**SCIENTIFIC DISCUSSION**

## I. INTRODUCTION

Porcilis AR-T DF is an inactivated vaccine against progressive atrophic rhinitis in pigs. It is an aqueous vaccine, in which the immunogenic components are mixed in a dl-α-tocopherol based adjuvant. The active substances in the vaccine is Protein dO, a non-toxic derivative of *Pasteurella multocida* dermonecrotic toxin produced by a genetically modified *Escherichia coli* strain and inactivated strain Bb7 *Bordetella bronchiseptica* cells.

The dermonecrotic toxin produced by *Pasteurella multocida* is the causative agent of progressive atrophic rhinitis and the Protein dO is capable of inducing neutralising antibodies against the dermonecrotic protein. *Bordetella bronchiseptica* causes respiratory diseases in different animal species and it preferentially multiplies on the surface of the nasal mucosa. Infection of the surface of the nasal mucosa by *Bordetella bronchiseptica* promotes colonisation of *Pasteurella multocida*, but is not a requirement.

The vaccine Porcilis AR-T DF is a modification of two other Intervet vaccines, Nobi-vac AR-T and Porcilis Atrinord dO, which are already approved in several EU-member states. The Nobi-vac AR-T vaccine contains inactivated *Bordetella bronchiseptica* cells and detoxified *Pasteurella multocida* dermonecrotic toxin in a water in oil emulsion. The Porcilis Atrinord dO vaccine contains protein dO in an Al $(OH)_3$ adjuvant.

In the Porcilis AR-T DF vaccine the Protein dO is produced by a modified *Escherichia coli* strain according to the same method as the Protein dO in Porcilis Atrinord dO. In the vaccine Porcilis AR-T DF the inactivated *Bordetella bronchiseptica* cells have not been altered and are identical to the cells in Nobi-vac AR-T. The adjuvant of Porcilis AR-T DF is an aqueous dl-α-tocopherol, which is different from the two former vaccines.

The indication for Porcilis AR-T DF is reduction of clinical signs of progressive atrophic rhinitis in piglets by passive oral immunisation with colostrum from dams hyperimmunised with the vaccine.

The volume of a single dose is 2 ml and the vaccine is administered by intramuscular injection to pregnant sows and gilts. The basic vaccination consists of administration of 2 doses: the first dose is given 6 weeks before expected farrowing and the second is given 2 weeks before expected farrowing. Revaccination is recommended prior to each subsequent farrowing.

 

## II- OVERVIEW OF PART II OF THE DOSSIER: ANALYTICAL ASPECTS

### *II.A. QUALITATIVE AND QUANTITATIVE PARTICULARS OF THE CONSTITUENTS*

#### *A.1. Composition*

| | Name of Ingredient(s) | Quantity per dose | Function | Reference to Standards |
|---|---|---|---|---|
| Active ingredients | Protein dO<br>*Bordetella bronchiseptica* cells | ≥ 5.9 $\log_2$ TN. titre[1]<br>≥ 4.2 $\log_2$ Aggl.titre [2] | Induction of immunity | In-house<br>In-house |
| Constituents of the adjuvant | dl-α-Tocopherol acetate | 150 mg | Adjuvant | Ph.Eur. |
| Preservative | Formaldehyde | | Preservative | Ph.Eur. |
| Constituents of the diluent | Water for injections | | Diluent | Ph.Eur. |

[1] Mean Toxin neutralising titre obtained after repeated vaccination of a half dose in rabbits.
[2] Mean agglutination titre obtained after a single vaccination of a half dose in rabbits.

#### *A.2. Presentation*

Each bottle contains 20 or 50 ml of the vaccine corresponding to 10 or 25 doses of 2 ml.

#### *A.3. Container*

The vials are of Ph.Eur. hydrolytic class type I glass or polyethylene terephthalate (PET) closed with a Ph.Eur. type I halogenobutyl rubber stopper and sealed with a coded aluminium cap.

Acceptable certificates of analysis and descriptions for each size and type of containers and for the closures were presented.

#### *A.4. Development Pharmaceutics*

*Choice of strain*
The choice of antigens included in the vaccine is justified since the causative agent of progressive atrophic rhinitis (PAR) is a dermonecrotic toxin produced by *Pasteurella multocida*. Infection with *Bordetella bronchiseptica* is an important, though not necessary, predisposing factor of PAR.

The two antigens included in the vaccine are used in two other marketed Intervet vaccines: Nobivac AR-T and Porcilis Atrinord dO. The former vaccine consists of detoxified *Pasteurella multocida* dermonecrotic toxin and inactivated *Bordetella bronchiseptica* cells blended in a w/o emulsion with paraffin oil as the adjuvant. Porcilis Atrinord dO contains Protein dO (50 μg/dose) in an aluminium hydroxide based adjuvant suspension.

Porcilis AR-T DF is a modified version of Nobivac AR-T in which the detoxified *Pasteurella multocida* dermonecrotic toxin and the paraffin oil have been replaced with Protein dO and an aqueous dl-α-tocopherol based adjuvant, respectively.

The toxicity of Protein dO was tested under the development of Porcilis Atrinord dO and a toxic concentration of >189 μg of Protein dO was established. The ability of Protein dO induced antibodies to neutralise the native *Pasteurella multocida* toxin *in vivo* was also investigated during development of Porcilis Atrinord dO.

*Choice of adjuvant*

The aqueous dl-α-tocopherol based adjuvant was developed by Intervet to replace the mineral oil based adjuvants previously used in some of Intervet's vaccines. The choice of adjuvant is justified as less local reactions are observed and a good seroresponse against the two antigens is demonstrated in target animal and in the laboratory animals used in the potency test.

*Choice of preservative*

The vaccine contains 0.04-0.05% formaldehyde as preservative. Formaldehyde is used to inactivate the *Bordetella bronchiseptica* cells and is also added during preparation of the final product. The concentration is according to Ph.Eur. monograph 0062. The microbial efficacy of the preservative is tested using the bacteria and fungi prescribed in Ph.Eur. 5.1.3 and complies with the criteria of EU Note for guidance III/3469/92 and the criteria A of Ph.Eur.

*Overages*

The dose-response relationship study performed during validation of the batch potency test showed that a formulation of 0.25 µg/ml of Protein dO and 0.625 x $10^9$ cells/ml of *Bordetella bronchiseptica* (≈ 0.5 µg Protein dO/dose and 1.25 x $10^9$ *Bordetella bronchiseptica* cells/dose) provided a satisfactory protection in target animals after challenge. A standard formulation of 4 µg/dose of Protein dO and $10^{10}$ cells/dose of *Bordetella bronchiseptica* has been chosen for vaccine production. From the control of the filling volume it appears that a volume overage of up to 3 g are applied for both dose presentations (10 and 25 doses/vial). The volume overage is acceptable in order to extract the total amount of doses per vial.

*Choice of the potency testing of final product*

This test is based on the potency test for the already existing water-in-oil vaccine Nobi-vac AR-T. The batch potency of Protein dO is determined after two administrations of a half pig dose to rabbits by measuring the mean dermonecrotic toxin neutralising antibody titre. After the first vaccination the sera of the same rabbits are tested for antibodies against *Bordetella bronchiseptica* by an agglutination test (Blobel test). The dose-response relation in target animals has been studied and correlation with the potency test in rabbits performed. Protection of piglet from vaccinated sows against challenge infection with *Pasteurella multocida* and *Bordetella bronchiseptica* were obtained using full dose, a 1/4 and a 1/8 dose vaccine. A dose-response relation could be established for *Pasteurella multocida* dermonecrotic toxin (PMT) neutralising antibodies in the sera from sows and piglets and in the colostrum. No dose-response relation could be established for *Bordetella bronchiseptica* agglutinating antibodies.

In the batch potency test a dose-response relation could be observed for *Bordetella bronchiseptica* agglutinating antibodies after the 1st vaccination and for PMT neutralising antibodies after the 2nd vaccination of the rabbits. The minimum antibody titres for each antigen included in the vaccine are established using the average serum titres obtained from the rabbits vaccinated with a 1/8 vaccine dose which is proven protective in the target animal.

 

## *II B DESCRIPTION OF METHOD OF PREPARATION OF THE FINISHED PRODUCT*

### *B.1. Flow Charts*

Acceptable flow charts were presented, describing respectively the production of inactivated *Bordetella bronchiseptica* antigen, Protein dO antigen, adjuvant concentrate and production of the final product.

### *B.2. Detailed descriptions of the stages of manufacture*

The production steps are performed in accordance with GMP. Transfer of bulk is performed through sterile connections, under LAF or in a Grade A or B clean room (EEC air classification).

*Bordetella bronchiseptica* antigen
Following main culture fermentation in sterile medium until the optical density increase stops, the *Bordetella bronchiseptica* cells are inactivated using formaldehyde in a final concentration of 0.5% for 3 hours at Room Temperature. Maximum number of subculture of *Bordetella bronchiseptica* strain is Master Seed (MS) + 9. Production stage is maximally MS + 10. It was considered that the inoculation level range has no influence on the batch to batch consistency.

The *Bordetella bronchiseptica* strain which is used in this vaccine is dermonecrotic toxin (DNT) positive. However, the final amount of any present active DNT per dose vaccine will be far below the established minimal necrosing dose. Any possible present detoxified DNT should not be considered as an antigen component.

*Protein dO antigen*
The non-toxic derivative of *Pasteurella multocida* dermonecrotic toxin, Protein dO, is prepared from the genetically modified *E. coli* strain PTO-1. Following main culturing in sterile medium, the *E. coli* is cooled to 2-10°C. Maximum number of subcultures of the *E. coli* strains is MS + 7. Production stage is maximally MS + 8. It was considered that the inoculation level range has no influence on the batch to batch consistency.

The Protein dO containing suspension is purified by hydrophobic interaction. The final antigen concentrate is Protein dO in PBS.

Adequate details have been given concerning the conditions of use, reuse and sterilisation for the various filters used during the production of the Protein dO antigen. The reuse, cleaning process and storage conditions of the columns have been satisfactory described. Adequate information have been provided on how the undesirable substances used during the purification process of Protein dO are excluded from the final product to avoid negative effects on the target animal species. The determination of the protein content and the specific activity have been included as in process control tests and are performed on each production run of Protein dO antigen.

The maximum storage time for both antigen components has been fixed to 12 months.

*Adjuvant concentrate*
The method of preparing the concentrated adjuvant has been described in detail.

*Vaccine production*
An antigen concentrate, containing 20 µg/ml of Protein dO, 5 x $10^{10}$ *Bordetella bronchiseptica* cells/ml, 0.5% w/v formaldehyde is mixed with the other ingredients of the final product and the homogeneous mixture is filled into vials aseptically. Batch blending details are provided for the preparation of 100 kg of vaccine.

The glass vials are washed and sterilised (at least 1 min at ≥ 250°C) on the tube glass filling line.
Pumps and hoses used during filling are autoclaved (≥15 min at 121°C).

### *B.3. Validation*

*Inactivation kinetics for the Bordetella bronchiseptica cells*
Kinetics for the inactivation of the *Bordetella bronchiseptica* cells by incubation with 0.5% formaldehyde at RT were presented. The study was conducted using a production batch and two passages in production media were performed to determine the viable count in the culture after various time intervals. No growth was observed after 2 hours of incubation with formaldehyde. The 3-hour incubation period used during routine production complies with the requirements of GRIMV 1.9.2.1. Validation of the method used to control the inactivation has been provided. The study showed that culturing in TPB is of acceptable sensitivity and that the sensitivity is not affected by the presence of 0.005% formaldehyde. The control method used complies with Ph.Eur. monograph 0062.

Purification of the Protein dO antigen and consistency of the Protein dO antigen production
Remaining viable germs of the *E.coli* PTO 1 production strain are removed by sterile filtration. The removal of viable *E. coli* germs is controlled in line with Ph.Eur. monograph 0062.

The results of the test for Protein dO, total protein, specific activity and LPS have been presented for 3 consecutive production batches of Protein dO. Inconsistency between the three batches have been noted.

Inactivation of ampicillin used during production of Protein dO
The level of ampicillin at the end of fermentation of the *E. coli* PTO-1 production strain has been investigated using the agar diffusion test with *Bacillus stearothermophilus var. calidolactis* as test organism. The ampicillin content was found to be below the detection limit (<0.01 μg/ml).

Residual plasmid in Protein dO bulk antigen
Documentation regarding residual plasmid DNA in Protein dO bulk antigen was presented. It was found that the presence of bulk antigen reduces the transformation efficacy 3-4 times and the detection limit is 10 pg/μl. It was concluded that final products contain less than 380 pg of pSPE857 plasmid/dose. No residual plasmid were observed in the batch containing the highest amount of impurities.

## *II.C. PRODUCTION AND CONTROL OF STARTING MATERIALS*

### *C.1. Starting materials listed in a pharmacopoeia*

The adjuvant and the preservative are mentioned in the Ph.Eur. The excipients are of non-animal origin and they are all mentioned in either the Ph.Eur or the USP.

The microbial purity of the starting materials in general have been controlled and adequate details were provided.

### *C.2. Starting materials not listed in a pharmacopoeia*

*2.1 Starting materials of biological origin*

*Bordetella bronchiseptica* production strain Bb7 92932
The strain was isolated in 1973 by Berovic of the Dutch CVI, ID-DLO Lelystad from a piglet, and found positive in the guinea pig skin test. Intervet obtained the freeze-dried strain at an unknown passage level in 1979. This strain has been used in other vaccines for many years without complaints that could have been related to any possible biological contamination of the production strains.

Master Seed (MS):

The MS was established after one passage in TPB medium, freeze-dried and stored below -15°C. 92 vials were produced on 13 December 1983 and labelled "*Bordetella bronchiseptica* Bb7, 92.932 CDI 131283, 0.5 ml". The freeze-drying medium (autoclaved, ≥15 min at 121°C) used for preparation of MS and WS contains gelatin.

Controls and tests performed on the MS:
Purity and identification by incubation on blood agar plates an in TPB, gram stain (neg. rods) and API NE test: 1200025 (tested with WS charge 2).

Working Seed (WS):
SOP 5613.298.01 that describes the preparation of the *Bordetella bronchiseptica* WS was provided. The WS is produced by 2 passages of MS on blood agar plates. Colonies are rinsed off in freeze-drying medium (20 ml/plate) and freeze-dried and stored at below -15°C. 125 vials (0.5 ml/vial) were produced on 25 January 1991, labelled "WORK. SEED *Bordetella bronchiseptica* type 7, 25-01-91, charge 1, 0.5 ml" and controlled as described for the MS.

Batch protocols which include quality control testing were presented for the MS and the currently used WS.

*Escherichia coli* production strain PTO-1
This genetically modified strain has been prepared by the Genetic Engineering Group at the Technical University of Denmark (Lyngby, Denmark). It consists of an *E. coli* host strain. The source materials used to prepare the production strain, the *E. coli* and the gene of interest were described.

The segregational stability of the strain has been evaluated. Plasmid stability during fermenter culture was also demonstrated.

Constructional stability was demonstrated as well.

Intervet obtained the strain at an unknown passage level. This strain has been used in other vaccines for many years without complaints that could have been related to any possible biological contamination of the production strains.

**Master Seed (MS):**
The preparation of the MS was presented.

Controls and tests performed on the MS are listed below:
Purity (gram-stain and microscopically examination) and production of Protein dO. The results show that Protein dO remains in the cells during culture and that it is released after sonification.

**Working Seed (WS):**
The production of *E. coli* PTO-1 WS was adequately presented.
Controls and tests performed on the MS are listed below:
Purity, viable count and production of Protein dO.

Batch protocols, which include quality control testing, were presented for the MS and the currently used WS.

Starting materials of biological origin are of bovine, porcine or ovine source, except the yeast extract, which is of non-animal origin.

The starting materials of bovine or ovine origin used in the production of Porcilis AR-T DF are sourced from TSE free or low incidence countries fulfilling the requirements of EMEA/CVMP/145/97 paragraph 3.1. All components obtained from low incidence countries are categorised as EMEA/CVMP/145/97 paragraph 3.2 category IV. All of these products are used at the start of the production process resulting in > 6 $\log_{10}$ reduction at the end of the production process of the active components. The obtained reduction is in accordance with Ph.Eur. 5.2.5.

*2.2 Starting materials of non-biological origin*

Antifoam
A sample certificate of analysis is presented for polyalkylene glycol.

*2.3 In-house preparation of media*

The various media and buffers were adequately described. The purified water used to prepare different media and buffer meets the requirements of the Ph.Eur. The quality of the individual components of the various media and solutions is stated. Storage details are also specified. All buffers and media are controlled for the required pH at preparation and visually inspected just before use. Further, the growth media used for production of the main cultures are tested for sterility. The media and solutions are autoclaved or sterile filtrated.

## *II.D. CONTROL TESTS DURING PRODUCTION*

### *D.1. Flow charts*

The flow charts presented in Part II.B.1. are applicable to this part too.

### *D.2. Tests performed*

All the in-process tests are carried out on every production batch.

**Production of *Bordetella bronchiseptica* antigen**
Sterility of medium, purity, identity of cells, cell counts, inactivation and sterility are tested.

**Production of Protein dO antigen**
Sterility of the medium, purity, identity of cells, cell counts, inactivation and final sterility are tested.

**Production of final vaccine**
The filling volume for the 10 and 25 doses presentation is tested. The quantification of antigenic mass of Protein dO produced by *E. coli* is presented. The test has been satisfactorily validated for sensitivity, reproducibility, repeatability and detection and determination limit. Each new reference antigen will be tested 10 times together with the old reference antigen.

### *D.3. Results for consecutive batches*

Results of the test for purity, identity, sterility, inactivation and determination of cell count are provided for production of 3 batches of *Bordetella bronchiseptica* cells. Results of the test for purity, removal of production strain, sterility and determination of antigen content were provided for production of 3 batches of Protein dO antigen. The results presented were acceptable.

## *II.E. CONTROL TESTS ON THE FINISHED PRODUCT*

### *E.1. Specification*

Specification for sterility, safety, appearance, free formaldehyde, endotoxin, dl-α-tocopherol acetate and pH are given.

Provisionally and according to the current Ph.Eur. monograph 1361 "porcine Atrophic Rhinitis Vaccine (inactivated)" and the data submitted by the Applicant, the endotoxin specification has been set to ≤ $1x10^6$ I.U. per dose of vaccine.

Safety test:
Two pregnant sows obtained from herds that are free of PAR and not vaccinated against atrophic rhinitis should be used. The double dose of the first immunisation is injected on one site, in order to be able to comment on possible local reactions after overdosage. Each animal will be individually recorded. The requirements will be as following:
- Local reactions: mild temporary swelling (max 10 cm diameter), diminishing over maximum period of 14 days. No ulceration or abscesses should be observed.
- Systemic reactions: Rise in rectal temperature should not exceed 2.5 °C for a maximum period of 28 hours. Lack of appetite should be recorded for each animal.

| | |
|---|---|
| Potency Protein dO: | Serum from 10 rabbits inj. twice with 0.5 ml vaccine is tested for PMT neutralisation antibodies<br>Mean $\log_2$ antibody titre ≥ 5.9 |
| Potency *Bordetella bronchiseptica* | Serum from rabbits inj. once with 0.5 ml vaccine is tested for *Bordetella bronchiseptica* agglutinating antibodies<br>Mean $\log_2$ antibody titre ≥ 4.2 |

In the potency tests, minimum titres, which have to be achieved by at least 80% of the vaccinated rabbits, are titre ≥2 log 2.

SOP's for the control tests performed on the final product are provided.

Further to the Committee request, validation data for the sterility test have been provided. They were in compliance with Ph.Eur. monograph 2.6.1.

Validation of analytical methodologies
The validation of the potency tests performed on the final product is presented. The batch release requirements are established in a dose-efficacy study. Significant protection ($p<0.001$) against Progressive Atrophic Rhinitis was obtained in piglets born from sows vaccinated twice with a full dose, 1/4 dose and 1/8 dose. Rabbits were vaccinated with full dose, 1/2 dose, 1/4 dose and 1/8 dose and a good dose-response relationship was found for the Bb agglutinating antibodies after 1 vaccination and for the PMT neutralising antibodies after 2 vaccinations. The release requirements were based on statistical analysis of the potency results of rabbits vaccinated with a full dose.

Further to a question raised by the Committee, the use of and requirements for the internal standard have been described by the applicant. The *Bordetella bronchiseptica* suspension is tested against a standard serum with a known titre and a negative control serum. The suspension is diluted in such a way that when the standard serum is tested the right titre is obtained.

### *E.2. Results for 3 consecutive production runs*

Data were provided for 3 batches of vaccine. The results were satisfactory and showed acceptable consistency. A template of the format intended for use for the Manufacturer release document and Manufacturer batch protocol was presented. The template is in line with III/5372/93 and is acceptable for further use. Further to the Committee's request, a new proposed format batch protocol was issued by the Applicant.

## *II.F. STABILITY*

### *F.1. Stability of the Finished Product*

- After the initial assessment of the dossier, only glass vials were authorised. The assessment of stability is as follows.

Two years stability data were presented for 3 batches of final product stored in glass type I at 2-8°C. The parameters studied were the potencies of the two antigen components, pH and the formaldehyde and dl-α-tocopherol acetate content.

The results presented for the potency test of the Protein dO antigen showed some fluctuation but there was no indication towards a loss of the component's antibody inducing capability.

The titres obtained against the *Bordetella bronchiseptica* antigen of one batch at storage time 0, 9 and 15 months and of one other batch storage time 0 and 9 months were excluded because the stability study was initiated before the validation of the batch potency test was completed. Again some fluctuation in the results could be observed, but the results remained above the limit set in the finished product specification.

The pH and the formaldehyde and dl-α-tocopherol acetate content remained satisfactorily stable and within the limits set in the finished product specification.

A stability study of three batches of vaccine stored in glass type I vials has been initiated and the first results were submitted.

The Applicant proposed an in-use shelf life of 10 hours. An in-use shelf life of 10 hours seems reasonable for batches stored in glass type I vials on the basis that the product is preserved and the Ph.Eur. criteria A is satisfied. A broached vial test was performed on one batch, using 18 vials. Six vials were kept at 2-8°C as untreated samples; for 6 other vials the rubber stoppers were removed and the vials were stored at 2-8°C until testing. The last 6 vials were stored at 30 °C for 3 days. The results obtained justify the proposed in-use stability of 10 hours.

- A variation was submitted in 2001, and accepted, to add 20 and 50 ml multidose polyethylene terephthalate (PET) vials as alternative containers to the already approved 20 and 50 ml multidose hydrolytic Ph.Eur. Type I glass. The assessment of stability is as follows.

The approved shelf life for the vaccine stored in glass vials was 24 months at 2-8°C.

27 months stability data were presented for 3 batches of final product stored in glass type I and in PET vials at 2-8°C. (There was no transfer of the vaccine content from one type of vial to the other.) The parameters studied were the potencies of the two antigen components, pH and the formaldehyde and dl-α-tocopherol acetate content.

The results presented for the potency test of the Protein dO antigen and the *B. bronchiseptica* antigen showed some fluctuation but there was no clear indication of a loss of the component's antibody inducing capability. The potency results remained above the limit set in the finished product specification.

The pH and the formaldehyde and dl-α-tocopherol acetate content remained satisfactorily stable and within the limits set in the finished product specification.

It was demonstrated that the stability of the vaccine stored in either glass vials or PET vials was comparable and a shelf life of 24 months was appropriate for either vial.

*Post-authorisation note:*

In support of the variation to extend the shelf-life of the product in PET vials, further stability data from three production batches, stored up to 54 months at 2-8°C, were provided. The results justified the proposed shelf-life of 48 months for PET vials (20ml & 50ml). Note – the shelf-life of the product in glass vials remains unchanged at 24 months.

An in-use shelf life for vaccine stored in PET vials of 10 hours was accepted as identical contents of free formaldehyde were found in the batches stored in both types of vial for the study period of 27 months at 2-8°C.

 

## III SAFETY ASSESSMENT

### *A. INTRODUCTION*

**General information**
The presentation of the results from the study reports are largely in conformity with the "Requirements for immunological veterinary medicinal product" of Commission Directive 92/18/EEC, title II, parts 7 and 9.

**Relevance of the data to the product to be marketed**
The studies which document the safety under laboratory and field conditions were performed with Porcilis AR-T DF with the same formulation as the proposed product to be marketed.

**GLP/GCP**
The laboratory safety trials were conducted in compliance with GLP regulations and GLP statements are provided. The field safety trials were performed according to the E.U. guidelines of "Good clinical practice for the conduct of clinical trials for veterinary clinical products" but no GCP statement was provided.

**European Pharmacopoeia**
A new monograph for "Porcine progressive atrophic rhinitis vaccine (inactivated) 1999:1361" is published in the 1999 supplement to Ph. Eur. Some of the trials in the dossier were not performed in accordance with the demands of this new monograph.

**Completeness of safety data for each of the classes and species for which the product is intended**
The only target animal is the pig. All experiments were performed with gilts or sows treated with Porcilis AR-T DF by the recommended intramuscular route. The youngest gilts treated were 18 weeks of age and this has been specified in the SPC as the minimum age for first vaccination.

The product is mainly intended for use in sows or gilts in the second half of pregnancy and the trials include investigation of sows and gilts vaccinated up to 2 weeks prior to farrowing. The product has therefore been investigated in the most sensitive class of animals for which it is recommended.

**Evaluation of risk to non-target species and operator**
Operator safety is not discussed in the dossier. The expert concludes that with such an inactivated vaccine, no specific problems should be anticipated with respect to its use, other than the possibility of local reactions to self-injection. A warning to the operator in case of self-injection has been included in the SPC.

There is no potential risk of spreading to other animals of target or non-target species, since the vaccine is inactivated.

### *C. LABORATORY TRIALS*

Four laboratory safety studies have been performed. The first trial investigated the safety of administration of a single dose, double dose and a repeated single dose in non-pregnant gilts. The second trial investigated the safety of a repeated single dose in pregnant sows and gilts including examination of reproductive performance. The third and the fourth trials were performed further to the request of the Committee in order to provide information lacking with the studies submitted originally.

**Potency of the vaccines used in the safety trials**
The vaccines used were two production batches, with the same formulation as the one that will be introduced on the market.

***C.1 Safety of the administration of one dose***
***C.2 Safety of the administration of an overdose***
***C.3 Safety of the repeated administration of one dose***

**Safety trial in non-pregnant gilts at 18-19 weeks of age.**
Two groups of 10 gilts were used. The animals were housed in a fattening unit with 72 gilts at a farm in Boxmeer. The gilts were allocated to the groups at random after a veterinary examination. No placebo group was included. Group 1 was vaccinated once on day 0 with 4 ml of the vaccine. Group 2 was vaccinated on day 0 with 2 ml of the vaccine and revaccinated in the other side of the neck at day 21. None of the animals used were vaccinated before against atrophic rhinitis. The interval between the two vaccinations was shorter than the one recommended. However, there is no indication that this will decrease the chance of systemic reactions due to the vaccine. Therefore, the 3 weeks interval does not devalue the investigation of the safety of the vaccine.

The parameters observed for were rectal temperature (18 hours before vaccination, 0, 6, 30 and 54 hours after vaccination), systemic and local reactions as well as post mortem investigation of the injection site.

Rectal temperatures were recorded 18 hours before vaccination, 0, 6, 30 and 54 hours after vaccination. This observation-schedule was not in compliance with the Ph. Eur. Observations should have been recorded at least the day before vaccination, at the time of vaccination, 4 hours after vaccination and on the following 4 days according to Ph. Eur. 5.2.6 "Evaluation of safety of veterinary vaccines". There was an increase in rectal temperature of approximately 1°C during the first 30 hours after vaccination in all pigs. The temperatures had returned to normal after two days. This was considered acceptable.

Systemic reactions were observed from day –1 to day 7 and at day 19 post first vaccination. In both groups, after the first vaccination, all pigs had normal appearance and no abnormalities were recorded. The observations after the first vaccinations should have been continued until day 14 post vaccination according to Ph. Eur. and Commission Directive 92/18/EEC, title II, part 7, C1.

In group 2, systemic reactions have been observed daily from day –1 to day 16 after the second vaccination. The observation period for the systemic reactions after revaccination is acceptable. Many systemic reactions and abnormalities have been observed (pigs less active, abnormalities in respiration rate, one pig depressed). On day 11 one pig died that was housed in the same fattening unit as the test animals. The post mortem diagnosis was pleuropneumonia and all pigs including the test animals were treated with oxytetracycline during 6 days. On day 13 and 14 one of the test animals had additional treatment because of respiratory problems. It was impossible to conclude anything from the trial about the safety of a repeated injection of the product. The pleuropneumonia outbreak and treatments are deviations from the protocol that weakens the trial and it should have been avoided by housing the test animals separately from other pigs.

Local reactions have been investigated by inspection and palpation for size in cm and type of reaction, i.e. “hard”, “diffuse”, “warm” or “painful”.

In the double dose group (group 1), further to the first vaccination, daily observation and palpation on days 0, 1, 2, 3, 7 and 14 post vaccination revealed no visible or palpable reaction. In group 2, further to the first vaccination, daily observation and palpation on days 0, 1, 2, 3, 7 and 14 post vaccination revealed only at day 7, two pigs with local reactions at the injection site. The reactions were hard and respectively 0.5 and 0.7 cm.

Post mortem investigation was only performed in the single dose group and only on the left side of the neck, that is at the site of the first injection, 46 days after the injection and only by macroscopic inspection. Each slice of tissue was inspected for visible local reactions. No reaction was found. No microscopic investigation was performed.

In the group vaccinated twice, daily observation for local reactions on days 0, 1, 2, 7 and 14 post the second vaccination revealed no visible or palpable reaction.

The observation schedule with observations only on day 0, 1, 2, 3, 7 and 14 post vaccination (and for the single dose, second vaccination only on day 0, 1, 2, 7 and 14 post vaccination) was not in compliance with the Directive 92/18/EEC or the Ph. Eur. (5.2.6. Evaluation of safety of veterinary vaccines). Furthermore, it is not acceptable that post mortem investigation was only performed in the single dose group 46 days after the injection. The investigations should have been performed in a period of time where local reactions can be expected, i.e. 10-14 days post vaccination, so the nature and extend of the reactions will be known. The product is intended for use until 2 weeks before farrowing. This means that a sow with poor reproduction results, e.g. a stillborn litter, could be sent for slaughter 2 weeks post vaccination.

**Safety trial in pregnant sows and gilts.**
A group of 27 sows was observed for one week prior to the date of first vaccination. Fifteen animals in their first to ninth pregnancies were then selected after a veterinary health-check and an ultrasound scan to confirm pregnancy. This group of sows was selected in such a way that sows in all relevant stages of pregnancy and parity contributed to the study. Sows were vaccinated twice with 2 ml of vaccine at day 0 and day 28. None of the animals used had previously been vaccinated against atrophic rhinitis.

Before revaccination, one sow was withdrawn from the study because of an inflamed hind leg and her results were not included in the revaccination data. At term she gave birth to 10 healthy piglets.

The injection site of the first vaccination was shaven and marked with ink before injection of the vaccine. The injection site of the second vaccination was not shaven, and was marked after the injection.

Rectal temperature was measured 18 hours before vaccination and at 0, 4, 6, 30 and 54 hours after vaccination. The mean rectal temperature rise of 1.5°C during the first hours post vaccination was considered acceptable. The observation schedule was not in compliance with the Ph. Eur.5.2.6. “Evaluation of safety of veterinary vaccines”, even though an additional observation 4 hours post revaccination was included. The rectal temperatures should have been recorded 4 days following each vaccination. This is however a minor fault, since the temperatures were back to normal after 2 days.

The sows were observed for systemic reactions from 1 day before to 14 days after each vaccination. On the day of vaccination an additional observation 6 hours post vaccination was performed. In the clinical observations the sows were scored as normal, less active or depressed.

Clinical observations of the first vaccination showed that only at the observation point 6 hours post vaccination were there any systemic reactions. One sow was depressed, one was less active and did not consume the total amount of food and one sow was normal but also did not consume the total amount of food. The remaining 12 sows were normal. No systemic reaction in any of the sows was recorded on the other days.

At revaccination, clinical observations again showed that only 6 hours post vaccination were there any systemic reactions. Four sows were less active and one was depressed. The rest of the observations on all other days were normal.

The extent of systemic reactions to the vaccination was considered acceptable. The results of the clinical observations showed that most sows are unaffected by vaccination. Only during the first 24 hours after vaccination 20-36 % of the sows showed systemic reactions. The animals that were scored depressed or less active were in most cases the same as the animals which had a high rectal temperature.

Local reactions were recorded after inspection and palpation of the injection site 6 hours after vaccination and the following 3 days and then again on day 6, 10 and 14 post vaccination. The type of reaction is scored either "hard", "diffuse", "warm" and/or "painful". After the second vaccination three additional scores are listed in the table, called "red", "deep" and "spot". The "spot" is described as "a hard spot in the skin less than 1 cm". This size of less than 1 cm can, however, not be totally definite, since one "spot" appears in a sow which the previous day had a reaction size of 3 cm and the following day had a reaction size of 4 cm. It is therefore not plausible that this "spot" was less than 1 cm. The reason for not recording these spots after the first vaccination was that the investigator considered the spots to be caused by irritation of the skin at the injection site and not by the vaccine since the vaccine was administered deep in the neck muscle.

The trial showed a certain amount of local reactions at the injection site. The reactions were mainly superficial and in all but one case disappeared after 14 days. A few reactions consisted of an inflammation described as hard, diffuse, warm, painful, deep and/or red with a size up to 5 cm at the skin surface. There was no investigation of the macroscopic or microscopic lesions in the muscle and fat at the injection site. It is not acceptable that the investigator excluded some of the findings during the trial like the "spot", which was only described after the second vaccination. Even minor reactions should be recorded or it should be clearly stated in the protocol which findings that will not be recorded. The local reactions in this trial are not consistent with the findings in the previous trial where the non-pregnant gilts had hardly any reactions recorded at all after the vaccinations.

Further to the request of the Committee, another GLP safety study with the applied product was performed to provide documentation for the safety of an overdose in pregnant animals and another GLP study was also performed according to the Ph.Eur. 5.2.6 "safety of administration of one dose" with the applied product, to provide documentation for lack of local reactions after intramuscular vaccination.

**Safety of an overdose in pregnant animals**
Twenty healthy, pregnant sows, not previously vaccinated against Atrophic Rhinitis, seronegative to *Pasteurella multocida* dermonecrotic toxin (PMT) and with low *Bordetella bronchiseptica*-titres were vaccinated with a double dose and revaccinated with a single dose 4 weeks later as recommended in the Ph.Eur. monographs 1995:62 and 1999:1361. The animals were observed with the required intervals for systemic and local reactions, rectal temperature and reproductive results.

The sows showed no local reactions, and only one sow was less active and had lack of appetite on the day following the booster vaccination. Rectal temperatures showed a general increase of 0.5-2.5 ºC (two animals: 3.2 ºC) in the day following double dose vaccination. All temperatures were back to normal after 30 hours. After the single dose booster vaccination, rectal temperatures raised less, average 1 ºC (0 – 2.8 ºC).

Reproduction results were measured as number of piglets, divided into live, stillborn and mummies and compared to farm average. The size of the 3 mummies was used for calculation of approximate mummification date. The results were very close to the farm average, and within normal expected reproductive results. Two of the mummies were calculated mummified near the date of the first vaccination, but all together this does not raise concern, since the number of mummies was so low.

The experiment shows that a double dose injection is safe in pregnant sows, but rectal temperature may increase significantly after single and overdose vaccination, as mentioned in the SPC.

**Safety of administration of one dose:**
Ten commercial fattening pigs of 18 weeks of age were vaccinated with a single dose and observed for local and systemic reactions. At slaughter, 14 days later, the injection sites were investigated.

No clinical abnormalities were observed after vaccination apart from a temperature increase of less than 2 ºC. However, rectal temperatures before vaccination were high, most probably due to stress to the animals, not being used to handling. No local reactions were observed by palpation during the 14-day period following vaccination. A skin "spot" was not observed after vaccination. At post mortem two injection sites with pale discoloration were seen, with a size of 1 x 0.5 x 0.5 cm (0.25 $cm^3$). No vaccine remnants were found, nor significant tissue damage was observed. Therefore, no histological examination was performed. The results of the study indicate that sows with poor production results can be sent for slaughter safely at 2 weeks after vaccination.

### *C.4. Examination of Reproductive performance*

**Safety trial in pregnant sows and gilts.**
This trial included 15 sows and corresponds also to the second trial summarised in the previous section. Three gilts in their first pregnancy and 12 sows in different parities, ranging from second to ninth were included in the trial, to exclude a parity bias.

The course and outcome of pregnancy were recorded by counting the number of liveborn and stillborn piglets and mummies. These data were compared to the reproduction history of each multiparous sow and to farm data over the previous 2 years for gilts.

For the multiparous sows, it was stated that there was no statistically significant difference in the number of liveborn and stillborn piglets when compared to the mean of the previous litters of these sows. However, no statistical calculations were provided.

For the three gilts the number of piglets (6, 10 and 13 liveborn and 1 stillborn piglet) is claimed to be within the normal range of the reproduction results for the farm (4 – 15 liveborn piglets). The original farm data have been provided.

The size of the mummies was recorded in order to establish the time of death and compare that to the time of vaccination. Four of the mummies had died more than one week before the first vaccination. Two mummies were calculated to have died within the week before the first vaccination and 1 mummy was calculated to have died about 3 weeks after the first vaccination (and more than 1 week before the second vaccination). It was concluded that the date of death of the mummies during the pregnancies could not be connected with the vaccination dates.

Cause of death of stillborn piglets was investigated by dissection. Samples of blood, body fluids and/or lungs were taken from dead piglets and tested for Porcine Parvo Virus (PPV) and for Porcine Respiratory and Reproductive Syndrome Virus (PRRS). The study report states that no cause of death could be found at necropsy of the piglets and that there was found no indication for the presence of PPV or PRRS-V in the lung, body fluid or blood samples tested. Necropsy reports and laboratory results have been provided.

It was concluded from this study that vaccination does not seem to have a negative influence on the reproduction results of the animals. The number of liveborn and stillborn piglets appears to be equal to the previous litters of the sows. For the gilts, the results are more difficult to interpret, but again the numbers are within normal limits for porcine reproduction.

### *C.5. Examination of immunological functions*

The effect of vaccination with Porcilis AR-T DF on the immunological functions was examined by simultaneous vaccination of Porcilis AR-T DF and the non-related immunogen tetanus toxoid. Four groups of five pigs at the age of 10 weeks were injected once with either Porcilis AR-T DF, a tetanus toxoid containing vaccine or both (on one or both sides of the neck). The pigs were seronegative to PMT and TT and only 2 pigs had a *Bordetella bronchiseptica*-titre before vaccination.

It was demonstrated that the vaccine had no effect on the immunological functions i.e. the anti-tetanus toxoid titres of pigs that were vaccinated with tetanus toxoid and Porcilis AR-T DF at the same time were equivalent to the anti-tetanus titres of pigs that were vaccinated with tetanus toxoid only.

### *C.6. Special requirements for live vaccines*

Not applicable.

### *C.7. Study of Residues*

The starting materials dl-α-tocopherol acetate, polysorbate 80 and formaldehyde are included in Annex II to Commission Regulation 2377/90. The antigens are inactivated bacterial and protein material. Hence, the vaccine does not contain components, which might be a risk to human health. It is therefore concluded that a withdrawal period of zero days is acceptable.

### *C.8. Interactions*

Interactions with other products have not been investigated. Therefore the Committee has proposed that the SPC "5.6 Interactions…" should be worded as "No information is available on the safety and efficacy from the concurrent use of this vaccine with any other. It is therefore recommended that no other vaccines should be administered within 14 days before or after vaccination with the product."

Section 6.1 of the SPC "Incompatibilities" is worded "In the absence of incompatibility studies this vaccine must not be mixed with other veterinary medicinal products."

## *D. FIELD STUDIES*

Two field safety trials in pregnant sows have been provided.

**Field safety trial Porcilis AR-T DF *vs* Placebo**
The first trial was blinded and was performed on 4 commercial swine farms in Hungary. The sows and gilts were randomly assigned to the treatment or the placebo group, and injected in accordance with the suggested vaccination schedule of the product, i.e. the first injection was made 6-8 weeks prior to farrowing and the second one 28 days later. The four farms were selected because of good management and accurate recording of reproduction data. In one of the farms atrophic rhinitis was considered to be a relevant problem by the farmer. The vaccination schedule varied between the farms but none of them was vaccinating breeders against atrophic rhinitis. A production batch of the Porcilis AR-T DF and a placebo of sterile phosphate buffered saline solution (diluent for freeze-dried vaccines) were used.

Groups of 30-40 pregnant sows and gilts were selected on each farm. Gilts had been vaccinated against Porcine Parvo Virus before breeding. Randomisation was achieved by using a random table after having listed the selected animals in ascending order by their eartag or tattoo number. To achieve blinding, the persons performing the observations were not aware of the treatments of the animals.

Parameters observed in this trial were rectal temperature, local and systemic reactions including feed intake, reproductive performance and serological response to vaccinations to confirm the vaccine taking (2 of the farms are included in the efficacy field trial described in Part IV).

The statistical analyses were performed with the individual sow or gilt as the statistical unit. The level of significance was set at 0.05. Rectal temperature and antibody titres were tested by two sample and paired T-test. Local and systemic reactions were compared between the trial groups by a chi square test.

In all included animals rectal temperature was measured at both vaccinations at the following points in time: 24 hours before vaccination, just prior to vaccination, 6 hours post vaccination and 24 and 48 hours post vaccination. At 6 hours post first and post second vaccination the rectal temperature was significantly higher in the Porcilis AR-T DF than in the Placebo group ($p< 0.0001$). At no other time did the mean temperature show significant difference. Four sows treated with Porcilis AR-T DF had a temperature > 40.0 °C at 6 hours after the first vaccination. None of the other animals showed a temperature above 40.0 °C at any time. The rise in temperature of approximately 1°C in the first 24 hours post vaccination was considered acceptable.

Systemic reactions/general health or attitude was looked for 1 and 6 hours post vaccination and then daily for 14 days in all animals. The reactions were classified in 4 categories (0: no abnormalities- 1: tendency to lie down, minor signs of disease – 2: listless, drowsy, shivering, signs of disease- 3: signs of severe disease). Feed intake was recorded together with the systemic reactions and scored "Normal", "Reduced" or "Does not eat". Further to the first vaccination, all animals were "normal" after 24 hours, except one placebo treated sow that died from pulmonary oedema on day 13 post vaccination after having shown signs of disease from day 8 post vaccination and one Porcilis AR-T DF treated sow that was slaughtered on day 3 post-vaccination because of lameness resulting from an accident. Further to the second vaccination, 1% and 2% of the animals showed systemic reactions in the vaccinated and Placebo groups respectively. The small amount of systemic reactions was considered acceptable.

All animals were inspected daily for local reactions for 14 days post each vaccination. The injection site was palpated 6, 24 and 48 hours, and 7 and 14 days after vaccination, and when visually any reaction was observed. The reaction size / the reaction type were scored (from 0: no observable reaction - to 3 : > 10 cm diameter / painful, warm swelling, redness). Local reactions were seen in 22-43% of the Porcilis AR-T DF treated animals compared to 6-10% of placebo treated. The number of animals with local reaction was significantly different between product- and placebo treated groups after both first ($p< 0.05$) and second ($p< 0.001$) vaccination. The reactions had in the majority of cases disappeared after 4 days. In a few animals local reactions were visible for a longer period, up to 12 days after vaccination with the product. The size and type of local reactions were largely acceptable.

Evaluation of reproduction results was performed by recording the number of liveborn piglets, the number of stillborn piglets, the number of mummies and abortions. The results were not statistically different between the two treatment groups. No mummies were observed in any farm.

Reasons for trial withdrawal (5 sows) have been adequately documented.

The field trial supports the results from the laboratory trials. The trial was well designed and thoroughly performed and all results have been adequately discussed

Another large safety field trial comparing Porcilis AR-T DF with another vaccine against progressive atrophic rhinitis (PAR) was conducted on 25 commercial pig farms. The other vaccine contains detoxified dermonecrotic toxin of *Pasteurella multocida* type D and inactivated *Bordetella bronchiseptica* cells in a water in oil emulsion.

**Comparative field Safety trial Porcilis AR-T DF *vs* another vaccine against PAR.**
Pregnant sows were randomly assigned to one of the groups and 637 received an injection with Porcilis ART-DF and 638 received the other vaccine against PAR (Water in oil emulsion). Observations included rectal temperature from 5 animals per group per farm 6-8 hour post vaccination, local and systemic reactions and feed intake up to 5 days post vaccination, and reproductive data: parity, date of breeding/abortion/farrowing, number of piglets born alive/dead/mummified.

Systemic reactions were observed in 19% of the Porcilis AR-T DF group and in 2 % of the other vaccine group. The difference was statistically significant. These systemic reactions were mainly inactivity, listlessness, drowsiness and shivering.

Local reactions were observed in 15 % and 24 % of the Porcilis AR-T DF and the other vaccine groups respectively. The difference was statistically significant.

The reproductive results were equivalent or above the expected means, based on historical data for the individual farms. The number and distribution of abortions do not raise concerns.

The field data provided indicate that vaccination with Porcilis AR-T DF leads to a significantly higher percentage of animals showing reduced activity, reduced or absent feed intake and a significantly higher mean temperature increase than vaccination with the other vaccine on the day of immunisation.

Although the Applicant has demonstrated that with regard to fertility results no significant differences between the two vaccines can be expected, the increased incidence of systemic reactions and the higher temperature increase after vaccination with Porcilis AR-T DF has been clearly specified in the SPC.

It may be speculated that the difference in reactions is due to the kind of adjuvant used in the vaccines resulting in different endotoxin effects. It is well known that water in oil adjuvants tend to cause local reactions, but this type of adjuvant incorporates endotoxin. In contrast, the DF adjuvant is obviously much better tolerated at the injection site, but leads to a significant higher incidence of systemic reactions due to a quicker release of endotoxin. The adverse reactions reported are typical for endotoxin induced effects. Due to the difference in endotoxin release into circulation after vaccination, a comparison between the endotoxin content of the two vaccines seems impossible. To avoid pharmacovigilance problems in the future, it has been proposed to limit the endotoxin content of Porcilis AR-T DF to the maximum content shown to be safe in the safety studies. Since the endotoxin content of the batch used in this field trial was $1.3 \times 10^5$ IU, and two other production batches had similar or higher endotoxin content, the Committee would have preferred to limit the maximum endotoxin content per dose for future batch release to $1.5 \times 10^5$ IU per dose. However, in the current Ph.Eur. monograph for Porcine Atrophic Rhinitis, a limit of $1 \times 10^6$ is considered as acceptable. Therefore, the Committee accepted the argumentation of the Applicant but suggested that a request for a modification of the monograph should be made.

## *III.E. ECOTOXICITY*

Following the guidance for the environmental risk assessment for IVMPs (EMEA/CVMP/074/95) the possible ecotoxicity for using Porcilis AR-T DF is evaluated.

The product is a liquid suspension containing formaldehyde inactivated *Bordetella bronchiseptica* cells and purified Protein dO, a non-toxic derivative of *Pasteurella multocida* dermonecrotic toxin produced by a genetically engineered *E. coli* strain, as active components together with an dl-α-tocopherol based adjuvant. It is intended to be administered by injection to individual pigs. Direct exposure of the environment to the product does not take place.

As no live microorganisms are present in the product, hazards and risks from the active ingredients are likely to be negligible. Toxic effects of the product compounds are considered not to present a hazard. An assessment of the risk to the environment including hazard identification, assessment of likelihood, assessment of consequence, and assessment of level of risk is included in the application. The overall risk to the environment is assessed to “Effectively zero”. This was considered acceptable by the Committee and a second phase evaluation was not considered necessary.

## PART IV EFFICACY ASSESSMENT

### *A INTRODUCTION*

Indication of tests carried out

Four laboratory trial reports and one field trial report have been included in the efficacy documentation of Porcilis AR-T DF. The presentation of the reports conforms with the "Requirements for immunological veterinary medicinal products" (Commission Directive 92/18/EEC, Title II, parts 8 and 9).

Justification of the choice of antigens

Progressive atrophic rhinitis is caused by dermonecrotic toxin producing strains of *Pasteurella multocida*. Current opinion is that infection with *Bordetella bronchiseptica* is an important, but not necessary, predisposing factor. The toxin has a bone resorbing effect. The osteogenesis of the osteoblasts of the nasal turbinates is inhibited by the toxin and the osteoclastic osteolysis of the nasal turbinates is stimulated by the toxin. This may result in total atrophy of the turbinate structures in the snout and alterations of the nasal bones. Clinical symptoms of the disease are deviation of the nose, nasal bleeding, shortening of the upper jaw and growth retardation. The pigs are then more sensitive to lung infections because of the effect on the nose barrier. It is very important to give the piglets the earliest possible protection after birth because infection only results in atrophic rhinitis if the infection takes place at an early age,. This can be achieved by vaccination of the pregnant animals, thereby providing maternally derived antibodies to the piglets via the colostrum.

The current application for Porcilis AR-T DF includes both the colonising and toxogenic antigens of atrophic rhinitis. The *Bordetella bronchiseptica* component is a formaldehyde-inactivated strain, Bb 7, originally obtained from an atrophic rhinitis problematic farm. The *Pasteurella multocida* toxin component is a non-toxic Protein dO, which is a 1281 amino acids long derivative of *Pasteurella multocida* dermonecrotic toxin, capable of inducing neutralising antibodies against the native toxin. It is produced by genetically engineered *E. coli*. The adjuvant is dl-α-tocopherol based and has been developed to replace the mineral oil based adjuvants in a number of vaccines. It induces good seroresponses against the antigens and causes less local reaction at the injection site.

The vaccine is a development and combination of two other atrophic rhinitis vaccines produced by Intervet. The following table shows the contents and the marketing authorisation status in the EU of the three vaccines:

| Contents of | PM-toxin component | *Bordetella bronchiseptica* component | Adjuvant | Authorisation status in EU |
|---|---|---|---|---|
| Porcilis AR-T DF | Protein d O<br>4 µg/dose | Bb strain 7<br>$10^{10}$ cells/dose | dl-α-tocopherol | (Current application) |
| Nobi-vac AR-T | PM-toxoid,<br>1,8 µg/dose | Bb strain 7<br>$10^{10}$ cells/dose | Paraffin oil | BE, FR, DE, NL, IR, IT, PT, ES, UK |
| Porcilis Atrinord dO (=Protech AR) | Protein d O<br>50 µg/dose | | $Al(OH)_3$ | AT, BE, DK, FI, DE, NL, NO, PT, ES, SE, UK. |

Relevance of the data to the product to be marketed

All studies are carried out with vaccine batches of Porcilis AR-T DF with the same formulation as the proposed one and are therefore relevant to the product to be marketed. In order to establish a dose-response relation and to verify the choice of dose, trial IV.D.2 also includes testing of diluted vaccine batches.

The vaccination/challenge test performed in target animal deviates from the potency test prescribed in the Ph. Eur. 3rd Ed. (1999 suppl.) monograph 1361 "Porcine progressive atrophic rhinitis vaccine

(inactivated)". This is acceptable as the monograph was implemented after development of the vaccine.

Relevance of the data for recommended uses
The trials were conducted with the recommended route of injection in the recommended classes of animals, and the vaccination scheme was tested with regard to basic vaccination and revaccination. The presented data are relevant for the vaccine.

## *B GENERAL REQUIREMENTS*

Data required from laboratory and field trials on

Each category of each target species
The target animals of the vaccine are piglets receiving colostrum from vaccinated sows. The vaccine is tested with vaccination of pregnant sows, followed by challenge of the piglets and investigation of antibodies in sows, colostrum and piglets.

Recommended route of administration
The recommended intramuscular route of administration has been used in all trials.

Proposed schedule of administration
All efficacy data were obtained after basic vaccination with a 4 weeks interval of 2 ml vaccine. In 2 studies the animals received a single revaccination 2-4 weeks before the subsequent farrowing(s) after the basic vaccination. The proposed schedule of administration is in accordance with the schedule used in the trials.

Effect of passively acquired and maternally derived antibodies
The effect of maternally derived antibodies was investigated in the piglets from vaccinated sows. The vaccine is intended for use in pregnant or non-pregnant breeder gilts or sows. These animals are too old to have maternally derived antibodies remaining that could influence the effect of vaccination. The trials include serological testing of antibody titre against the two antigen components of the vaccine prior to vaccination. The included animals had negative PMT-titre, and negative or low *Bordetella bronchiseptica*-titre, and they had not been vaccinated previously against atrophic rhinitis.

Claims regarding onset and duration of protection
The onset of protection in the piglets takes place after the ingestion of colostrum, and is demonstrated by challenge and serology at 3 days of age. The serological investigation of the immune response in the sows showed antibody titres against both antigen components in the vaccine 2 weeks after the second vaccination of the basic vaccination schedule. This is in accordance with the recommended vaccination schedule, where revaccination should be performed 2-4 weeks prior to each farrowing.

The duration of immunity was investigated by serological testing of the sows, the colostrum and the piglets after revaccination of the sows 2-4 weeks prior to the subsequent farrowing(s). The revaccination induced equal or higher levels of antibody than the basic vaccinations.

Each component
The vaccine consists of two antigen components – the Protein dO, a derivative of the progressive atrophic rhinitis causing *Pasteurella multocida* toxin, and the important predisposing, but not necessary factor, *Bordetella bronchiseptica*. Serological investigation of both antigens was performed in the trials. Challenge experiments were performed with a combined challenge mimicking the natural conditions of the disease, where *Bordetella bronchiseptica* affects the nasal mucosa after which *Pasteurella multocida* is able to colonise.

GLP/GCP:
No GLP statements were provided in any of the laboratory trials, and no GCP statement was provided in the field trial, although it is claimed to be performed according to the E.U. guidelines of "Good clinical practice for the conduct of clinical trials for veterinary clinical products".

Potency of the vaccines used in the efficacy tests:
The vaccines used were from two production batches with the same formulation as the proposed one, except in one trial where 3 diluted batches and a placebo batch also was used. A sterile phosphate buffered saline solution (diluent for freeze-dried vaccines) was used as placebo in the field trial. The potency of the batches was tested in rabbits according to an SOP. The results have been provided. As the vaccine contains standard amounts of antigen, no minimum or maximum antigen batches were tested.

## *C. LABORATORY TRIALS*

**Challenge trial in piglets**.
The objective of this trial was to provide documentation for the efficacy of the vaccine when administered as recommended to sows in the second half of pregnancy, followed by challenge of the piglets. Two groups of three sows were constituted. Sows were obtained from a farm that had been free of atrophic rhinitis for 10 years, although *Bordetella bronchiseptica* had been isolated on several occasions. They were not vaccinated against atrophic rhinitis before the start of the experiment. Group 1 was vaccinated twice according to the recommended scheme and group 2 was not vaccinated. All piglets from these sows were challenged with *Bordetella bronchiseptica* at 3 days of age and with *Pasteurella multocida* at 7 days of age. Challenge was performed by intranasal infection with 1 ml of a *Bordetella bronchiseptica* culture, strain Bb92932 (which is the same as the production strain) at day 3, followed by intranasal infection with 1 ml of a *Pasteurella multocida* culture strain Pm47459 into each nostril of all piglets. Minimum two piglets per litter randomly selected were then investigated at 70 days of age, and the rest of the piglets were investigated at 174 days of age.

One sow aborted 2 days after the first vaccination and was excluded from the trial and replaced by another. Seven piglets died during the study, 3 from the vaccinated group and 4 from the control group. There were no comments or necropsy results provided.

Blood sampling was performed on sows at each vaccination and 2 weeks post second vaccination. Colostrum was sampled immediately after farrowing. Blood sampling was performed on piglets on day 3 of age. The blood samples and colostrum were analysed for antibodies against *Bordetella bronchiseptica* and PMT using ELISA methods and for neutralising antibodies against PMT using Vero-cells. The vaccinated sows show good seroconversion against PMT and an increased titre against *Bordetella bronchiseptica* after the second vaccination. In both the vaccinated and the control group, the colostrum titres reflect the serum titres. One of the vaccinated sows had lower titres for both PMT and *Bordetella bronchiseptica* than the other two, and this sow also had lower colostrum titres. As expected the piglets from this sow also showed lower titres than piglets from the 2 other vaccinated sows.

Clinical observation of sneezing frequency in a 5-minute period was counted for each litter daily from day 14 to day 39. No sneezing at all was observed in the litters of the vaccinated sows. The 3 control litters all had sneezing observed every day varying from 1 to 16 sneezes during the 5-minute period of observation. A marked difference between the groups was seen: the vaccinated group seemed unaffected while the control group was clearly affected.

Piglets were weighed at birth, at day 28, day 70 and at slaughter. Daily weight gain was calculated for all litters and for all intervals and clearly showed the better performance by the vaccinated group. The differences between the groups in average daily weight gain ($0.66 \pm 0.05$ kg in the vaccinated group versus $0.44 \pm 0.09$ kg in the control group) was statistically significant as determined by a student's paired t-test ($p < 0.005$).

Nasal swabs from the left nostril were taken at 3, 14, 28, 42 and 70 days of age. The right nostril was not swabbed in order to avoid damaging at least one nostril. Toxin producing *Pasteurella multocida* was isolated in 4 of the 19 piglets from the experimental group and only at the sampling at day 14 of age. In contrast it was isolated from all 26 piglets in the control group at 14 days of age and in approximately 50 – 75 % of the control piglets at the other sampling days. *Bordetella bronchiseptica* was isolated in the experimental group with a frequency of 15-63 % at the samplings at day 14, 28 and 42 of age, but not at day 70. In the control group *Bordetella bronchiseptica* was isolated at day 14, 28, 42 and 70 in 19-81% of the piglets. Quantification of the isolated bacteria showed a higher number in the piglets of the control group.

The degree of shortening of the upper jaw was estimated by determining the distance between the cutting edges of upper and lower jaw in millimetres. In the normal pig, the cutting of the upper jaw is approximately 1 mm in front of the cutting edge of the lower jaw. If the upper jaw is shorter than the lower jaw the value is preceded with a minus sign.

| Distance in mm | Day 40/43 | Day 70 |
|---|---|---|
| Average, vaccinated group | 0.7 (±1.0) | 0.1 (±1.1) |
| Average, control group | - 6.4 (±2.6) | - 11.1 (±4.2) |

The results were statistically significant between the groups as determined by a student's paired t-test ($p < 0.001$).

Conchae atrophy and septum distortion were investigated as follows: The snouts of the piglets were sawn transversally between the first and second premolar teeth. The scores were set on a scale ranging from 0 – 4 starting with 0 = "no distortion and no atrophy" to 4 = "very severe atrophy, distortion of snout or nasal septum and discrepancy between upper and lower jaw". The investigations were performed at day 70 of age for the piglets sacrificed at this time and at slaughter at day 174 for the remaining pigs.

For the piglets sacrificed at 70 days of age, the experimental group had no atrophy observed at all. The control group had atrophy in every piglet ranging from score 2 to 4, i.e. all severe atrophy.

For the piglets examined after slaughter at day 174 of age, minor deformations of the conchae and septa were found in some of the piglets of the experimental group. The control group showed atrophy in all pigs, with scores ranging from 3 to 4, i.e. very severe atrophy and some septum distortion. This showed a marked difference between the two groups with almost normal noses in the experimental group and almost total atrophy in the noses of the control group.

Signs of pneumonia and pleuritis was investigated post mortem in all piglets at 70 and 174 days of age respectively. At 70 days of age no lesions or pneumonia was seen in any of the groups. At 174 days of age 4 of 14 control pigs showed signs of pneumonia, while none of the 11 experimental pigs had pneumonia signs.

From this trial, it was concluded that the tested vaccine showed very good protection against challenge of the piglets from vaccinated sows compared to the control animals. All together this trial indicates that the vaccine has a pronounced protective effect.

**Challenge trial for validation of the potency test**.

To establish a dose-response relation and to correlate this response to the potency assay in rabbits, the vaccine was tested in different doses, i.e. full dose(group A), ¼ dose (group B), 1/8 dose (group C) and placebo (group D).

Three sows per group were vaccinated 6-7 weeks prior to farrowing and revaccinated 4 weeks later. Blood samples were taken at each vaccination and 2 weeks after the second vaccination. At first

vaccination all sows were negative for both PMT and *Bordetella bronchiseptica* antibodies, (titres of 1 or <1). Colostrum samples were taken at farrowing. The piglets were blood sampled at 3 days of age.

There was seroconversion in the vaccinated sows for *Bordetella bronchiseptica* 4 weeks after the first vaccination. There was no clear dose-response relation found. In the control sows a minor, but unexplained titre-rise was seen in 2 sows.

For PMT neutralising antibodies the seroconversion does not happen until 2 weeks after second vaccination. A dose-response relation was seen for PMT in group A, B and C both in the serum of the sows, in the colostrum and partly in the serum of the piglets.

Piglets were challenged intranasally with *Bordetella bronchiseptica* culture at 3–6 days of age, followed by intranasal challenge with *Pasteurella multocida* culture 4 days later. Descriptions of strains, production and strength of the cultures were provided. Piglets were sacrificed and necropsied at approximately 42 days of age. The lungs were examined for the presence of pneumonia and pleuritis, and the degree of conchae atrophy was determined. The scale used for scoring of conchae atrophy ranged from 0 to 4, with 0= "no atrophy or distortion" and 4= "very severe atrophy, distortion of snout or nasal septum and discrepancy between upper and lower jaw". There was no precise description of the methods for lung examination or necropsy of the snouts.

There was almost full protection of the piglets in all the 3 vaccinated groups. There was no significant difference found between the vaccinated groups on conchae atrophy, but all vaccinated groups were significantly different from the placebo group ($p<0.0001$). Seven piglets died during the trial, 5 of these for causes unrelated to the challenge, 1 (full dose) for unknown reasons on day 8 of age, and 1 (placebo) died on day 11 of age of necrotizing pneumonia after challenge.

Rabbits were vaccinated twice with the same preparations as the sows, plus a ½-dose group, with 4 weeks interval. Similar results as for the sows were found. A dose-response relation was found for the PMT-titres 2 weeks after the second vaccination. For *Bordetella bronchiseptica* a dose-response relation was found after the first vaccination but not after the second where titres in the groups were similar.

From this trial, it was concluded that the vaccine gave almost full protection against challenge of the piglets in all 3 vaccinated groups. This means that even 1/8 of a normal dose will provide good protection. There was no statistically significant difference between the 3 vaccinated groups, but they were all significantly different from the placebo group. The serum titres of the rabbits show similar results and it can be concluded that this test can be used in the release criteria for the batch potency test.

**Efficacy trial on duration of immunity**

The objective of this trial was to determine the effect of revaccination in the following pregnancies by means of serology. No group of control animals was included. Each vaccination was performed with 2 ml administered by intramuscular injection in the neck. Eight healthy gilts or sows without detectable PMT antibody level and with low *Bordetella bronchiseptica* antibody level were used. They were vaccinated according to the recommended scheme prior to a first farrowing, and then revaccinated 2-4 weeks prior to a second and third farrowing. One sow died during the trial of unknown reason and 2 sows were sold by the farmer. All sows that farrowed had normal sized litters.

Blood sampling of the sows was performed 12 times during the trial: at each vaccination and approximately 2 weeks after the vaccinations (except for the first vaccination), and approximately 2, 5 and 11 weeks after the first farrowing, and 5 and 12 weeks after the second farrowing. Colostrum samples were taken at each farrowing.

The serum titres for both *Bordetella bronchiseptica* and PMT are at similar level after second, third and fourth vaccinations. No statistical analysis has been made, but the titres correspond well to the

titres found in the potency validation trial, that provide almost full protection against challenge of the piglets.

The colostrum titres have increased in the following farrowings for both *Bordetella bronchiseptica*-titres and PMT titres, when compared to the first farrowing. No statistical analysis has been made, but the colostrum titres also correspond well to the titres found in the potency validation trial, that provided almost full protection against challenge of the piglets.

It was concluded from this trial that there was a good effect of revaccination 2-4 weeks prior to farrowing in the following pregnancies, as recommended in the Summary of Product Characteristics. Serum titres and colostrum titres for both antigens are at similar level as those found in the potency validation trial, where the vaccine provided almost full protection against challenge in the offspring.

**Efficacy trial on comparison of Porcilis AR-T DF with another vaccine against PAR, antibody response.**

The objective of the trial was to compare antibody levels in serum and colostrum of sows and in serum of piglets after vaccination with one of two atrophic rhinitis vaccines. Randomisation methods for distribution and selection of sows and piglets have been provided. Each vaccination was performed with 2 ml administered by intramuscular injection in the neck.

Healthy gilts or sows coming from a herd without signs of atrophic rhinitis without detectable PMT antibody level and with low *Bordetella bronchiseptica* antibody level were used. Group A (6 sows) was vaccinated with one existing vaccine against PAR at 8 and 14 weeks of pregnancy; group B (6 sows) was vaccinated with Porcilis AR-T DF at 10 and 14 weeks of pregnancy; Group C (6 sows) was vaccinated with Porcilis AR-T DF at 4, 8 and 14 weeks off pregnancy, and group D (3 sows) was not vaccinated. Group B corresponds to the product and vaccination schedule in the application of Porcilis AR-T DF. The vaccination schedule tested in group C was not in compliance with the recommendation for Porcilis AR-T DF.

Colostrum samples were taken from the vaccinated groups at farrowing. Blood was sampled from the sows at the time of first vaccination and 2-5 days post farrowing. Blood was sampled from 3 piglets from each litter at 2-5 days of age and at 4 weeks of age.

Samples were tested for *Bordetella bronchiseptica* antibodies in the Blobel agglutination test, as in the previous trials. All samples were tested for PMT neutralising antibodies in the Verocell-neutralisation test. Sera from piglets were also tested for PMT in a more sensitive ELISA system because several of the piglets showed results under the detection limit.

No statistical analysis has been provided, but looking at the titres for *Bordetella bronchiseptica*, the following can be stated. Group B, Porcilis AR-T DF used as recommended on the label, has lower mean *Bordetella bronchiseptica*-titres than both group A and group C (Porcilis AR-T DF) administered 3 times during pregnancy. The titres found in group B were not much different from the titres obtained in the Potency validation trial where almost full protection against challenge of the piglets was shown.

No statistical analysis has been provided, but looking at the titres for *Pasteurella multocida* toxin, the following can be stated: In group B, the serum PMT-titres of both sows and piglets and the colostrum titres were lower than seen in the previous trials. The titres of the young piglets and in the colostrum are, however, not lower than those which provided almost full protection in the Potency validation trial.

The colostrum titres are reflected in the titres of the young piglets in the majority of the animals.

Group B, Porcilis AR-T DF used as recommended on the label, had higher mean PMT- titres than group A. This is mainly because 3 of 6 sows in group A do not seroconvert after the vaccinations.

Their piglets do, however, show a slight seroconversion at 2-5 days of age, detectable with the sensitive ELISA method. Group B shows lower PMT-titres than group C, which is Porcilis AR-T DF vaccinated 3 times during pregnancy.

In this trial the optimal seroresponse for both PMT and *Bordetella bronchiseptica* comes from Porcilis AR-T DF injected 3 times during pregnancy. This is, however, not the vaccination schedule of this application. The applied vaccination schedule for Porcilis AR-T DF, as used in group B of this trial, shows equal results to the other vaccine at the PMT-titres, but lower *Bordetella bronchiseptica*-titres.

In group B, the serum PMT-titres of both sows and piglets and the colostrum titres are lower than seen in the previous trials. The titres for group B are, however, not much different from those, which provided almost full protection in the Potency validation trial.

No statistical analysis was provided in this non-GLP trial and therefore the results can only be regarded as indicative.

## *D FIELD TRIALS*

**Efficacy field trial, duration of immunity IV.D.3.**.

This trial was also part of the safety field trial described in Part 3.

This was a blinded trial performed on 2 commercial swine farms in Hungary. The sows and gilts were randomly assigned to the treatment or the placebo group, and injected in accordance with the suggested vaccination schedule of the product and reinjected prior to the following farrowing (approximately 6 months later). The farms were selected because of good management and accurate recording of reproduction data. On farm 1 atrophic rhinitis was present; on farm 2 there were no clinical signs of atrophic rhinitis. The animals were healthy reproduction swine between 6 and 8 weeks before expected date of farrowing at the time of first vaccination. Pregnancy was confirmed by a Doppler effect pregnancy detector between day 28 and 49 after the last breeding. None of the animals had previously been vaccinated against atrophic rhinitis. Gilts had been vaccinated against Porcine Parvo Virus before breeding.

Randomisation was achieved by using a random table after having listed the selected animals in ascending order by their eartag or tattoo number. To achieve blinding, the persons performing the laboratory analysis of the blood samples were not aware of the treatments of the animals.

Parameters recorded for this study were antibody titres against *Bordetella bronchiseptica* and *Pasteurella multocida* toxin (PMT). Results have been given in a $\log_2$-scale. Blood sampling was performed at the following times: the day before first vaccination, the day before second vaccination, after first farrowing, at weaning (4 weeks post partum), 4-6 weeks after conception (=last service), at revaccination, and after second farrowing.

There was a statistically significant difference for *Bordetella bronchiseptica* titres ($p<0.01$) between vaccinated and controls at all timepoints except at first vaccination, i.e. bloodsamples 2-7. In the vaccinated group the increase seen from the first to the second vaccination and again to post the first farrowing was statistically significant ($p<0.01$). So also was the increase from revaccination to post the second farrowing. In the control group the increase seen from the second vaccination to post the first farrowing was also statistically significant ($p<0.05$). It is explained with the circulation of *Bordetella bronchiseptica* in farm 1. The decrease in both groups seen from the first farrowing to weaning was also statistically significant.

There was a statistically significant difference for *Pasteurella multocida* toxin titres ($p<0.01$) between vaccinated and controls at samplings 3, 4, 5, 6 and 7, i.e. from after the second vaccination. In the vaccinated group there was a significant increase ($p<0.01$) from sample 2 to 3, i.e. after second vaccination, and from 6 to 7, i.e. after revaccination. The decrease seen from sample 3 to 4 and 4 to 5, i.e. post the first farrowing to weaning and on to post conception was also statistically significant. The increase seen in the placebo group at sampling 7, though not statistically significant, came from 2 animals with a very sharp rise in titres. These animals had been practically seronegative in the previous samples. The investigator explains the results with that the samples by mistake, during handling or in the laboratory, could have been mixed up with 2 samples from vaccinated animals. Looking at the individual sample results, this explanation is plausible.

Seroconversion was defined as a fourfold or higher increase in the antibody titre (log2-scale). For *Bordetella bronchiseptica*, seroconversion in the vaccinated animals was seen in 100% after first farrowing and in 97% after second farrowing. For *Pasteurella multocida* toxin seroconversion in the vaccinated animals was seen in 87% after first farrowing and in 94% after second farrowing.

The trial supports the findings from the laboratory trials, regarding serology. There was a good seroconversion in the vaccinated animals after second vaccination and after revaccination in the following pregnancy. The antibody titres are at similar levels seen in the laboratory trial for potency validation. The trial was well performed and statistical analysis shows significant differences between

vaccinated and placebo treated animals. It would, however, have been a very strong field trial if the trial design had included data from the performance of the piglets at the farm where atrophic rhinitis was present with clinical signs.

## RISK-BENEFIT ASSESSMENT AND CONCLUSION

The data submitted in the dossier and in response to questions confirm the acceptability of the proposed formulation and presentations, the suitability of the specification for the active ingredient, the method of manufacture of the product and the validity of the test methods applied to the product, given that the Applicant has committed to provide some complementary data to answer deficiencies identified by the Committee within a specified time frame. The stability tests provided for the finished product show that the product is stable for 24 months. The retained in-use shelf life is 10 hours.

The studies on the safety of the administration of one dose and on the safety after a repeated administration of one dose showed that the administration of the vaccine can induce in up to 36% of the animals systemic reactions such as lack of appetite, reduced activity, depression and increase in body temperature during the first 24 hours post vaccination. Local reactions can also occur. These reactions are mainly superficial and should disappear after 14 days. A few reactions consist of inflammation described as hard, diffuse, warm, painful, deep and/or red with a size up to 5 cm at the skin surface. Taking into account the importance of the disease, the benefits of the vaccination should outweigh the risks and such lesions are therefore acceptable.

The safety of the administration of an overdose has been performed and is considered acceptable. No other side effects than those recorded using one dose have been recorded.

The vaccine has been administered to gilts and sows according to the recommended vaccination scheme. No negative effects on reproductive performance have been recorded.

Porcilis AR-T DF does not contain live organisms, consequently there is no risk for the environment associated with the use of the vaccine.

Efficacy trials with Porcilis AR-T DF showed good protection against clinical signs of atrophic rhinitis after challenge of the piglets from vaccinated sows compared to the control animals. The challenge testing was performed with the combined challenge, which mimics the natural conditions of the disease. Seroconversion for *Bordetella bronchiseptica* takes place in the sows after the first vaccination and for *Pasteurella multocida*-toxin after the second vaccination. The good seroconversion in the sows is reflected in the antibody titres in the colostrum, which again corresponds well to the titres of the piglets. Toxin producing *Pasteurella multocida* is eliminated sooner and does not colonise in as many piglets in the vaccinated group, as in the control group. *Bordetella bronchiseptica* does not show the same clear differences. The weight at slaughter and the daily weight gain of the piglets are markedly different in favour of the vaccinated group. The clinical signs of atrophic rhinitis measured such as frequency of sneezing, shortening of the upper jaw, turbinate atrophy and nasal septum distortion are also clearly different, with severe signs in the control animals compared to almost no signs in the piglets from the vaccinated sows.

The choice of dose was investigated in a trial where sows were vaccinated with a full dose, a 1/4 dose or 1/8 dose. The vaccine gave almost full protection against challenge of the piglets in all 3 vaccinated groups. There was no statistically significant difference between the 3 vaccinated groups, but they were all significantly different from the placebo group. However, the potency at the end of the shelf life has been adequately documented for the retained dose.

Effect of revaccination 2-4 weeks prior to farrowing in the following pregnancies, as recommended on the label, was investigated serologically. The titres were equal or higher after revaccination compared to basic vaccination.

In conclusion, the product is efficacious demonstrating very good protection against the clinical signs of atrophic rhinitis in the piglets by passive oral immunisation with colostrum from dams hyperimmunised with the vaccine.

Based on the original and complementary data presented and in view of the commitments provided by the Applicant, the Committee for Veterinary Medicinal Products concluded that the quality, safety and efficacy of the product were considered to be in accordance with the requirements of Council Directive 81/852/EEC.

 