MINISTERIO
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SUBDIRECCIÓN GENERAL
DE MEDICAMENTOS
DE USO VETERINARIO

Agencia Española de Medicamentos y Productos Sanitarios

C/Campezo 1, Edificio 8
28022 – Madrid
España
(Reference Member State)

MUTUAL RECOGNITION PROCEDURE

DRAFT PUBLICLY AVAILABLE ASSESSMENT REPORT FOR A VETERINARY MEDICINAL PRODUCT

ICTHIOVAC-LG LACTOCOCOSIS TRUCHA

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MODULE 1

PRODUCT SUMMARY

EU Procedure number	ES/V/0171/001/MR
Name, strength and pharmaceutical form	ICTHIOVAC-LG LACTOCOCOSIS TRUCHA
Applicant	LABORATORIOS HIPRA S.A
Active substance(s)	*Lactococcus garvieae* inactivated, strain TW-446.B3
ATC Vet code	QI10BC
Target species	Trout (*Oncorhynchus mykiss*)
Indication for use	For active immunization of trouts to reduce mortality caused by infection by *Lactococcus garvieae.*

MODULE 2

The Summary of Product Characteristics (SPC) for this product is available on the Heads of Medicines Agencies website (http://www.hma.eu).

MODULE 3

PUBLIC ASSESSMENT REPORT

Legal basis of original application	Mutual Recognition application in accordance with Article 12(3) of Directive 2001/82/EC as amended.
Date of completion of the original mutual recognition procedure	23rd of March 2011
Date product first authorised in the Reference Member State (MRP only)	13 rd of July 2005
Concerned Member States for original procedure	EL, FR, IT, PL, PT

I. SCIENTIFIC OVERVIEW

The product is produced and controlled using validated methods and tests, which ensure the consistency of the product released on the market.
It has been shown that the product can be safely used in the target species.
The product is safe for the user, the consumer of foodstuffs from treated animals and for the environment, when used as recommended. Suitable warnings and precautions are indicated in the SPC.
The efficacy of the product was demonstrated according to the claims made in the SPC.
The overall risk/benefit analysis is in favour of granting a marketing authorisation.

II. QUALITY ASPECTS

A. Composition

The product contains:

- Active substance:

  - inactivated *Lactococcus garvieae*, strain TW-446.B3… RPS* ≥ 75%

* RPS = Relative Percentage of Survival

- Adyuvant:

  - Montanide ISA-763 A

- Components of the excipient (PBS solution):

  - Disodium phosphate dodecahydrate
  - Potassium dihydrogen phosphate

- Sodium chloride
- Potassium chloride
- Water for injections

The container consists of 500 ml of high density polyethylene bottles, bromobutyl stoppers and aluminium caps. The particulars of the containers and controls performed are provided and conform to the regulation.

The choice of the adjuvant, vaccine strain, formulation, inactivating agent, absence of preservative are justified.

The inactivation process and the detection limit of the control of inactivation are correctly validated.

The product is an established pharmaceutical form and its development is adequately described in accordance with the relevant European guidelines.

B. Method of Preparation of the Product

The product is manufactured fully in accordance with the principles of good manufacturing practice from a licensed manufacturing site.

Process validation data on the product have been presented in accordance with the relevant European guidelines.

C. Control of Starting Materials

The active substance is *Lactococcus garvieae* inactivated; strain TW-446.B3 an established active substance.

Biological starting materials used are in compliance with the relevant Ph. Eur. Monographs and guidelines and are appropriately screened for the absence of extraneous agents according to the Ph. Eur.

The master and working seeds have been produced according to the Seed Lot System as described in the relevant guideline.

D. Specific Measures concerning the Prevention of the Transmission of Animal Spongiform Encephalopathies

Scientific data and/or certificates of suitability issued by the EDQM have been provided and compliance with the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products has been satisfactorily demonstrated.

E. Control tests during production

The tests performed during production are described and the results of 3 consecutive runs, conforming to the specifications, are provided.

F. Control Tests on the Finished Product

The tests performed on the final product conform to the relevant requirements. The tests include in particular appearance, bacterial and fungal sterility, residual formaldehyde, viscosity, particle size, emulsion stability, inyectability, safety, potency and volume control.

The demonstration of the batch to batch consistency is based on the results of three batches produced according to the method described in the dossier.

G. Stability

Stability data on the finished product have been provided in accordance with applicable European guidelines, demonstrating the stability of the product throughout its shelf life when stored under the approved conditions (24 months at 2-8ºC).

The in-use shelf-life of the broached vaccine is supported by the data provided.
Shelf life after first opening the immediate packaging: 5 hours

III. SAFETY ASSESSMENT

Three different vaccine batches were used.

Laboratory trials

The safety of the administration of one dose, an overdose and the repeated administration of one dose in the target animal is demonstrated.

PE-2002-CB-024: Study of the efficacy and innocuousness of the administration of one dose of ICTHIOVAC-LG in trout.

This study has been conducted in accordance to Good laboratory Practice Principles.
In this trial, 400 trout sized 20-30g were distributed in four groups of 100 trout each ones. Three groups were vaccinated according to the recommended vaccination programme with three different vaccine batches. The vaccination dose was 0,1 ml per fish by intraperitoneal route. The fourth group was kept as control group and was vaccinated with sterile PBS.

Fish were observed for 28 days after vaccination. General clinical signs (anorexia, abnormal swimming behaviour, abnormal external appearance and death) were recorded from D-2 to D28, local clinical signs from D0 to D14 and fish weighing was evaluated three times: D0, D14 and D28. Dead animals during this period were necropsied to establish the cause of death and the strains that had caused the deaths were reisolated

Results:
- No abnormal external appearance, anorexia, abnormal swimming behaviour were observed either in vaccinated or in control animals from D-2 to D28
- The applicant encloses results of the mean weight evolution of the different trout Groups at day 0 (vaccination day), day 28 (challenge infection) and day 49 (21 days after challenge). The weigh evolution showed no significant differences among survivors in control and vaccinated fishes. These results proved that vaccination does not affect the production parameters of fish and it is innocuous and safe.
- Necropsies carried out in survival fishes after challenge showed that no lesions in the site of inoculations could be found at 49 days after vaccination. Presence of rests of vaccine in the abdominal cavity of some fish vaccinated with 6CD3 could be found.

PE -2002-CB-025: Study of the innocuousness of the administration of an overdose of ICTHIOVAC-LG and the repeated administration of one dose of ICTHIOVAC-LG in trout.

This study has been conducted in accordance to Good laboratory Practice Principles.
It was carried out using 200 trout distributed in four groups of 50 fish, sized approximately 20g (minimum recommended size). Three groups were vaccinated with three different vaccine batches with an overdose whereas the fourth group was submitted to a mock vaccination in order to maintain a control group. The administration route was intraperitoneal inoculation, as required in E. Pharm., with an overdose consisting on a two-fold dose of the inactivated product (0.2 ml). The observation period was 14 days. At 14 th day, trout were revaccinated with one dose and were observed for 21 days.
General clinical signs, which included anorexia, abnormal external appearance,

abnormal unprovoked swimming behaviour, abnormal swimming behaviour in response to stimuli and death were recorded from D-2 to D35. Local clinical signs were recorded from D0 to D35 and fish weighing was evaluated three times: D0, D14 and D35.

At D35, the necropsy were carried out in a 5% of fishes of groups 1, 2 and 3 to observe any local reaction (degree of marking or adhesion on the peritoneum and melanotic spots in the site of injection), external and internal appearance, bacteriological analysis of internal organs and presence of vaccine residues at the peritoneal cavity.

Results:

- No mortalities were recorded during the study.
- No clinical signs were observed in any of the groups after vaccination and revaccination.
- Weight evolution was also recorded for the four groups. No significant differences were found among these groups.
- The necropsies carried out in a 5% of the survival fish showed no internal lesions, but some rests of vaccine in the abdominal cavity of the vaccinated groups. However this is not considered as relevant, since these trout were sacrificed when weighted approximately 25 g, much time before what is usual in the fish farms, where the trout reach weights of approximately 200-300g. Additionally, bacterial analyses were carried out from the abdominal contents of the survival necropsied fish and the results were negative, which confirms the safety of the vaccine.

Thus, the administration of an overdose of vaccine and the repeated administration of one dose of the vaccine showed to be innocuous when administered at the target species (trout) at the minimum recommended size.

The investigation was performed according to the recommendations of Directive 2001/82/EC as amended and the relevant guidelines.

In the SPC it is stated that: “No adverse reaction has been described and No adverse reactions have been observed after the administration of a double vaccine dose”.

No investigation of effect on reproductive performance was conducted because the vaccine is not intended for this category of animals.

There are no data suggesting that this product might adversely affect the immune system of the vaccinated animal or its progeny therefore a specific study was not carried out.

The vaccine is inactivated and thus the specific tests to be performed for live vaccines are not applicable.

The adjuvant used is included in Annex II of the European Council Regulation nº470/2009. Based on this information, no withdrawal period is proposed.

No specific assessment of the interaction of this product with other medicinal product was made. Therefore, an appropriate warning in the SPC is included.

Field studies

One multicentric field trial, referenced as **EC-2006-CB-005** has been carried out to assess both the safety and efficacy of ICTHIOVAC-LG LACTOCOCOSIS TRUCHA under field conditions.

EC-2006-CB-005
See IV efficacy

Ecotoxicity

The applicant provided a first phase environmental risk assessment in compliance with the relevant guideline which showed that no further assessment is required.
The vaccine is inactivated: there are not biohazard risks for the environment. In case of improper use of the vaccine, none of the components of the formulation is known to cause environmental problems.
The method of administration is injection. This method does not allow the direct dispersion of the product into the environment, at least in a significant quantity.

No Warnings are therefore required.

IV. CLINICAL ASSESSMENT (EFFICACY)

IV.B Clinical Studies

Laboratory Trials

The efficacy of the product has been demonstrated in laboratory studies in accordance with the relevant requirements which show that this vaccine reduces mortality caused by infection by *Lactococcus garvieae.*

In the laboratory **study PE-2002-CB-024**, four hundred fish were divided in four groups. Three groups were vaccinated with one dose of the vaccine Icthiovac LG Lactococosis trucha (0,1ml) with three different batches of vaccine, whereas a mock vaccination (same procedure but with PBS instead of vaccine) was carried out in the fourth group. All four groups were challenged at day 28 post-vaccination, and fish were observed for further 21 days.

The parameters recorded were the following:

- General clinical signs
- Local clinical signs
- Fish weighing
- Necropsies of diseased fish.
- Necropsy 5% of survivors of group 1, 2 and 3 to observe any local reaction.

The assessment of efficacy of the vaccine was calculated following the RPS (Relative Percentage of Survival).

Results:
No clinical signs were observed either in vaccinated or in control group from D-2 to D28.
RPS and specific mortality met the required established specifications. Results were the following: 96% in group 1, 92% in group 2 and 92% in group 3. It was considered that vaccination with ICTHIOVAC-LG LACTOCOCOSIS TRUCHA is an efficacious method in preventing death by Lactococcus.

PE-2002-CB-026: **Study of the duration of the immunity of Icthiovac LG lactococosis trucha in trout.**

This study was carried out in 600 trout distributed in four groups of 150 fish, sized approximately 20 -30 g (minimum recommended size). 3 groups were vaccinated with 0.1 ml of vaccine with 3 experimental batches. The fourth group was submitted to a mock vaccination in order to maintain a control group. The administration route was intraperitoneal.

The observation period was until day 180 (6 months), date in which 100 of the 150 fish of each group were submitted to a challenge. On the other hand, the 50 fish left of each group were maintained in observation until day 240 (8 months), date in which they were submitted themselves to a second challenge. All the survivors were sacrificed in an ethical way and the necropsy of 6 animals from each group (6%) was done.

The RPS values obtained in each time point (6 and 8 months) ranged between 88 and

91% at 6 months after vaccination and between 83 and 86% at 8 months after vaccination. Thus, the protection conferred by the administration of Icthiovac LG lactococococosis trucha lasts until at least, 8 months after vaccination.

Field Trials

The objectives of the clinical trials were to determine the safety and efficacy of Icthiovac- Lg lactococococosis trucha in rainbow trout (*Oncorhynchus mykiss*) on industrial fish farms, when administered to animals of the recommended minimum age, by the recommended administration route and following the recommended vaccination programme.

Two farms were selected based on previous outbreaks of lactococococosis.

Two groups of rainbow trout (vaccinated and control) were used in each fish farm. They were housed in different tanks. There were approximately 30.000 fish in each vaccinated group and 5.000 fish in each control group, at the start of the trial, weighing on average 30 grams.

The vaccination was carried out with 0.1 ml/fish, by intraperitoneal route. Fish were kept under observation until the end of the test at 201 days post-start with the aim of detect any abnormality due to the vaccination.

The control group is negative. It did not receive any treatment. No comparative product was administered, because there is no equivalent product on the national market.

To assess the safety
- General reaction (mortality, anorexia, and behavioural changes such as abnormal swimming in response or not to stimulus).
- Local reaction

The following parameters were used to assess the efficacy of the vaccine:

1. Mortality due to the pathogenic agent *Lactococcus garvieae*. The efficacy of the vaccine was assessed based on the mortality obtained and by calculating the RPS (Relative Percentage of Survival).

2. Relative morbidity to Lactococococosis: the morbidity observed, directly related to, *Lactococcus garvieae* in the vaccinated and the control groups was computed. The diagnosis of Lactococcosis must be confirmed by laboratory analysis.

RESULTS:

Safety
- No adverse side effects were detected. The behaviour and appetite were normal and no differences with the control group were recorded.
- Mortalities recorded in the vaccinated groups did not show any external symptoms that could be related to vaccine administration
- Necropsy of all mortalities was carried out with the aim of evaluate possible visceral reactions, adhesions and intraperitoneal absorption. No internal lesions that represent lack of safety such as adhesions between viscera and abdominal, pigmentation of the visceral peritoneum, laceration of the peritoneum…were observed.

Efficacy

In one of the fish farms selected no outbreaks of lactococcosis occurred during the course of the test. The efficacy of the vaccine could not be confirmed in this farm.

A natural outbreak of Lactococosis occurred at the second location causing 23.74% mortality in the control group whereas vaccinated fish only suffered a mortality percentage of 1.83%. These mortality percentages gave a RPS value of 92.29% that represents a high level of protection conferred by the vaccine administration

V . OVERALL CONCLUSION AND BENEFIT– RISK ASSESSMENT

The data submitted in the dossier demonstrate that when the product is used in accordance with the Summary of Product Characteristics, the risk benefit profile for the target species is favourable and the quality and safety of the product for humans and the environment is acceptable.

MODULE 4

POST-AUTHORISATION ASSESSMENTS

The SPC and package leaflet may be updated to include new information on the quality, safety and efficacy of the veterinary medicinal product. The current SPC is available on the veterinary Heads of Agencies website (www.hma.eu).