisms

### D.1. In the case of genetically modified organisms other than upper plants

1. Likelihood of the genetically modified organism to become persistent and invasive in natural habitats under the conditions of the proposed release is very low. The GMO will not be excreted by inoculated animals, and in the case of accidental release, a prolonged viability of the GMO in the environment is not expected.

2. No selective advantage or disadvantage conferred to the genetically modified organism and the likelihood of this becoming realised under the conditions of the proposed release is expected. The GMO

presents two deletions in its genome so it does not present any competitive advantages with regard to wild-type IBRV strains. The deletions have proved to remain stable after the carrying out of serial passages in cell cultures and in target animals, so the GMO is not expected to recover both deleted genome sequences.

3. There is no potential for gene transfer to other species under conditions of the proposed release of the genetically modified organism and any selective advantage or disadvantage conferred to those species. The GMO does not incorporate any additional genome sequence so the possibility of gene transfer to other species is zero.

4. No potential immediate and/or delayed environmental impact of the direct and indirect interactions between the genetically modified organism and target organisms is expected.

5. No potential immediate and/or delayed environmental impact of the direct and indirect interactions between the genetically modified organism and non-target organisms is expected, including impact on population levels of competitors, prey, hosts, symbionts, predators, parasites and pathogenic organisms.

6. No possible immediate and/or delayed effects on human health resulting from potential direct or indirect interactions between the genetically modified organism and persons working with, coming into contact with or in the vicinity of the release of the genetically modified organisms are expected.

7. No possible immediate and/or delayed effects on animal health and consequences for the human or animal food chain resulting from consumption of the genetically modified organism and any product derived from it if it is intended to be used as animal feed are expected.

8. No possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the genetically modified organism and target and non-target organisms in the vicinity of the release of genetically modified organisms are expected.

9. No possible immediate and/or delayed, direct or indirect environmental impact of the specific techniques used for the management of the genetically modified organism, where these are different from those used for non-genetically modified organisms is expected.

## *5. CONCLUSION*

**Assessment of risk to humans**

Generally, Bovine Herpesviruses are reported to be host-specific and not to affect other species different from cattle. Therefore the handling of the CEDDEL strain does not pose any risk for humans.

The IBRV strain CEDDEL consists of a gene-deleted vaccine strain that does not incorporate any foreign genomic sequence likely to be transmitted, and has demonstrated to be safe for the target species, genetically stable when submitted to serial in vivo and in vitro passages and not to spread to the environment. These properties guarantee that the GMO contained in the vaccine Hiprabovis IBR Marker Live would not come in contact with the person handling the product, unless it is handled improperly. In such a case, no risk is expected to occur, as human beings are not reported to be affected by IBRV strains.

**Assessment of the risk to the environment**

The gE- tk- IBRV strain CEDDEL is not transmitted from vaccinated to unvaccinated target species as it does not disseminate within the body organism of vaccinated animals. Therefore no risk of virus shedding into the environment is expected to occur.

The GMO consists of a gene-deleted virus strain in which no foreign genomic sequences have been inserted. Therefore no risk of transmission of extraneous genomic sequences are expected to occur. In

the same way the stability of the gene deletions constructed has been demonstrated by the performance of five serial *in vitro* passages in GBK cells and comparison between the pattern bands obtained in the unpassaged and passaged strain. In case of improper use of the vaccine, i.e. breakage of the glass vials containing the antigen fraction and release into the environment, OGM has demonstrated not to revert to virulence when submitted to serial *in vivo* passages by intranasal administration (the main infection route for wild-type IBRV strains). The results of the study on reversion to virulence also demonstrated that lower virus titres are obtained among passages, so in case of an unexpected release into the environment less than five natural passages in target animals would be expected to occur.

Additionally, in case of an unexpected release into the environment, the survival and propagation of IBRV strains are affected by a wide series of specific agents such as temperature, ultraviolet radiation, pH, environmental humidity and common disinfectant agents (formaldehyde, ß-propiolactone, etc.). Therefore the survival rate can be considered as very low.

**Assessment of the overall risk**

According to the intrinsic properties of wild-type IBRV strains, such as host-specificity and low capability of surviving into the environment, together with the specific properties of the gene-deleted CEDDEL strain (no virus dissemination in the treated animal's body, no transmission from vaccinated to unvaccinated animals, genetic stability, absence of foreign genomic sequences and absence of reversion to virulence), the overall risk can be established as negligible.

The environmental risk assessment supplied enabled confirmation of the conclusion and the argumentation is reasonable to support the view that the release of the GMO would not present a risk to humans, other non-target species or the environment.

## *OVERALL CONCLUSION ON QUALITY*

The methods used to create the defined deletions in the glycoprotein E (gE) and thymidine kinase (tk) genes of IBRV to create the vaccine strain CEDDEL (IBRV gE- tk-) double deletion mutant were well described. The applicant used a complex restriction pattern and hybridisation analysis to ascertain that the modifications that were intended have been achieved.

Sufficient detail has been provided to describe the method of production. The starting materials were also described in detail. Since this is a live viral bovine vaccine the risk of transmission of bovine adventitous agents such as BVD virus are maximum. Therefore, particular attention was taken to confirm compliance with the relevant guidelines and regulations in relation to the control of these agents. Information was provided in support of compliance with the bovine serum monograph and the relevant guidelines. The applicant presented a thorough assessment of the risk of transmission of TSE of the different starting materials and manufacturing phases of the vaccine. In addition, laboratory testing was conducted on the MSV and MCS to confirm that they are negative for the presence of prion proteins. Confirmation was provided that the antiserum used to neutralise the vaccine strain prior to extraneous agents testing did not contain antibodies to the full list of agents listed in the Ph. Eur. The applicant was requested to develop a specific test for testing Brucella abortus in the Master Cell Seed MCS and MSV within a presice timetable (6-9 months post authorisation).The in-process and finished product testing comprised of an adequate range of tests, most of which have been the subject of validation.

Consistency of production was demonstrated from results of 3 consecutive batches.

A comprehensive environmental risk assessment was conducted in accordance with Directive 2001/18/EC; sufficient information to enable the environmental risk assessment for the release of the GMO to be considered acceptable was provided.

Stability data were provided from consecutive batches of both proposed presentations which showed reasonable stability and which appeared sufficient to support the proposed 24 month shelf-life as packaged for sale.

# 3. Safety assessment

Hiprabovis IBR Marker Live is a live attenuated vaccine which includes a live gene-deleted Infectious Rhinotracheitis Virus, strain CEDDEL as the active ingredient. The vaccine is intended for active immunisation of calves from 3 months of age and adult cows, for the prevention and control of respiratory processes caused by IBRV in cattle and to reduce the virus shedding.

The onset of protection is 21 days and duration of protection 6 months after completion of the basic vaccination scheme. The recommended vaccination program is the administration of one dose of 2 ml by intramuscular route and revaccination 3 weeks after with the same dose. A single booster dose every 6 months is recommended.

The safety of Hiprabovis IBR Marker Live was evaluated on the basis of the requirements for immunological veterinary products as described in the legislation.

The safety studies were conducted in commercial breeds of cattle at the minimum age recommended for vaccination (3 months old). There was a safety study conducted in pregnant cows at different stages of pregnancy and a field trial including dairy cows and fattening calves.

Safety laboratory studies were conducted according to GLP standards and field studies were conducted according to GCPv guidelines.

The batches used in the safety studies contained vaccine virus at the least attenuated passage level that will be present in a batch of vaccine.

## *A. Safety assessment*

### *Laboratory tests*

### *Safety of the administration of one dose, safety of an administration of an overdose, and safety of the repeat administration of vaccine doses*

**Safety of Hiprabovis IBR marker live vaccine: safety of the repeated administration of vaccine doses**

This GLP compliant study was designed following the recommendations in Ph. Eur. monograph 0696 for IBR vaccines (live) and general monograph 50206 for evaluation of safety of veterinary vaccines and immunosera; also VICH guideline on target animal safety for veterinary vaccines.

**Animals:** Bovine Holstein, males and females, at 3 months of age (±2 week at vaccination on day 0), free of anti-IBR antibodies were enrolled.
**Vaccine:** Hiprabovis IBR marker live, Titre 107.0 TCID50/dose.

**Administration:** Intramuscular (IM) route.

**Vaccine scheme:** One vaccine overdose: 4 ml containing a titre of 108.3 TCID50 and one single dose of 2 ml with titre 107.3 TCID50, 21 days apart

**Follow-up:** Fourteen days after each vaccination (42 days) the following were monitored:

- Abnormal local reactions observed daily, i.e. abnormal swelling, abscesses (diameter in cm)
- General signs observed daily.
  Relevant signs: dyspnoea, coughing, ocular secretion, nasal secretion, prostration, anorexia.

- Rectal body temperatures taken daily from D-3
- Blood taken for serology on D0, D14, D28 and D42. Tested by ELISA
- Nasal swabs taken for assessment of vaccine spread on D0, D7, D14, D21, D28 and D35. Tested by virus isolation.

All samples were analysed for virus isolation and virus titration. Serum samples tested for antibodies anti-IBR by ELISA.

**Results**

Local reactions: no abnormal local reactions were observed after repeated vaccination in 7 of the animals.

General clinical signs: a few clinical signs were recorded in the animals throughout the observation period, most of them being isolated episodes of slight coughing.

Rectal temperatures: slight increases in rectal temperature (0.13°C – 0.93°C) were observed in most of the calves, between 4-48 hours post-inoculation, after some inoculations.

Rectal temperatures in some calves were slightly higher than the ones considered as normal, all of them slight and transient and at days 7-14 post vaccination, not related to any other clinical signs and were therefore considered unrelated to vaccination.

Virus shedding: all swab samples were negative for the presence of IBRV after analysis by a validated isolation-titration method.

Serology: all animals were seronegative on Day 0 before inoculation for gB antibodies. All calves apart from one had seroconverted by 14 days post-inoculation, as detected by ELISA Herdcheck IBRgB. They had all seroconverted by day 28 of the study. At the end of the study, day 42, all calves remained positive for gB antibodies, whilst they were all gE seronegative, indicating the absence of a natural infection with IBRV.

**Conclusions:** The CVMP considered that this repeated administration study complied with the requirements stated in the relevant monographs and supported satisfactorily the safety profile of the vaccine after repeated administration.

## *Examination of reproductive performance*

**Assessment of the safety/abortigenicity of a BoHV-1 vaccine administered to pregnant cattle**

This GLP compliant study was designed according to the specifications in Ph. Eur. Monograph 0696.

**Animals:** Bovines free of anti-IBR antibodies, at approximately 4, 5 and 6-7 months pregnant (± 7 days)were enrolled and animals were allocated on each group.

**Vaccine:** Hiprabovis IBR marker live, Titre 106.9 TCID50/dose.

**Administration:** Intramuscular (IM)

**Vaccine scheme:** One vaccine overdose (10X): 4 ml containing a titre of $10^{8.3}$ $TCID_{50}$

**Follow-up:** Animals were followed up until the end of the pregnancy for the parameters below:

- General health observed twice daily
- Clinical observations before vaccine administration and 3 hours after, for adverse events
- Blood taken for serology on D0 and final day of study and at approximately 4-weekly intervals tested by ELISA (ELISA, to detect antibodies against "BoHV1 viral antigen")
- Nasal swabs taken 2 days after arrival to confirm free of IBR status. Tested by virus isolation.

- Procedures following birth of live calf: blood sample from calves before colostrum intake and physical examination. Blood sample from dam after partition (also 14 days after if calf was abnormal).

Safety was evaluated according to the following criteria: birth of live, healthy, full-term calves; lack of IBRV antibodies in calf sera and absence of IBRV antigen in samples from aborted foetuses or placenta.

**Results:**

Adverse events: there were no adverse events or suspected adverse product reactions after vaccination.

General clinical observations: all cows remained in good health during the study except for two abortions. All were confirmed to be negative for IBR on D0.

Abortions/calving: A number of healthy calves were produced . A very small number of abortions occurred during the study, being both cows just over 4 month pregnant. Samples collected from foetuses were negative for virus isolation, no significant histopathological changes were found and were also negative for immunohistochemistry. One of the samples from placenta presented changes consistent with bacterial infection.

IBRV antibody in calf sera: from the 23 blood samples tested two of them were positive. It was noticed however that both calves may have suckled colostrum before the samples were collected.

**Conclusions:**

This study demonstrated the safety of the vaccine during pregnancy and showed that the vaccine strain does not disseminate to the reproductive organs of female cattle.

### *Special requirements for live vaccines*

## Spread of the vaccine strain

**Safety of the Hiprabovis IBR marker live vaccine: safety of the administration of an overdose, of the repeat administration of vaccine doses, and assessment of the spread from vaccinated to unvaccinated target animals**

The spread of the vaccine strain from vaccinated to unvaccinated animals was tested in this GLP compliant laboratory trial where safety of a single dose, overdose and repeat dose administration were also studied. Bovines at 3 months of age (±1 week at vaccination on day 0) were enrolled and divided in vaccinates and controls (not vaccinated). The details of the study design were described earlier in the report under safety. Nasal swabs taken for assessment of vaccine spread on D0, D4, D7, D11, D14, D18D21, D25, D28, D32, D35, D39 and D42. Tested by virus isolation.

The applicant tested the swabs that were frozen at -80°C after collection and were confirmed negative by two specific PCR tests, s known to be able to detect viral DNA from frozen samples. Therefore absence of virus shedding in this trial was shown. The absence of general signs also supports this conclusion.

## Dissemination in the vaccinated animal

**Safety study to test for dissemination in vaccine animals of the IBRV strain CEDDEL**

This was a GLP compliant study.

**Animals:** Bovines of approximately 2 months of age and free of antibodies anti-IBR were enrolled.

**Vaccine:** Hiprabovis IBR marker live, titre $10^{8.41}$ $TCID_{50}$/ml.

**Administration**: Intramuscular (IM)

**Vaccine scheme:** 2 ml containing a titre of $>10^{8.3}$ $TCID_{50}$ (>10 times maximum dose)

**Follow-up:** Six days after vaccination the following took place

- Day -4: nasal swabs
- Day -1: blood collection for serum
- Day 0: nasal swabs
- Days 2, 4 and 6: two animals killed each day and the following samples taken: nasal swabs, nasal mucosa, ocular swabs, ocular conjunctiva, faeces, urine, blood (whole and serum), trachea, lungs and trigeminal ganglions, salival swabs, vaginal or balanal swabs, testis, seminal vesicle, prostate, ovaries, uterine mucosa and vaginal mucosa.

All samples were analysed for virus isolation and virus titration. Two specific PCRs were used to confirm negativity of the samples. Serum samples tested for antibodies anti-IBR by ELISA.

**Results:**

- All samples collected before vaccinations were negative for IBRV by serology and virus isolation.

- All samples collected from the necropsied animals on days 2,4 and 6 were negative for IBRV by virus isolation and PCR.

**Conclusions:**

The study design was based on the requirements on the Ph. Eur. monograph 206 for general safety evaluation of veterinary vaccines. The number of animals seems sufficient and the type and number of samples analysed appropriate for the assessment of potential dissemination of the vaccine virus within the body. The applicant included reproductive organs and semen within the sampling regime to evaluate the potential dissemination of the vaccine virus. Furthermore, as indicated, it also included the assessment of virus presence in target body organs and fluids by PCR to exclude the fact that the vaccine virus could establish latency in target organs, especially trigeminal ganglions.

The titre and dose of vaccine administered to the animals was appropriate, as well as the age and serological status of the animals prior to vaccination, according to the requirements on the Ph. Eur. monograph 206 for general safety evaluation of veterinary vaccines.

The fact that no virus was isolated from any of the samples analysed indicated that the vaccine virus does not reach any of the body fluids and target organs when administered by the recommended route. It is especially relevant that the virus could not be isolated or detected by specific PCR from the trigeminal ganglions, which is crucial for the establishment of latency.

## Reversion to virulence of attenuated vaccines

**Safety study to test the increase in virulence of the IBRV, strain CEDDEL**

**Animals:** Bovines of 3 months of age free of antibodies anti-IBR were enrolled.

**Vaccine:** Titre $10^{8.41}$ $TCID_{50}$/ml

**Administration**: Two series of passages were carried out; calves were vaccinated in each passage with 2 ml of suspension containing $10^{8.3}$ $TCID_{50}$/calf by intramuscular route.

**Follow-up:** Twenty-one days after administration of suspension material the following activities took place:

- Abnormal local reactions were observed daily.
- General signs were observed daily.

- Relevant signs: dyspnoea, coughing, ocular secretion, nasal secretion, prostration, anorexia
- Rectal body temperatures at D-3, D-2, D-1, D0, D0+4h, D1 to D21
- Blood taken for serology on D0 and D21.
- Nasal swabs were taken on D0 and D1-D14

All samples were analysed for virus isolation and virus titration. Two specific PCRs were used to confirm negativity of the samples. Serum samples tested for antibodies anti-IBR by ELISA.

**Results:**

Local reactions: no abnormal local reactions were observed in any animal during the 21 days observation period except for one calf that showed slight swelling at the inoculation site 24 hours after vaccination, for 72 hours.

General clinical signs: no general clinical signs related with the inoculations were recorded during the observation period.

Rectal temperatures: slight increases in rectal temperature were observed in most of the calves from both series of passages between 4-48 h post vaccination, with increases between 0.27 and 0.88 °C during the 21 days post-inoculation. Rectal temperatures in some calves were slightly higher than the ones considered as normal, all of them slight and transient and at days 13-14 post vaccination, not related to any other clinical signs and were therefore considered unrelated to vaccination.

Serology: all animals were seronegative on Day 0 before inoculation for IBR glycoprotein B (gB) antibodies. All calves had seroconverted by 21 days post-inoculation, as detected by ELISA Herdcheck IBRgB. They were all gE seronegative on day 21 (ELISA Herdcheck IBRgE), which indicated the absence of a natural infection with IBRV.

Virus isolation:

First inoculation: no vaccine virus was detected in any calf at any day post-vaccination, all swabs were negative for IBRV by virus isolation. Further confirmation was done by testing of samples with validated PCR tests, which gave also a negative result.

Second inoculation: following the monograph recommendations, a second series of passages were initiated. All samples collected from the inoculated animals were also negative by virus isolation and confirm by PCR.

**Conclusions**

The study confirmed the innocuousness, regarding local reactions at the inoculation site, general clinical signs and evolution of rectal temperatures, of the administration of Hiprabovis IBR Marker Live to calves at minimum age by the recommended route. All vaccinated calves were seropositive for gB antibodies against IBRV by day 21 post inoculation. In contrast, no gE antibodies were raised, confirming the suitability of the strain as marker vaccine. The inoculated virus was not recovered in any sample neither in the first nor the second series of passages. The virus was also no detected by validated specific PCRs. The impossibility of in vivo passages was therefore demonstrated.

The study design followed the recommendations on the Ph. Eur. monograph 0696 for IBR live vaccines. Vaccine virus could not be recovered from any of the samples taken. Therefore, according to the relevant monograph, a second series of passages were conducted and calves were inoculated again in the same conditions. Monograph 0969 states that 'if virus is not recovered at any passage level in the first and second series of passages, the vaccine virus also complies with the test'. Therefore, it is accepted that the impossibility of *in vivo* passage has been demonstrated.

The samples taken were confirmed to be negative by specific validated PCR methods, which take into account the comments made to the original study.

Monograph 0696 states that the vaccine virus complies with the test if 'no calf shows signs attributable to the vaccine virus and if no indication of increased virulence of the maximally passaged virus compared with the unpassaged virus is observed'. No indication of reversion to virulence can be seen, as the virus could not be recovered from any clinical sample. Regarding clinical signs, mild local reactions lasting for 72 hours were seen only in one animal, although the size of the swelling was not recorded.

A few mild general signs were seen in some animals, which appeared more than 10 days after vaccine administration and were considered not related to vaccination due to the fact that they were isolated and not associated to other symptoms or temperature increase.

Some temperature increase was seen in most of the animals, which was mild and transient in most of the cases. This increase in temperature was also seen in the field studies presented in the original dossier and is covered by the comments made to the relevant section of the SPC.

The use of the gE ELISA confirmed that the antibody response was due to vaccination and natural IBR infection did not occur. All methods used appeared to be fit for purpose.

Overall, compliance with the relevant Ph. Eur. monograph for IBR live vaccines regarding reversion to virulence of the vaccine strain was considered as demonstrated.

## Recombination or genomic reassortment of strains

The applicant provided extensive information concerning the potential recombination events. The probability and possible outcome of the recombination of the CEDDEL strain with other herpesviruses have been investigated based on published literature and the results from the safety studies conducted for this application. Together with the information provided in the quality part of the report the conclusion that the probabilities of problems arise from potential recombination between the CEDDEL strain and other herpesvirus is likely to be negligible is reasonable.

### *Interactions*

The interactions of the vaccine with other products have not been studied, since no known interactions between live vaccines against IBR and other vaccines commonly administered to herds (such as inactivated BVD vaccine) have been reported. A specific warning in the SPC has been included.

### *Field Studies*

**Evaluation of the safety and efficacy of the live vaccine Hibrabovis IBR marker against IBR under field conditions**

One GCP compliant multi-centred field trial was carried out to assess both the safety and efficacy of Hiprabovis IBR Marker Live under field conditions.

**Animals:** Adult cows and calves, above 3 months (calves) and less than 2 years (cows) were enrolled. Only healthy, without showing clinical signs of any type of disease animals were included. There were no serological criteria for selection.

**Vaccine**: Vaccinates: Hiprabovis IBR marker live.

**Controls:** Blank vaccine containing the same excipient than the vaccine but without antigen.

**Administration**: Intramuscularly (IM)

**Vaccine scheme:**

Cows :
**Group A** : 2 doses of 2 ml of vaccine on D0 and D21
**Group B** : single dose of 2 ml on D0
**Group C** : control; 2 doses of 2 ml of blank vaccine on D0 and D21
Calves :
**Group A** (168 calves): 2 doses of 2 ml of vaccine on D0 and D21
**Group B** (148 calves): control; 2 doses of 2 ml of blank vaccine on D0 and D21

**Follow-up**

Dairy cows: from 14 days post-vaccination (D35) gestating cows were observed until calving to note any reproductive problems.

Fattening calves: from 180 days pots-vaccination, until slaughter animals were observed

Schedule of tasks performed in adult cows:

Abnormal local reactions observed on D0+4 hours, D1 to D14, D21, D21+4 hours and D 22 to D35.

General signs/adverse secondary reactions observed daily.

- Rectal body temperatures were taken from 15% of the animals, at D-1 to D4 and D20 to D25. The product was considered safe when the average temperature increase for all animals did not exceed 1.5°C from the one observed in the control group.
- Necropsy and samples from dead animals daily.
- Respiratory clinical signs observed daily.
- Abortions and reproductive parameters observed in gestating cows daily until calving. They should not be significantly different than that observed in the control group.

Schedule of tasks performed in fattening calves:

- Abnormal local reactions observed on D0+4 hours, D1 to D14, D21, D21+4 hours and D 22 to D35. Recorded according to the scoring system described for cows.
- General signs/adverse secondary reactions observed on D-1 to D14 and D20 to D35. Recorded according to the scoring system described for cows.
- Rectal body temperatures were taken from 15% of the animals, at D-1 to D4 and D20 to D25. The product was considered safe when the average temperature increase for all animals did not exceed 1.5°C from the one observed in the control group.
- Necropsy and taking samples from dead animals daily.
- Respiratory clinical signs observed daily.
- Blood sampling on D0, D21 and D45 to sacrifice. Samples were analysed using IDEXX kits.

Samples that were gB positive (i.e. vaccination or clinical infection) positive were retested for the presence of gE (infection only). The results were part of the criteria to assess vaccine efficacy.

**Results:**

Results in dairy cows:

Adverse events: no severe or unexpected adverse events attributable to vaccination were observed in any of the cows.

General clinical signs: none of the vaccinated animals or control group showed general clinical signs after first or second vaccination.

Rectal temperatures: temperature was measured in a sample of 33.6% animals distributed in different groups.

First vaccination: no significant differences were observed after first vaccination between groups A (double dose) and C (control), or between groups B (single dose) and C (control), in primiparous or

multiparous animals. Differences were only observed at punctual days in the decrease of temperature between groups, but this was considered of no clinical relevance.

Second vaccination: no significant differences were observed after second vaccination between groups A and C, in primiparous or multiparous animals. Significant differences were observed in the increase of temperature 4 hours of vaccination in group C (primiparous animals) and one day post-vaccination in group A (primiparous animals). In both cases the rise of temperature was not greater than 1°C (in relation to the mean pre-vaccination value).

Local reactions:

No significant differences in the percentage of local reactions and their severity were observed between the control and vaccinated groups.

Reproductive parameters: no significant differences were observed in any of the reproductive parameters between treatment groups. This ratio of abortions was the usual of the farms included in the trial. For the small number of abortions observed it was concluded after relevant investigations that the cause of abortion was not attributed to the vaccination with Hiprabovis IBR.

Results in fattening calves:

Adverse events: neither the vaccinated calves nor the control calves showed any adverse event attributable to the vaccination.

General clinical signs: none of the vaccinated animals or blank group showed general clinical signs after the first or second vaccination.

Rectal temperatures: temperature was measured in a sample of 18% of animals distributed in different subgroups.

First vaccination: no significant differences were observed after first vaccination between vaccinated animals and control group. Significant differences were observed 4 hours post-vaccination and 1 day post-vaccination in the vaccinated group when comparing the temperature difference between the day of control and the mean of the days prior to vaccination. This was also observed 4 hours post-vaccination in the control group. The rise of temperature was not greater than 0.39°C in any case, in relation to the mean pre-vaccination value.

Second vaccination: significant differences were observed 3 days after second vaccination between vaccinated animals and control group. The difference was 0.29°C and the rise of temperature was 0.13°C in relation to the mean pre-vaccination value.

Local reactions: no local reactions at the inoculation site were observed in any of the calves included in the study after first or second vaccination. None of the animals showed inflammation, pain or nodules after any of the administrations.

Serology: Close to 30% of the animals were positive for IBR on D0 by the gB ELISA. The number of gE positive animals in both groups decreased progressively, no animals were gE positive by day 135, indicating the absence of infection by a wild BoHV-1 strain.

**Conclusions:**
The field trial presented was a well designed study with a representative number of animals, including both dairy and fattening cattle from eight different sites. According to the serology data the study included a percentage of seropositive animals close to 30%. The CVMP considered that Hiprabovis IBR Marker is safe when administered to dairy cows and fattening calves under field conditions, when administered according to the recommended vaccination program.

## B. Residue assessment

### Study of residues-MRL

Hiprabovis IBR Marker Live includes a live gene-deleted IBRV, strain CEDDEL as the active ingredient. It contains no adjuvant and the rest of the excipients used 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. As a result of the composition of the vaccine no specific residue studies were considered necessary.

***Withdrawal period***: Cattle: Zero days

### Environmental Risk Assessment

The assessment of environmental risk was based on the fact that the vaccine does not include hazardous components and the main component is the live gene-deleted IBR virus. The vaccine is intended to be administered individually by intramuscular route to cattle and not release into the environment. It was demonstrated in the laboratory studies that the strain does not spread from vaccinated to unvaccinated animals or the environment and does not contain agents capable of disseminating within the target animal. Therefore, its possible release and exposure to the environment is effectively zero.

An Phase I risk assessment study was performed, which demonstrated that the product has an estimated risk for the environment as effectively zero and therefore, a Phase II study was considered unnecessary.

#### Phase 1 Assessment

E.1.1 Hazard identification

- Capacity of live organisms to transmit to non-target species: the results from the safety studies demonstrated that the vaccine antigen does not disseminate within the organism of the target host and is not shed into the environment. Therefore, the likelihood of target and non-target species to come in contact with the antigen is effectively zero.
- Shedding of live product organism (route, numbers and duration): the antigen component of Hiprabovis-IBR Marker Live is not shed by the vaccinated target animals into the environment.
- Capacity to survive, establish and disseminate: non-applicable, as the antigen is not shed into the environment.
- Pathogenicity to other organisms: non-applicable, since IBR strains are host-specific and do not affect other organisms than bovines.
- Potential for other effects of live product organisms: non-applicable, since strains are host-specific and do not affect other organisms than bovines.
- Toxic effects of excreted metabolites: non-applicable concerning the biological materials contained in the vaccine, since the antigen component does not disseminate within the vaccinated animals and no metabolites are produced. No other active ingredients are used at any stage of the manufacture process. The administration route does not allow the antigen release into the environment.

- E.1.2. Assessment of likelihood

The likelihood of a hazard occurring is negligible. Moreover, the individual method of administration avoids the possibility of releasing of the vaccine into the environment.

E.1.3. Assessment of the consequence of a hazard occurring

The consequences of the occurrence of any hazard can be considered as negligible.

E.1.4. Assessment of level of risk

Considering the consequences of a hazard occurring being negligible and the likelihood of hazard occurring being negligible, the estimation of risk can be considered affectively zero.

E.1.5. Selection and assignment of appropriate control measures (risk management)

Non applicable.

**Assessment of the overall risk to the environment**

In line with the matrix of estimation of risk of Annex I of the Note for Guidance EMEA/CVMP/074/95, and the experience in the use of similar vaccines, the risk to the environment when using the vaccine Hiprabovis IBR Marker Live was estimated as effectively zero.

## *User safety*

Considering the composition of the vaccine there is no specific safety concern in relation to the user.

## *Overall conclusions on safety*

The safety of the vaccine Hiprabovis IBR Marker Live was demonstrated in accordance with the requirements of the Ph. Eur. Monograph on Infectious Bovine Rhinotracheitis vaccine (live) and evaluation of safety of veterinary vaccines and immunosera. All calves used in the safety studies were at minimum age and all were seronegative for antibodies against IBR, tested by ELISA. The vaccine was administered intramuscularly in the neck muscles, following the recommended schedule stated in the SPC. The safety of the vaccine was demonstrated in a study including single dose, double dose (10 times the maximum titre) and repeat dose.

Safety in pregnant animals was tested using animals at three different states of pregnancy, according to Ph. Eur. requirements and using vaccine at maximum titre. The lack of effect on the reproductive parameters and the lack of seroconversion on newborn calves were demonstrated.

Specific studies required for live vaccines were also conducted. The spread of vaccine from vaccinated to unvaccinated animals was assessed including a sentinel group in the single/double/repeat dose study. The lack of spread was demonstrated showing the seroconversion in the vaccinated animals and the lack of seroconversion and clinical signs in the sentinel group.

The dissemination study showed that the vaccine virus could not be isolated from any of the samples tested, which strongly suggests that the virus does not reach any of the target organs.

The lack of reversion to virulence was tested in a study designed according to the specifications in Ph. Eur., apart from the use of a different administration route for the inoculum, which can be considered as appropriate as it seems to be the most susceptible route for recovery of the virus and the inclusion of an in vitro passage, which was allowed by the relevant monograph at the time the study was conducted. The study complied with the requirements and showed the lack of reversion to virulence of the vaccine virus.

Results from the laboratory studies were supplemented by data from a multi-centric field trial, which included dairy cows and fattening cattle. Slight increase in rectal temperature and transient swelling at injection site were observed and a warning has been included in the SPC. The study demonstrated that Hiprabovis IBR Marker Live vaccine is safe when administered to dairy cows and fattening calves under field conditions, when administered according to the recommended vaccination programme.

Due to the composition of the vaccine no specific residue studies were considered necessary.

A Phase I environmental assessment was conducted, which estimated the risk to the environment when using the vaccine Hiprabovis IBR Marker Live as effectively zero, based on the studies that demonstrated that the vaccine antigen does not disseminate within the organism of the target host, is not shed into the environment and does not revert to virulence. Considering the composition of the vaccine there is no safety concern in relation to the user.

In general the safety of the vaccine was tested in appropriate studies. The adverse reactions appeared mild, restricted to an elevated temperature with no major safety concerns.

# 4. Efficacy assessment

## *Introduction*

Infectious Bovine Rhinotracheitis (IBR) is a disease of major economic importance. Eradication programmes are in place in a number of countries, but this is hampered by the latency of the virus. Existing vaccines serve to reduce infection, lessening clinical signs and therefore economic consequences, and reducing viral excretion, leading to a reduction in spread, although they do not prevent latency. Animals vaccinated with traditional vaccines cannot be differentiated from those that have been naturally infected, leading to problems in eradication. Marker vaccines are obtained from structurally modified viruses. The antibody profile raised by a marker vaccine can be distinguished serologically from that raised by wild type virus, making it possible to distinguish between vaccinated animals and previously infected animals.

The associated claims for this vaccine are to reduce the clinical signs of IBR and field virus excretion. Onset of immunity is 21 days after completion of the basic vaccination scheme and duration of immunity: 6 months after completion of the basic vaccination scheme. The first vaccination is recommended at 3 months old, 2nd injection 21 days later.

The challenge strain was chosen following information published by Kaashoek et al 1996, Vet. Rec. 139. In Kaashoek's experiment the chosen Iowa strain produced severe clinical disease, including nasal ulceration and death in calves.

## *General requirements*

There is an Ph. Eur. monograph relating to this product (Infectious bovine rhinotracheitis vaccine (live) 01/2008:0696).

In line with the requirements of Annex I of Directive 2001/82 as amended, the applicant provided information on the available diagnostic tools in order to differentiate infected animals from vaccinated ones. In order to support the marker claim the applicant presented a validation report for a kit which is a serological test used to differentiate between vaccinated and infected animals. The suitability of this kit to differentiate between vaccinated and infected animals was demonstrated in the validation report. The kit appeared fit for purpose, for differentiating between Hiprabovis IBR Marker vaccinated animals and infected animals. Laboratory trials

## *Establishment of a Challenge Model*

Challenge was at 21 days after completion of vaccination and by intranasal instillation of 7.0 log10TCID50 IBR virus strain Iowa.

No specific study was provided to describe the establishment of the challenge model. Whilst the design of the model is intended to provide challenge by the most appropriate route.

## *Determination of the Vaccine Dose*

Three early studies were done using vaccine administered using a 5.5 log10TCID50 dose. The results of these early studies pointed to the convenience of a slight increase in the minimum vaccine dose. Consequently the applicant raised the minimum titre to 6.3 log10TCID50 per dose. The applicant has provided a study using a dose of 6.3 log10TCID50. Based on this study the applicant proposed a minimum titre of 6.3 log10TCID50 per dose which was acceptable.

## *Onset of protection*

***Efficacy of HIPRABOVIS IBR MARKER LIVE at $10^{6.3}$ $TCID_{50}$/dose in front of an experimental infection. Efficacy of the basic vaccination scheme (vaccination/revaccination) in seronegative calves.***

This was a GLP compliant study.

***Study design:***

Calves: calves, seronegative and antigen negative for IBRV were enrolled and divided in two groups. Calves were minimum age, 3 months old, at inoculation. They were free of both IBR antigen and antibodies to IBR at the start of the study.

Vaccine: A batch with an initial titre 7.0 $\log_{10}TCID_{50}$ /dose, diluted at point of injection to provide 6.3$\log_{10}TCID_{50}$ per 2ml dose was used. The virus used for the production of this batch was of the most attenuated passage level that can be present in a batch of vaccine.

Inoculation schedule: each vaccination consisted of 2ml given intramuscularly

| Group | Age of calves at V1 | N | Vaccination: D0 & D21 | Follow Up (till day 63) |
|---|---|---|---|---|
| B | 3 months | 5 | 6.3 $\log_{10}TCID_{50}$ | Clinical signs, virus isolation |
| A | 3 months | 5 | placebo | |

Challenge: Intranasal instillation of IBR virus.

Follow-up:

Serology was carried out pre-trial, at Vaccination Day 1 (V1), at V2, at challenge and 21 days post challenge, at the end of the study. Nasal swabs were collected pre-trial, pre-V1, pre-V2, pre-challenge and for each of 21 days post challenge. Rectal temperatures were measured pre and post each vaccination and daily post challenge. Clinical observations were recorded daily post challenge, and after each vaccination.

Results:

Clinical & Rectal temperature post-vaccination:

There were no general clinical signs seen in the calves during the vaccination phase of the trial. Rectal temperatures were elevated pre-vaccination in 20% of vaccinated calves. Slight pyrexia was seen on

some occasions in control calves post vaccination. At re-vaccination 20% vaccinates had had pyrexia at +4hrs and 24 hrs post V2. None of the control calves had elevated temperatures at revaccination.

Clinical signs post challenge:

Clinical signs of IBR were seen in both groups following challenge. Observations of moderate cough and/or nasal secretion were made in the vaccinated group, with signs being especially evident 9-10 days post-challenge, when they were accompanied by a mild pyrexia. The controls were more severely affected. By day 3 post challenge all animals were clearly affected, and the pyrexia was long lasting and severe at times. Dyspnoea, cough, nasal secretion, anorexia, rhinitis and ocular secretion were all seen. The signs were statistically significantly more severe in the control group. Statistical differences were seen between vaccinates and controls for most clinical signs where vaccinates were observed with significantly lower scores.

Rectal temperatures post challenge:

There were a few incidences of pyrexia pre-challenge. The control group were all pyrexic by day 45, 2 days after challenge. Seventy- five percent of vaccinates were pyrexic on day 51, 8 days post challenge. The duration of pyrexia was longer in controls.

The degree of pyrexia was also greater in controls. Differences were statistically significant on thirteen individual days ($p<0.01$), and for the period ($p<0.01$), with vaccinated animals having significantly lower rectal temperatures post challenge than controls.

Nasal virus shedding post challenge:

Virus was isolated from all animals in each group after challenge. Virus was shed from animals in the controls group from days 1-10 post challenge, and from days 1-6 post challenge in the vaccinates. Therefore the controls excreted virus for 4 days more than the vaccinates.

In 80% of vaccinates the maximum virus titre was more than 2 logs lower than the mean of the maximum titres of the control calves. Twenty percent of calves showed a reduction of 1.90 log.

When the data were analysed by period a statistically significant association ($p<0.01$) between treatment and nasal shedding was found. There were also numerous days when the vaccinated group had statistically significantly ($p<0.01$) lower virus isolation titres than controls.

Serology

Serology was based on the detection of anti-gB immunoglobulin (IBRgB), an antigen still present in this marker vaccine. gE antibody (IBRgE) titres were also tested. All calves were seronegative at the start of the study and control calves remained seronegative until challenge. The surviving control calves seroconverted following challenge.

Four of the five vaccinates were clearly seropositive by day 21 (re-vaccination). By challenge (day 42) all vaccinates were clearly seropositive, and titres did not increase significantly thereafter.

The vaccine is a gE negative marker vaccine. All pre-challenge samples, including those from vaccinated animals were gE negative. Following challenge 40% of controls were gE positive and 60% were suspect. Eighty percent of vaccinates were similarly positive, and 20% suspect.

Conclusion:

This was a pivotal study, that supported the onset of immunity claim (21 days post second vaccination), in antigen and antibody free calves of minimum age, given a revised minimum titre vaccine by the recommended route. The controls showed "typical" signs. Vaccinates showed no more

than mild clinical signs. This is in compliance with the EP monograph. The nasal virus shedding results were in compliance with EP monograph requirements.

The percentage of vaccinates with a maximum virus titre more than 2 logs lower than the mean max titres of the control calves was 80%, therefore this is in compliance with EP monograph requirements.

Overall the study was clearly compliant with the EP monograph, with the controls showing classical signs that were statistically significantly worse than vaccinated The requirements of the EP monograph were met, and it was concluded that a 2ml dose of 6.3 log10 TCID50 Hiprabovis IBR Marker Live has an onset of immunity in 3 month old susceptible cattle of 21 days post second vaccination.

## *The Influence of Maternal Antibody on the Efficacy of the Vaccine*

***Study of the influence of maternally derived antibodies (MDA) on vaccine efficacy of Hiprabovis IBR Marker Live at $10^{6.3}$ $TCID_{50}$/dose***

This was a GLP compliant study.

Calves: calves were enrolled. Calves were of minimum age at 3 months old (±2 weeks) at inoculation were enrolled. One third of the animals were seronegative for IBRV, and the remaining two thirds which were enrolled in two groups were seropositive with MDA to IBRV at the start of the study.

Vaccine: 6.3log10TCID50 per 2ml dose.

The inoculation schedule: each vaccination consisted of 2ml given intramuscularly

| Group | Age of calves at V1 | N | Sero-status | Vaccination : D0 & D21 | Challenge Day 64 |
|---|---|---|---|---|---|
| A : control (placebo+MDA) | 3 months | 5 | MDA +ve | placebo | |
| B : vac, no MDA | 3 months | 5 | seronegative | 6.3 $log_{10}TCID_{50}$ | |
| C : vac, + MDA | 3 months | 5 | MDA +ve | 6.3 $log_{10}TCID_{50}$ | |

Challenge: Intranasal instillation of IBR virus.

Follow-up: Serology, nasal swabs, rectal temperatures and clinical observations were carried out at frequent intervals.

Results:

Clinical & Rectal temperature post-vaccination:

There were no general clinical signs seen in the calves during the vaccination phase of the trial.

Some slight elevations were seen pre-vaccination, and post vaccination. See elsewhere for safety evaluation.

Clinical signs post challenge:

Some clinical signs of IBR were seen in all groups following challenge. Every animal in the study showed some: nasal secretion, ocular secretion and coughing

Only control animals showed: Rhinitis, Dyspnoea, Anorexia, Prostration.

A persistent nasal secretion was observed in all groups, but it was more severe in the controls. There were no records of severe secretion in group B animals. The differences were statistically significantly different on several days, and by period, with higher secretion in the control group than either of the vaccinated groups. More persistent ocular secretion, more severe, was also seen in controls, and again this was statistically significantly worse than in the vaccinates. Statistical differences were seen

between groups. Both vaccinated groups had significantly lower scores on all clinical signs than controls.

Rectal temperatures post challenge:

There were no incidences of pyrexia pre-challenge.

Following challenge the control group were all pyrexic by 3 days after challenge, where only some vaccinates of group B were pyrexic on day 4. Most animals of group C were pyrexic on day 4 post challenge. The duration of pyrexia was longer in controls and the degree of pyrexia was also greater in controls.

Nasal virus shedding post challenge:

Virus was isolated from all animals in each group after challenge.

Virus was shed from animals in the controls group from days 1-13 post challenge and from days 1-7 post challenge in the vaccinates without MDA and days 1-9 in the vaccinates with pre-existing MDA at vaccination.

The controls excreted virus for 4.6 or 3.2 days more than the vaccinates.

In most seronegative vaccinates (80%) the maximum virus titre was more than 2 logs lower than the mean of the maximum titres of the control calves.

In 60% of seropositive vaccinates the maximum virus titre was more than 2 logs lower than the mean of the maximum titres of the control calves. Controls shed significantly more than groups B or C. There were also several days when the vaccinated groups had statistically significantly ($p<0.01$) lower virus isolation titres than controls. Vaccinate groups were not statistically significantly different from one another.

Serology

Serology was based on the detection of anti-gB immunoglobulin, an antigen still present in this marker vaccine. Seronegative vaccinated animals seroconverted by 21 days following first vaccination with Hiprabovis IBR Marker Live. Revaccination increased the titres in both seronegative and seropositive vaccinated animals. At the time of challenge all vaccinated animals were IBR gB positive, with seronegative vaccinated animals with slightly higher (but not statistically significantly so) titres.

**Conclusion:**

The study was conducted with reference to the Ph. Eur. monograph on live Infectious Bovine Rhinotracheitis vaccines (01/2008:0699). More than the number of animals required were used. They were the correct age (3 months), were seronegative (one vaccinated group, group B) or seropositive (controls group A and vaccinates group C). They were vaccinated with minimum titre (6.3 log10 TCID50/dose), according to the recommended vaccination regime.

Overall, there was a very clear diminution in the effects of challenge following vaccination. Statistical tests consistently gave significant results when comparing the control group and either vaccinated group. There were no statistically significant differences in signs between the two vaccinated groups. Control calves shed more virus and for longer than either vaccinated group, and the differences were statistically significant.

Overall on the basis of information provided it was confirmed that the study followed the recommendation outlined in the CVMP referenced reflection paper EMA/CVMP/43967/2007 and that vaccination of 3 months calves with MDA was efficacious, as shown by the significant reduction of clinical signs and virus excretion.

## *Duration of Immunity*

**Duration of the immunity of the Hiprabovis IBR Marker Live marker vaccine. Efficacy in front of an experimental infection carried out 6 months after the administration of the basic vaccination scheme (vaccination/revaccination)**

This was a pivotal GLP compliant study in support of duration of immunity claims (6 months post second vaccination), in antigen and antibody free calves of minimum age, given a minimum titre vaccine by the recommended route.

Calves: calves, seronegative and antigen negative for IBRV were enrolled and then divided in two groups. Calves were 3 months old at inoculation. They were free of both IBR antigen and antibodies to IBR at the start of the study. It was confirmed that calves were around 3 months of age (minimum age) when they were administered the 1st dose.

Vaccine: 6.0log10TCID50 per 2ml dose.

Inoculation schedule: each vaccination consisted of 2ml intramuscularly

| Group | Age of calves at V1 | Vaccination: D0 & D21 | Challenge Day 21+6months | Follow Up (challenge + 21d) |
|---|---|---|---|---|
| 1 | 3 months | 6.0 $\log_{10}TCID_{50}$ | | Clinical signs, virus isolation |
| 2 | 3 months | placebo | | |

Challenge: Intranasal instillation of IBR virus.

Follow-up:

Serology (ELISA gB/gE) was carried out pre-trial, at V1, at V2, monthly until challenge, at challenge and 21 days post challenge, at the end of the study. Nasal swabs were collected pre-trial, pre-V1, pre-V2, pre-challenge and for each of 21 days post challenge. Rectal temperatures were measured pre and post each vaccination and daily post challenge. Clinical observations were recorded daily post challenge, and after each vaccination.

**Results:**

Clinical observations and rectal temperature post-vaccination:

There were mild general clinical signs seen in the calves during the vaccination phase of the trial. Mild respiratory signs were seen in vaccinated and control calves. Similarly, rectal temperatures were elevated slightly in some calves, on occasion.

Clinical signs post challenge:

Clinical signs of IBR, but consisting solely of nasal secretion and cough, were seen following challenge. Statistical differences were seen between vaccinates in controls with the vaccinates showing signs such as nasal secretion and cough for significantly less days.

Rectal temperatures and virus isolation post challenge:

When rectal temperature data were analysed by period, a statistically significant difference was seen, with vaccinated animals have significantly lower rectal temperatures post challenge than controls ($p<0.01$).Virus was isolated from all animals in each group after challenge. When the data were analysed by period a statistically significant association ($p<0.01$) between treatment and nasal shedding was found. The vaccinated group also had statistically significant ($p<0.05$) lower virus isolation titres than controls.

Serology:

Serology was based on the detection of anti-gB immunoglobulin (and anti-gE as well). All calves were seronegative at the start of the study and control calves remained seronegative until challenge. The surviving control calves seroconverted following challenge, reaching anti-gB titres similar to vaccinates. All vaccinates seroconverted (to gB) on day 21, and remained seropositive until challenge. No seroconversion to gE occurred in any animal before challenge. After challenge, all animals seroconverted to gE.

**Conclusion:**

This study supported the claim applicant's claim for duration of immunity of 6 months. The data showed a statistically significantly difference between vaccinates and controls may be considered adequate to support a claim of reduction in clinical signs and reduction in virus shedding.

## *Field Trials*

**Evaluation of the safety and efficacy of the Hiprabovis IBR Marker Live vaccine against Infectious Bovine Rhinotracheitis under field conditions**

This was a multi-centric randomised double blind GCP compliant field study conducted in Spain.

Calves: calves, approximately 3 months old were enrolled. Of these, some received two-dose vaccination scheme, the others placebo.

Vaccine: A batch was used with a titre of7 $\log_{10}TCID_{50}$ /dose.

Inoculation schedule: each vaccination consisting of 2ml intramuscularly

| Group | Approx Age | V1: D0 | V2: D21 |
|---|---|---|---|
| 1 control | 3 months | Placebo | Placebo |
| 2 | 3 months | Vaccine | Vaccine |

Follow-up:

Serology was carried out at each vaccination (days 0 and 21) and every 45 days from the first vaccination thereafter (days 45, 90, 135 and 180).

**Results:**

There were no clinical events of IBR during the trial.

Serology

IDEXX kits were used. Samples that were gB positive (ie vaccination or clinical infection) were retested for the presence of gE (infection only). The number of gE positive animals in both groups decreased progressively. No animals were gE positive by day 135, indicating the absence of clinical infection, and that the gB profile reflected the efficacy of the vaccination. On a farm by farm basis there were big differences in the proportion of animals with MDA on day zero of the trial (p270). One farm had a group with 50% gB positive animals (mean across both groups 49.3%), and another a group with 8.2% (mean across both groups 10.1%).

**Conclusion:**

The only information related to efficacy provided by this field study was that the vaccine induced a good seroconversion, as measured by ELISA. Given that no IBR outbreak occurred, the clinical protection that the vaccine might have induced is not known.

### *Overall conclusion on efficacy*

The applicant presented a number of studies in support of this application. Efficacy data were provided to support the minimum titre and which confirmed compliance with the requirements of the specific Ph. Eur. monograph. The applicant's claim to reduce the clinical signs of IBR and field virus excretion was demonstrated in the studies. The vaccine was shown to be efficacious in the face of MDAs. The 6 monthly booster re-vaccination was considered as supported by data and the applicant provided laboratory data in support of an onset and duration of immunity of 21 days and 6 months respectively.

Serological data in support of the absence of antibodies to gE after vaccination with the product confirmed the function of the vaccine as a marker vaccine with potential for use to differentiate vaccinated and infected cattle in an eradication scheme. It was accepted that the product could provide a benefit by enabling differential testing of vaccinated and naturally infected cattle. In line with the requirements of Annex I of Directive 2009/9, the applicant has provided information on the available diagnostic tools in order to differentiate infected animals from vaccinated ones.

The field studies have provided limited support for the efficacy of the product. The farms and animals recruited to the field study were not challenged by wild-type virus during the study period and there was no evidence of exposure as measured by seroconversion.

# 5. Benefit risk assessment

### *Introduction*

Hiprabovis IBR Marker Live is a live attenuated vaccine which includes a live gene-deleted IBRV, strain CEDDEL as the active ingredient. The vaccine is intended for the active immunisation of calves from 3 months of age and adult cows, to reduce clinical signs of infectious bovine rhinotracheitis (IBR) and field virus excretion. The onset of immunity is 21 days after completion of the basic vaccination scheme and the duration of immunity is 6 months after completion of the basic vaccination scheme.

### *Benefit assessment*

## Direct therapeutic benefits:

Vaccines are a well established and effective method to control the spread of the IBR virus.

The objective is to induce sufficient immunity to reduce clinical signs IBR and field virus excretion.

Clinical trials demonstrated that the product is capable of inducing an immune response which reduces clinical signs and reduces field virus excretions in cattle of minimum age (3 months). The effect is to reduce transmission and minimise the impact of clinical signs.

## Additional benefits

Hiprabovis IBR marker is a live marker vaccine which contains two structural changes. The CEDDEL strain (also referred to as PB-23) used in the vaccine is a double deletion mutant. The gene for glycoprotein E (gE) and that for thymidine kinase (tk) are both deleted. Being gE- and tk- deleted reduces the virulence of the vaccine strain, and allows the differentiation of animals with antibodies to gE (infected) and those without (vaccinated). This differentiation between vaccinated and infected animal is a significant parameter in the effective control of the disease, allowing the use of the vaccine in eradication programme.

Vaccination has been shown to be safe for use during pregnancy in cattle, which is important during a widespread vaccination programme usually necessary to control the spread of disease.

The vaccine has also been shown to be efficient in the face of MDAs which is useful as it includes a significant proportion of young cattle population during vaccination programmes.

### *Risk assessment*

## Main potential risks:

a) There is a risk of a slight increase in the body temperature up of  to 1° C is common within 4 days following vaccination. Occasionally, an increase in rectal temperature up to 1.63º C in adult cows and up to 2.18º C in calves may be observed. This transient rise in temperature is spontaneously resolved within 48 hours without treatment and it is not related to a febrile process. A transient inflammation at the inoculation site is common in cattle within 72 hours post-vaccination. This slight swelling lasts for less than 24 hours in most cases.

b) For the consumer there are no components which require an MRL, therefore there are no concerns over failure to observe an MRL.

c) For the user considering the composition of the vaccine there are no specific safety concerns.

## Specific potential risks, according to product type and application:

a) The vaccine contains a genetically modified organism (GMO). There is a negligible risk that the GMO may be released causing unexpected effects to the environment. Also, as this is a live vaccine there is a small risk of reversion to virulence. However, based on the studies provided it was demonstrated that the vaccine antigen does not disseminate within the organism of the target host, is not shed into the environment and does not revert to virulence.

b) Since this is a live viral bovine vaccine the risk of transmission of bovine adventitous agents such as BVD virus is present.

d) There is no specific test for testing *Brucella abortus* in the Master Cell Seed (MCS) and Master Seed Virus (MSV) which may result in contamination of the starting materials with *Brucella abortus.*

## Risk management or mitigation measures

a) Appropriate warnings have been placed in the SPC to warn of the potential risks to the target animal.

b) Starting materials are tested in compliance with the relevant requirements of the Ph. Eur. in order to minimise the risk of contamination with extraneous agents.

c) The applicant has been requested to develop a specific test for testing *Brucella abortus* in the Master Cell Seed (MCS) and Master Seed Virus (MSV) within a precise timetable (6-9 months post authorisation).

### *Evaluation of the benefit risk balance*

The product has been shown to have a positive benefit risk balance for use in cattle. The product has been shown to be efficacious for the indication of reduction of clinical signs and field virus shedding.

There are sufficient details of the production process to ensure consistency of production.

The applicant has provided sufficient information to enable the environmental risk assessment for the release of the GMO to be considered acceptable. Overall the quality of the vaccine was considered satisfactory.

The safety studies conducted by the applicant fully support the safety of the product in young and in pregnant cattle and in the face of maternally derived antibodies. Information provided in support of a lack of spreading and dissemination of the live GMO vaccine strain confirms the inability of the vaccine strain to disseminate and spread following immunisation. The absence of reversion to virulence has been adequately supported. Therefore, the safety of the vaccine administered as intended for use has been fully proven and is therefore acceptable.

Serological data in support of the absence of antibodies to gE after vaccination with the product do confirm the function of the product as a marker vaccine with potential for use to differentiate vaccinated and infected cattle in an eradication scheme. It is accepted that the product could provide a benefit through a role in enabling differential testing of vaccinated and naturally infected cattle, and acceptable claims and indications have been proposed.

Deficiencies include the current lack of a specific test for testing Brucella abortus in the Master Cell Seed (MCS) and Master Seed Virus (MSV). The applicant has been requested to develop a specific test for testing Brucella abortus in the MCS and MSV in order for the requirements for starting materials to be considered fully satisfactory.

Overall the benefits of this vaccine outweight the risks and therefore the balance is considered positive.

## *Conclusion on benefit -risk balance*

The information provided in the dossier and in response to points raised was sufficient to confirm an overall positive benefit-risk balance for this vaccine.

## *Conclusion*

Based on the data presented, the CVMP concluded that the quality, safety and efficacy of Hiprabovis IBR Marker Live were considered to be in accordance with the requirements of Directive 2001/82/EC as amended and the benefit-risk balance was favourable.

Based on the original and complementary data presented, the Committee for Medicinal Products for Veterinary Use concluded that the quality, safety and efficacy of the product were considered to be in accordance with the requirements of Directive 2001/82/EC as amended.