CLINICAL TRIAL REFERENCE EC-2006-VR-003 FOR THE ASSESSMENT OF THE VACCINE PB-23

SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

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A. General information

1. Details of notification

(a) Member State of notification	SPAIN
(b) Notification number	B/ES/07/44
(c) Date of acknowledgement of notification	07/06/07
(d) Title of the project	Assessment of the safety and efficacy of the live vaccine PB-23 against Infectious Bovine Rhinotracheitis under field conditions
(e) Proposed period of release	From November 2007 to May 2008

2. Notifier

Name of institution or company	LABORATORIOS HIPRA, S.A.

3. GMO characterisation

(a) Indicate whether the GMO is a:

(a) Indicate whether the GMO is a:	Viroid	☐
	RNA virus	☐
	DNA virus	☒
	Bacterium	☐
	Fungus	☐
	Animal	☐
	- mammals	☐
	- insect	☐
	- fish	☐
	- other animal	☐ specify phylum, class
Other, specify (kingdom, phylum and class)		
(b) Identity of the GMO (genus and species) Infectious Bovine Rhinotracheitis Virus, strain CEDDEL.		

(c) Genetic stability – according to Annex IIIa, II, A(10)

Considering those factors involved in the genetic stability of the parental microorganism, the Infectious Bovine Rhinotracheitis Virus strain FM, the DNA viruses have generally a greater genetic stability rather than RNA viruses. DNA viruses have corrective mechanisms for those genoma replication mistakes that may be produced. Those mechanisms reduce the generation of mutations and therefore the generation of new variants.

On the other hand the strain FM of the IBRV shows a characteristic restriction pattern, which corresponds to that expected for a Bovine Herpesvirus type 1 (BHV-1). Such a patter was described in 1983 by Mayfield et al. and constitutes a characteristic "fingerprint" for this virus subtypes, as well as a good way to detect possible genoma modifications. This indicates that the IBRV has a very stable genetic structure. Similarly, no modifications in its restriction patters different from those expected after the introduction of the two genomic deletions gE and tk have been observed during the studies carried out on the FM strain.

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 6(1)), by the same notifier?

Yes ☐	No ☒
If yes, insert the country code(s):	

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?

Yes ☐	No ☒
If yes: - Member State of notification - Notification number	

6. Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier?

Yes ☐	No ☒
If yes: - Member State of notification - Notification number	

7. Summary of the potential environmental impact of the release of the GMOs.

No environmental impact attributable to the GMO release is expected to occur for the following reasons:

The IBRV strains are generally very host-specific and not reported to affect human being or other species different from bovines.

The IBRV strains are also very sensitive to warm temperatures, sunlight and disinfectants.

The IBRV strain CEDDEL has demonstrated not to disseminate to any body tissue or secretions of the inoculated animals and not to spread from inoculated to non-inoculated animals.

In the unexpected case of genomic recombination with field IBRV strains the strain CEDDEL would recover part of the deleted genomic material. The result obtained after recombination would not be different from a current IBRV field strain.

B. Information relating to the recipient or parental organism from which the GMO is derived

1. Recipient or parental organism characterisation:

(a) Indicate whether the recipient or parental organism is a:

(a) Indicate whether the recipient or parental organism is a :	
Viroid	☐
RNA virus	☐
DNA virus	☒
Bacterium	☐
Fungus	☐
Animal	☐
- mammals	☐
- insect	☐
- fish	☐
- other animal	☐ specify phylum, class
Other, specify	

2. Name

(i) order and/or higher taxon (for animals)
(ii) genus Herpesviridae
(iii) species Bovine Herpesvirus, Serotype 1 (BHV-1)
(iv) subspecies
(v) Strain CEDDEL
(vi) pathovar (biotype, ecotype, race, etc.)
(vii) common name Infectious Bovine Rhinotracheitis Virus (IBRV)

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:

Yes ☒ No ☐ Not known ☐

(b) Indigenous to, or otherwise established in, other EC countries

(i) Yes ☒

If yes, indicate the type of ecosystem in which is found:

- Atlantic ☒
- Mediterraean ☒
- Boreal ☐
- Alpine ☐
- Continental ☒
- Macaronesian ☐

(ii) No ☐

(iii) Not known

(c) Is it frequently used in the country where the notification is made?

Yes ☒ No ☐

(d) Is it frequently kept in the country where the notification is made?

Yes ☒ No ☐

4. Natural habitat of the organism

(a) If the organism is a microorganism

- water ☐
- Soil, free-living ☐
- Soil in association with plant-root systems ☐
- In association with plant leaf/stem systems ☐
- In association with animals ☒ (Bovine)

Other, specify

(b) If the microorganism is an animal: natural habitat or usual agroecosystem:

5(a) Detection techniques

Isolation in cell culture (GBK cell line) and hibridation *in situ*

5(b) Identification techniques

Serumneutralisation, Immunoflorescence, IPMA, ELISA, PCR

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?

Yes ☐	No ☒
If yes, specify	

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes ☒	No ☐	Not known ☐

If yes:
(a) to which of the following organisms:

humans	☐
animals	☒ (Bovine)
plants	☐
other	☐

(b) give the relevant information specified under Annex IIIA, point II. (A)(11)(d) of Directive 2001/18/EC

In a natural outbreak of IBRV in bovine herds the pathogenic virus reaches the nasal cavity and upper respiratory tract, where it replicates in the epithelial cells. Later on the virus colonises the ocular tissues, where an onset of conjunctivitis, multi-foci plaque formation, corneal oedema and deep vascularisation can be observed. In some cases the virus might spread from the nasal cavity to the trigeminal lymph node through the trigeminal nerve. In such a case a clinical onset of encephalitis (ataxia, ptialism, blindness, hyper-reactivity/depression and high mortality incidence) can be observed. A systemic spread could lead to the virus colonisation of different organs and tissues. The colonisation of the foetus and placenta in an acute way could lead to abortion, mummification, stillbirths, etc.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

5-6 days

(b) Generation time in the ecosystem where the release will take place:

5-6 days

(c) Way of reproduction

Not applicable. Sexual ☐ Asexual ☐

(d) Factors affecting reproduction:

Not applicable.

9. Survivability

(a) ability to form structures enhancing survival or dormancy:

- (i) endospores ☐
- (ii) cysts ☐
- (iii) sclerotia ☐
- (iv) asexual spores (fungi) ☐
- (v) sexual spores (fungi) ☐
- (vi) eggs ☐
- (vii) pupae ☐
- (viii) larvae ☐
- (ix) other, specify

Not applicable.

(b) relevant factors affecting survivability:

Temperature, UVA (sunlight), pH, environmental humidity.

10(a) Ways of dissemination

Airbone.
Contact between animals.

10(b) Factors affecting dissemination

Low density of bovines.

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers)

None.

C. Information relating to the genetic modification

1. Type of the genetic modification

(i) insertion of genetic material	☐
(ii) deletion of genetic material	☒
(iii) base substitution	☐
(iv) cell fusion	☐
(v) other, specify	

2. Intended outcome of the genetic modification

Deletion of the genomic sequences corresponding to the glycoprotein E (gE) and the enzyme timidin kinase (tk).

3(a) Has a vector been used in the process of modification?

Yes ☒	No ☐
If no, go straight to question 5.	

3(b) If yes, is the vector wholly or partially present in the modified organism?

Yes ☐	No ☒
If no, go straight to question 5	

4. If the answer to 3(b) is yes, supply the following information

(a) Type of vector plasmid ☐ bacteriophage ☐ virus ☐ cosmid ☐ transposable element ☐ other, specify
(b) Identity of the vector
(c) Host range of the vector
(d) presence in the vector of sequences giving a selectable or identifiable phenotype Yes ☐ No ☐ Antibiotic resistance ☐ Other, specify Indication of which antibiotic resistance gene is inserted
(e) Constituent fragments of the vector
(f) Method for introducing the vector into the recipient organism (i) transformation ☐ (ii) electroporation ☐ (iii) macroinjection ☐ (iv) microinjection ☐ (v) infection ☐ (vi) other, specify

5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification?

Not applicable.

(i) transformation ☐ (ii) microinjection ☐ (iii) microencapsulation ☐ (iv) macroinjection ☐ ((v) other, specify Not applicable.

6. Composition of the insert

Not applicable.

(a) Composition of the insert
(b) Source of each constituent part of the insert
(c) Intended function of each constituent part of the insert in the GMO
(d) Location of the insert in the host organism - on a free plasmid ☐ - integrated in the chromosome ☐ - other, specify
(e) Does the insert contains parts whose product or function are not known Yes ☐ No ☐ If yes, specify

D. Information on the organism(s) from which the insert is derived

Not applicable.

1. Indicate whether it is a:

viroid		☐
RNA virus		☐
DNA virus		☐
bacterium		☐
fungus		☐
animal		☐
- mammal	☐	
- insect	☐	
- fish	☐	
- other animal	☐	(please specify phylum, class)
other, specify		

2. Complete name

(i) order and/or higher taxon (for animals)
(ii) family name (for plants)
(iii) genus
(iv) species
(v) subspecies
(vi) strain
(vii) cultivar/breeding line
(viii) pathovar
(ix) common name

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes ☐	No ☐	Not known ☐
If yes, specify the following (a) to which of the following organisms?	humans animals plants other	☐ ☐ ☐ ☐
(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism?		
Yes ☐	No ☐	Not known ☐
If yes, give the relevant information under Annex IIIA, point II(A)(11)(d):		

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?

Yes ☐	No ☐
If yes, specify	

5. Do the donor and recipient organism exchange genetic material naturally?

Yes ☐	No ☐	Not known ☐

E. Information relating to the genetically modified organism

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

(a) is the GMO different from the recipient as far as survivability is concerned?
Yes ☐ No ☒ Not known ☐
Specify

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?
Yes ☐ No ☒ Not known ☐
Specify

(c) is the GMO different from the recipient as far as dissemination is concerned?
Yes ☒ No ☐ Not known ☐
Specify
The GMO shows no spread capability from inoculated to non-inoculated animals when compared with the parental strain.

(d) is the GMO different from the recipient as far as pathogenicity is concerned?
Yes ☒ No ☐ Not known ☐
Specify
The GMO shows a reduced pathogenicity when compared to the parental strain.

2. Genetic stability of the genetically modified organism

The genetic pattern remains stable up to 5 serial passages in cell cultures and does not revert to virulence after 6 serial passages in calves.

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?

Yes ☐	No ☒	Not known ☐
If yes, specify the following (a) to which of the following organisms?	humans animals plants other	☐ ☐ ☐ ☐
(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i) The parental IBRV strain was isolated from a clinical IBR outbreak diagnosed in Spain. Such a strain was identified as IBRV and referenced as BHV-1, strain FM. It is therefore a pathogenic strain. The gE and tk deletions diminished the virulence of the parental strain without affecting its immunogenic power. Moreover the gE deletion permits the use of the IBRV strain CEDDEL as marker vaccine in eradication programmes, as it does not induce the development of anti-gE antibodies. Thus the gene-deleted IBRV strain CEDDEL is less pathogenic than the parental strain FM. The laboratory studies carried out have demonstrated that the gene-deleted IBRV strain CEDDEL is genetically stable, does not cause any adverse effects in the inoculated animals, does not spread from inoculated to non-inoculated animals and does not revert to virulence.		

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment Hibridation *in situ*, isolation in cell cultures, serumneutralisation, IPMA, ELISA, immunofluorescence, PCR.
(b) Techniques used to identify the GMO Isolation in cell cultures, serumneutralisation, immunofluorescence, IPMA, ELISA, PCR.

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)

Assessment of the safety and efficacy of this GMO as marker vaccine against Infectious Bovine Rhinotracheitis.

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?

Yes ☐	No ☒
If yes, specify	

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference): Three farms sited in the towns of Llagostera, Gualta and la Vall de Bianya, in the province of Girona (Spain).
(b) Size of the site (m^2): (i) actual release site ($m^{2)}$: 7,200 m^2 approx. (ii) wider release area (m^2): 93,000 m^2 approx.
(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected: None.
(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO Growing of cereals, vineyards and fruit trees. Fauna: rabbits, birds, foxes and wild boars.

4. Method and amount of release

(a) Quantities of GMOs to be released: Maximum amount of $1.2 \times 10^{10.3}$ $TCID_{50}$ in each farm. The GMO will be administered by intramuscular injection to bovines.
(b) Duration of the operation: The GMO will be released 2 days (vaccination and revaccination days). The observation period will last for 8 months.
(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release No spread of the GMO is expected to occur, as it will be inoculated by intramuscular injection and the GMO has demonstrated not to spread from inoculated animals. In any case the animals will be housed in isolated farms.

5. Short description of average environmental conditions (weather, temperature, etc.)

The weather in Girona on average is mild. The average yearly temperature is 23 degrees Celsius. The temperature can reach as high as 40 degrees Celsius, on extreme occasions, but it would rarely be higher than this. In February it has been known to dip a couple of degrees below 0, although the average temperature for this time is usually 10 degrees Celsius. The average year of rainfall is 749 mm.

6. Relevant data regarding previous releases carried out with the same GMO, if any, specially related to the potential environmental and human health impacts from the release.

None.

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

1. Name of target organism (if applicable)

(i) order and/or higher taxon (for animals) *Vertebrae*
(ii) family name (for plants)
(iii) genus *Bos*
(iv) species *Bos taurus*
(v) subspecies
(vi) strain
(vii) cultivar/breeding line
(viii) pathovar
ix) common name Bovines (calves and adult cows).

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)

Replication of the GMO in the inoculated animal, without producing adverse reactions an inducing active immunity against Infectious Bovine Rhinotracheitis.

3. Any other potentially significant interactions with other organisms in the environment

None.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?

Yes ☐	No ☒	Not known ☐
Give details		

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established

None.

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO

None.

(i) order and/or higher taxon (for animals)
(ii) family name (for plants)
(iii) genus
(iv) species
(v) subspecies
(vi) strain
(vii) cultivar/breeding line
(viii) pathovar
ix) common name

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem: Recombination between the GMO and IBRV field strains is unlikely to occur. In such a case, the GMO would incorporate part of the deleted genoma sequences. This fact is not considered to have any negative impact for the environment.
(b) from other organisms to the GMO: None.
(c) likely consequences of gene transfer: None.

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.):

Different assays using the gene-deleted strain CEDDEL have demonstrated that such strain is less pathogenic than the parental one, does not interfere with the environment as it does not spread from inoculated animals, and are able to protect bovines against the disease

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)

None.

H. Information relating to monitoring

1. Methods for monitoring the GMOs

Detection of anti-gE antibodies in the inoculated and non-inoculated animals. Physical barriers in the release area. Absence of GMO sensitive wildlife.

2. Methods for monitoring ecosystem effects

Not applicable, as no effects on the environment are to be produced. The presence of the GMO in the wild fauna can be verified if considered necessary.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms

Not applicable.

4. Size of the monitoring area (m^2)

7,200 m^2 approx.

5. Duration of the monitoring

Eight months (duration of the whole trial).

6. Frequency of the monitoring

Daily.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

None, as GMO release into the environment is not expected to occur.

2. Post-release treatment of the GMOs

None.

3(a) Type and amount of waste generated

Glass vials containing the freeze-dried GMO, and plastic materials for inoculation and sample collection.

3(b) Treatment of waste

Glass vials, syringes, needles, tubes and other materials in contact with the GMO will be sterilized by incineration in the same farm.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread

Sacrifice and incineration of all the animals of the farm and disinfection of all the facilities.

2. Methods for removal of the GMO(s) of the areas potentially affected

Formaldehyde, phenols and UV.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread

All the animals will be sacrificed and incinerated immediately.

4. Plans for protecting human health and the environment in the event of an undesirable effect

The GMO is based on the Infectious Bovine Rhinotracheitis Virus, which it is reported not to affect human beings, other animal species or plants.