SCIENTIFIC DISCUSSION

Product name:	Neocolipor
Procedure No.:	EMEA/V/C/035
Applicant company :	Rhône Mérieux (Merial) 254 rue Marcel Merieux Lyon France
Active substances and strengths:	Adjuvanted inactivated porcine vaccine containing recombinant bacterial *E.coli* strains F4 and F5 and field strains F6 and F41.
(ATCvet code)	(QI09AB02)
Proposed International Non-proprietary Name:	n/a
Pharmaceutical form:	Suspension for injection.
Strength	per dose of 2 ml: adhesins F4 (F4ab, F4ac, F4ad), at least 2.1 SA.U* adhesin F5, at least 1.7 SA.U* adhesin F6, at least 1.4 SA.U* adhesin F41, at least 1.7 SA.U* *: 1 SA.U: quantity sufficient to obtain an agglutinating antibody titre of 1 $\log_{10}$ in the guinea pig. Adjuvant: Aluminium (as hydroxide*)* 1.4 mg
Presentation:	Single glass vials of 10, 20, 50 and 100 ml, containing 5, 10, 25 or 50 doses.
Package size:	Box of 5, 10, 25 and 50 dose vials
Target species:	Pigs (sows and gilts).
Withdrawal period:	Zero days
Route and method of administration:	Intramuscular injection
Product type:	Immunological
Therapeutic indication:	Reduction of neonatal enterotoxicosis of piglets, caused by *E. coli* strains, expressing the adhesins F4ab, F4ac, F4ad, F5, F6 and F41, during the first days of life.

SCIENTIFIC DISCUSSION

1. INTRODUCTION

Neocolipor is an inactivated vaccine for Neonatal Piglet Colibacillosis. It contains the *E. coli* antigens F4, F5, F6 and F41 and is presented in vials of 5, 10, 25 and 50 doses with aluminium hydroxide as adjuvant and thiomersal as a preservative. The target species is pigs (sows and gilts) and the route of administration is intramuscular. The vaccine is intended for use 5-7 weeks before farrowing with a second vaccination at 2 weeks prior to farrowing. A booster vaccination is given 2 weeks prior to each subsequent farrowing.

The vaccine which is to be administered by injection to sows/gilts thus enabling their progeny to derive a passive immunity during their first days of life against neonatal enterotoxicosis, caused by *E. coli* strains carrying the adhesins F4, F5, F6 and F41.

Neocolipor qualifies for the centralised system under Part A of the Annex to Council Regulation (EEC) No. 2309/93 as it has been developed using recombinant DNA technology.

2. OVERVIEW

2.1 QUALITATIVE AND QUANTITATIVE PARTICULARS OF THE CONSTITUENTS

The product contains:

Active Component:

For a 2-ml dose :

E. coli, recombinant strain F4 (Fab, Fac, Fad), at least	2.1 SA units[1]
E. coli, recombinant strain F5, at least	1.7 SA units[1]
E. coli, strain F6, at least	1.4 SA units[1]
E. coli, strain F41, at least	1.7 SA units[1]

[1]1 SA unit = q.s. to obtain a seroagglutinating antibody titre of 1 $\log_{10}$ in the guinea-pig.

Adjuvant :

Aluminium (as hydroxide) 1.4 mg

Other constituents :

Thiomersal
Saline solution

Container and Closure:

For the 10-ml, 20-ml, 50-ml and 100 ml vials: type I glass (Ph. Eur.). The closure is butyl elastomer with a non-tearable operculated aluminium cap.

Product Development Studies

The Applicant has chosen the standard way to use *E. coli* strains against neonatal colibacillosis. Recombinant strains were selected because the yield in antigens during production is better. The Manufacturer has justified the use of a preservative as the product is presented in multidose containers (thiomersal is included in Annex II of Council Regulation (EEC) 2377/90 for multidose use only).

2.2 METHOD OF PREPARATION

The plasmid carrying the concerned operon, derived from the *E. coli* field strain, is digested by a restriction enzyme. The fragment is separated on agarose gel and identified with a specific probe. The fragment is integrated into one site of the cloning plasmid. This inactivates the gene coding for resistance to tetracycline. This plasmid is then digested by restriction enzymes. The obtained fragment contains the concerned operon. It is integrated in the expression vector plasmid. The plasmid obtained is Tet^S Amp^r. The host organism is an *E. coli* strain, derived from a K12 strain not known for expressing any virulent characteristics.

The master seed bacteria was obtained for each strain after multiplication of the concerned strain in an appropriate agar medium and is stored freeze-dried. The working seed lots are obtained by culture of one ampoule of master seed bacteria (a maximum of two passages are carried out from the master seed bacteria) in an appropriate mediumand is stored freeze-dried. The active ingredient is obtained by inoculation of medium with one ampoule of working seed lot (5 passages at most from the working seed lot). Culture is stopped at the end of the growth phase. It is inactivated with formaldehyde. Bacterial cells are harvested and constitute the active ingredient.

2.3 CONTROL OF STARTING MATERIALS

The sequence of the ends of inserts surrounding the cloning sites and the sequence of the insert itself have been controlled to show that it contains the operons F4ab, F4ac, F4ad and F5. The protein expression of these factors, and F6 and F41, and the antigenicity have been checked with monospecific immune sera and by immunoprecipitation. The starting materials of both biological and non-biological origin are controlled. The various strains are checked with regard to identity and purity before and after inactivation.

The control tests carried out on culture medium components are satisfactory. The corresponding certificates of analysis are available and in accordance with the monographs presented by the manufacturer.

The absence of a sterility test during manufacturing of the active ingredient corresponds to the desire of the manufacturer not to open the circuit for sample analysis. The guarantee of sterility is obtained *a posteriori*, during the testing of the active ingredient and finished product. It should be noted that sterilisation is validated (in accordance with the Ph. Eur.) and that the parameters (time, temperature, pressure, bubble-point) are checked, ensuring sterilisation.

The control tests carried out on the different media, aluminium hydroxide and hydrochloric acid 1N are satisfactory. The corresponding certificates of analysis are available and in accordance with the monographs presented by the manufacturer. An applicant monograph for HCl was necessary because the manufacturer uses HCl 1N. The starting material hydrochloric acid used for the manufacturing of HCl 1N complies with the requirements of the European Pharmacopoeia, as indicated in the monograph of the manufacturer.

2.4 CONTROL AT INTERMEDIATE STAGES OF MANUFACTURE

After the mixing of the active ingredients with the various excipients, the following physico-chemical parameters have been monitored: appearance, pH, temperature, time and volume. The evolution of the

parameters of the sterilisation cycles is continuously monitored and must comply with the requirements of Ph. Eur. IX.1.

2.5 CONTROL OF THE FINISHED PRODUCT

In each subbatch[1], the appearance and pH are checked. The appearance of the vaccine should be a milky white suspension (after shaking) and the pH must be between 6.5 and 7.5.

The potency test on each batch of bulk is carried out as follows: after subcutaneous vaccination to 5 guinea-pigs at D0 and D21, a blood sample is collected two weeks after the second injection; titration is performed by the slow agglutination technique. The seroconversion index, calculated by the difference in titre between D0 (day at which the animals are seronegative) and D35, must be higher than or equal to :

F4	:	2.1 $\log_{10}$
F5	:	1.7 $\log_{10}$
F6	:	1.4 $\log_{10}$
F41	:	1.7 $\log_{10}$

The full batch protocols for three batches of finished product have been presented.

This approach is satisfactory and complies with the section "Batch Potency Test" of monograph 962 *"Vaccinum Colibacillosis fetus a partu recentis inactivatum ad suem"* of the European Pharmacopoeia. The use of guinea-pigs, the administration schedule and the definition of titres comply with the requirements of this section.

Assay of Al^{3+} ions is carried out on each subbatch, according to the method of the European Pharmacopoeia V.3.5.7: the concentration of Al^{3+} must be 0.7 ± 0.2 mg/ml.

Assay of thiomersal is carried out on each subbatch by a Rhône-Mérieux colorimetric technique; the concentration of thiomersal included is between 0.08 and 0.12 mg/ml.

Assay of free formaldehyde is carried out on each subbatch and according to a colorimetric technique: the concentration must be lower than or equal to 0.5 mg/ml (Ph. Eur. V.3.3.1).

Specific safety tests are carried out on each batch: intramuscular vaccination in two pigs weighing 20-30 kg with 2 doses of vaccine at D0, one dose at D14 per pig. Clinical signs and temperature are monitored for 28 days. Pigs must remain healthy, with no heat or abnormal local reactions (a post-mortem of the injection site is carried out). The test with pigs corresponds to the "safety' test of Monograph 962 *"Vaccinum Colibacillosis fetus a partu recentis inactivatum ad suem"* of the European Pharmacopoeia.

Bacterial and fungal sterility are carried out on each subbatch according to the requirements of the European Pharmacopoeia monograph 62 *"Vaccina ad usum veterinarium".*

Endotoxin content: the test is carried out on each subbatch according to the requirements of the European Pharmacopoeia V.2.1.9; the endotoxin content must be lower than or equal to $0.5\ 10^6$ IU/ml.

Inactivation control tests are carried out immediately after inactivation during the preparation of the active ingredient and batch-to-batch consistency has been demonstrated by the certificates supplied for three batches of Neocolipor vaccine.

[1] a bulk content is divided into several batches, each batch being divided into several subbatches; the subbatches are then delivered to the users.

2.6 STABILITY

The following parameters have been checked in two batches: appearance, pH, Al^{3+}, free formaldehyde, thiomersal, bacterial and fungal sterility, abnormal toxicity and potency in guinea-pigs, as described in part E2, identity and assay of active ingredients.

The seroconversion index, calculated based on the difference in titre between D0 and D35, must be higher than or equal to :

F4	:	2.1 $\log_{10}$
F5	:	1.7 $\log_{10}$
F6	:	1.4 $\log_{10}$
F41	:	1.7 $\log_{10}$

The stability studies were, however, undertaken 10 years ago before implementation of directive 92/18/EEC and before the establishment of definitive norms.

It should also be noted that the potency was tested in batches filled in type II glass. Since the quality of type II glass is inferior to that of type I glass (type II glass is neutral by surface treatment, while type I glass is neutral by nature), results obtained with batches in type II glass can be extrapolated to type I glass.

A shelf-life of 18 months is considered acceptable with the proviso that the Applicant demonstrates that the next three batches also satisfy the thiomersal acceptance limits. The Applicant has undertaken to provide the results of stability studies on the next three batches.

3. OVERVIEW OF PART III OF THE DOSSIER: SAFETY ASPECTS

3.1 SAFETY

Safety data has been presented for the recommendations for use currently proposed by the Applicant. The demonstration of the safety of Neocolipor, both under laboratory conditions and in the field, can be considered as satisfactory.

3.1.1 Safety of the Administration of One Dose

Vaccination with one dose by intramuscular injection into two pregnant sows at 6 and 2 weeks before farrowing showed that no macroscopic local or general reactions were seen prior to the second injection and up to 5 days after each injection.

3.1.2 Safety of One Administration of an Overdose

Vaccination with one double dose by intramuscular injection into 5 pigs of 35 kg average weight did not result in macroscopic local or general reactions. The rectal temperatures remained within the physiological norms. No notable lesions were seen at the site of injection.

3.1.3 Safety of the Repeated Administration of One Dose

Vaccination 3 times at a 2-week interval with one dose by intramuscular injection into 5 pigs of 35 kg average weight showed no macroscopic local or general reaction nor were there any notable lesions at the site of injection.

3.1.4 Examination of Reproductive Performance

Data from both laboratory tests and from field trials show that there is no effect on gestation following vaccination and the tests used were in accordance with the Ph. Eur..

3.1.5 Examination of Immunological Functions

In the absence of any data on undesirable effects of vaccination on the immunological functions, the manufacturer has not considered it necessary to carry out trials for this purpose.

3.1.6 Study of Residues

No study was undertaken for the following reasons: the vaccine is inactivated and there is no risk of having live organisms at the injection site; all the excipients are included in Annex II of Council Regulation (EEC) No. 2377/90; the quantity injected (2 ml) per animal is small for such large animals and the number of injections per animal is limited (1 or 2 injections per year). A withdrawal period of zero days is therefore acceptable.

3.1.7 Interactions

Two groups of 5 pigs were administered twice at a 28-day interval a simultaneous injection (in different sites) of 1 dose of inactivated vaccine in aluminium hydroxide adjuvant against atrophic rhinitis and 1 dose of Neocolipor, associated or not to 1 dose of inactivated vaccine against swine influenza and Aujeszky's disease. Various vaccines were tested. No local macroscopic or general reaction was seen at the injection site of Neocolipor. Six out of 45 animals (13.5%) had transient increased rectal temperatures (above 40°C). Whilst an increase in temperature might happen in some animals, the zootechnical performances of the vaccinated animals were not impaired and thus these associations can be considered safe.

3.2 ECOTOXICITY

An ecotoxicity study was not considered necessary and, as the vaccine is inactivated, no studies were required according to Council Directive 90/220/EEC on the deliberate release into the environment of genetically modified organisms.

4. OVERVIEW OF PART IV OF THE DOSSIER: EFFICACY ASPECTS

Neocolipor is indicated for the passive immunisation of piglets against neonatal colibacillosis through colostrum intake, by administration to pregnant sows and gilts.

A number of trials were carried out in both the laboratory and in the field in order to ascertain efficacy of the product. The main concern of the CVMP had been the lack of compliance with the European Pharmacopoeia Monograph *"Vaccinum colibacillosis fetus a partu recentis inactivatum ad suem"* with regard to potency data. Further to the oral explanation provided by the Applicant, the Committee agreed that the Applicant had used protocols written in accordance with the Ph. Eur. although the results were not in compliance. The Committee accepted that the results presented showed statistical significance between vaccinates and non-vaccinates and had thus demonstrated the efficacy of the vaccine. Concern was expressed by the Committee for the difficulty experienced by the Applicant in meeting the requirements of the European Pharmacopoeia monograph and it is understood that the data have been submitted to the Pharmacopoeia for review.

4.1 LABORATORY STUDIES

1) Efficacy by Serology

Potency tests were carried out using vaccination with one dose by intramuscular injection twice at a 4-week interval into 5 conventional piglets (i.e. not SPF); 5 other piglets served as controls. Blood samples were taken before each vaccination and 7 days after the second administration.
Seroconversion was observed in vaccinated animals after each injection whilst no seroconversion was observed in the controls.

This test corresponds to the "Batch Potency Test" of Monograph 962 *"Vaccinum Colibacillosis fetus a partu recentis inactivatum ad suem"* of the European Pharmacopoeia.
The mean results obtained may be presented as follows :

	D0/D42 ELISA OD ratio Batch reference H06101D		D0/D42 Agglutinating OD ratio Batch under test RMB 050 5A 050	
	Mean of 20 control sows	Mean of 20 vaccinated sows	Mean of 5 control sows	Mean of 5 vaccinated sows
F4	1.0	1.5	0.8	1.9
F5	0.8	1.7	1.9	3.4
F6	1.0	1.2	1.4	2.4
F41	0.9	1.2	2.0	2.7

The antibody titres obtained with the vaccine batch under test (RMB 050 5A 050) are higher than the antibody titres obtained with the reference batch H06101D. However, it is possible that the different analytical techniques used may have had an influence. There was no significant rise in the antibody titre of the controls during the trials. The trial met the requirements of the European Pharmacopoeia.

2) Efficacy by Challenge

a) Study 1
Vaccination was carried out with one dose by intramuscular injection twice at a 4-week interval into 2 specific pathogen free (SPF) pregnant sows, the second injection was administered 2-3 weeks before farrowing; 2 other SPF pregnant sows served as controls. Approximately 24 hours after birth, all the piglets (17 born to vaccinated sows and 17 born to controls) were challenged with an *E. coli* strain (O8 F4ac) different from the vaccine strain. Fifteen out of seventeen piglets of the control group and two out of seventeen in the vaccinated group died. *E. coli* re-excretion was evaluated by faeces sampling and 10/17 of the control group and 1/17 in the vaccinated group showed re-excretion. Therefore vaccination conferred protection and reduced excretion notably.

b) Study 2
Sixteen conventional (non SPF) sows were distributed into 4 groups of 4 animals each, then vaccinated with 1 dose by intramuscular injection twice at a 4-week interval into 2 sows per group, the 2 other sows serving as controls. Piglets born to all the sows were challenged with an *E. coli* strain per group (F4ac, F5, F41, F6 different than the vaccine strain), 12 hours after birth. Overall the results for *E. coli* reexcretion were better for the vaccinated animals, with a 24-hour shorter excretion duration for the piglets of the vaccinated group.

c) Study 3: ; Trial against porcine colibacillosis (Fac, F5/F41, F6)
Twenty four conventional (non SPF) sows were distributed into 6 groups of 4 animals each, then vaccination with 1 dose by intramuscular injection twice at a 4-week interval into the animals of the 3 groups, the 3 other groups serving as controls. Piglets born to all the sows were challenged with an *E. coli* strain per group (F4ac, F5/F41, F6 different than the vaccine strain), about 24 hours after birth.

This trial corresponds to the section "potency" of Monograph 962 *"Vaccinum Colibacillosis fetus a partu recentis inactivatum ad suem"* of the European Pharmacopoeia for the F4ac, F5, F41 and F6 components. The results may be summarised as follows :

	Piglets of the control group		Piglets of the vaccinated group	
	Mortality	Absence of clinical signs	Mortality Severe diarrhoea	Mild diarrhoea
Expected result	≥ 40% (6/15)	≤ 13.3% (2/15)	≤ 13.3% (2/15)	≤ 20% (3/15)
F4ac	27.5% (11/40)	0% (0/40)	3.1% (1/32)	68.7% (22/32)
F5/F41	80% (28/35)	0% (0/35)	8.6% (3/35)	91.4% (32/35)
F6	3.2% (1/32)	15.6% (5/32)	0% (0/37)	40.5% (15/37)

The requirements of the European Pharmacopoeia were not met. However, the statistical analysis evidently demonstrates the efficacy of the Neocolipor vaccine against a F4ac and F5/F41 challenge (prevalence and duration of diarrhoea) and against a F6 challenge (only for the prevalence of diarrhoea). In these conditions, using the chi^2 test on an appropriate scoring system in different categories (dead, severe diarrhoea, mild diarrhoea, no significant disease), a significant statistical difference was shown, which enabled the CVMP to conclude that satisfactory efficacy had been demonstrated.

The assay of post-vaccination serum antibodies with vaccine batch H06101D yields the following results :

	D0/D42 ELISA OD ratio	
	Mean of 12 control sows	Mean of 12 vaccinated sows
F4ac	1.03	1.5
F5	0.79	1.8
F6	0.98	1.4
F41	0.9	1.3

An ELISA with one F4ac strain as a coating antigen was used. However, since no F4ac specific monoclonal or purified antibodies were used, it is impossible for the serology to be "ac" : by construction it is F4, all serotypes taken together.

d) Study 4: Challenge trial against porcine colibacillosis (F4ab and F4ad)

Sixteen conventional sows were distributed into 4 groups of 4 animals each, then vaccination vaccinated with 1 dose by intramuscular injection twice at a 4-week interval into the animals of the 2 groups, the 2 other groups serving as controls. Piglets born to all the sows were challenged with an *E. coli* strain per group (F4ab and F4ad, different than the vaccine strain), about 24 hours after birth. The prevalence of diarrhoea was significantly lower for piglets born to the F4ab and F4ad vaccinated sows compared to the piglets of the control group. Compared to the control animals, the duration is shorter in the animals from the F4ad vaccinated group, and comparable for the animals from the F4ab vaccinated group. This trial corresponds to the "Potency" section of Monograph 962 *"Vacinum Collibacillosis Inactivatum ad Suem"* of the European Pharmacopoeia for the F4ab and F4ad components.

Data may be summarised accordingly:

	Piglets of the control group		Piglets of the vaccinated group	
	Mortality	Absence of clinical signs	Mortality Severe diarrhoea	Mild diarrhoea
Expected result	≥ 40% (6/15)	≤ 13.3% (2/15)	≤ 13.3% (2/15)	≤ 20% (3/15)
F4ab	21.4% (9/42)	4.8% (2/42)	5.7% (2/35)	71% (25/35)
F4ad	5.3% (2/38)	0% (0/38)	11.4% (4/35)	85.7% (30/35)

Whilst the requirements of the European Pharmacopoeia have not strictly been met, the statistical analysis demonstrates the efficacy of the Neocolipor vaccine against a F4ad challenge (prevalence and duration of diarrhoea) and against a F4ab challenge (only for the prevalence of diarrhoea). Using the chi^2 test on an appropriate scoring system in different categories (dead, severe diarrhoea, mild diarrhoea no significant disease), a significant statistical difference was shown, which enabled the CVMP to conclude that satisfactory efficacy had been demonstrated.

The assay of post-vaccination antibodies with vaccine batch H06101D yields the following results:

	D0/D42 ELISA OD ratio	
	Mean on 8 control sows	Mean on 8 vaccinated sows
F4	1.0	1.48
F5	0.87	1.49
F6	0.94	0.98
F41	0.95	1.04

Not all the results from laboratory studies on efficacy comply with European Pharmacopoeia monograph on *"Vaccinum Colibacillosis fetus a partu recentis inactivatum ad suem"*. However, the efficacy of Neocolipor was demonstrated in comparison with non-vaccinated animals.

4.2 FIELD STUDIES

1) Vaccination in a contaminated environment (probably *E. coli* F4) with one dose by intramuscular injection into 80 pregnant sows, was given twice at a 3-week interval (5 and 2 weeks before farrowing). Mortality was 4% in the vaccinated group and 10% in the control group.

2) Vaccination in a contaminated environment (*E. coli* F4) with one dose by intramuscular injection into 192 pregnant sows was given twice at a 3-week interval (5 and 2 weeks before farrowing); the zootechnical performance of 2 groups of 9 sows, one group vaccinated, the other serving as a control was assessed. Mortality and morbidity were comparable in both groups, however, at the age of 28 days piglets of the vaccinated group had a higher weight gain of 730 g compared to the piglets of the control group.

Conclusion

Despite difficulties inherent in the implementation of field trials, considering the importance of breeding factors involved in such a pathology, the efficacy of the Neocolipor vaccine was demonstrated. The trials have used several different batches of vaccine and all demonstrated a satisfactory efficacy even though only the trial designs were in compliance with the Monograph of the European Pharmacopoeia.

4.3 EFFICACY OF RE-VACCINATION

The Ph. Eur. monograph has been used as a basis for assessing the efficacy of re-vaccination, however the results of trials in a number of countries do not meet the criteria of the Pharmacopoeia. The results were obtained in countries where Neocolipor is already registered (Austria and Norway) and also using a similar product, Imocolipor, in Belgium.

The Applicant informed the Committee that a trial in France had begun and that the definitive results were due in March 1998. Preliminary results showed that there was a difference between vaccinates and controls at the end of first pregnancy and data on the second pregnancy would be available later.

Given the results so far, the Committee accepted that re-vaccination had been shown to be effective and, with the proviso that definitive results would be presented next year, allowed an appropriate claim in the SPC.

5. RISK-BENEFIT ASSESSMENT AND CONCLUSIONS

Based on the original and complementary data presented, 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 and supported the claims proposed by the Applicant.

The Applicant has given a commitment to provide a new stability study and also the results of a re-vaccination trial as soon as both are available.