PRODUCT PROFILE

Product name:	Locatim
Procedure No.:	EMEA/V/C/041/01/0/0
Applicant company :	Biokema Anstalt Aeulestrasse 38, 9490 Vaduz Furstentum Liechtenstein
Active substances	Bovine concentrated lactoserum containing specific IgGs against *E. coli* F5 antigen
Proposed International Non-proprietary Name:	N/a
Pharmaceutical form:	Oral solution
Strength	$\geq 2.8 \log_{10}$ /ml bovine concentrated lactoserum (microagglutination method)
Target Species:	Neonatal calves less than 12 hours of age
Presentation, Packaging and package size:	60 ml pharmaceutical glass bottles in a box.
Withdrawal period:	Zero days
Route of administration:	Oral use
Product type:	Immunological veterinary medicinal product
Therapeutic indication:	Reduction of mortality caused by enterotoxicosis associated with *E. coli* F5 (K99) adhesin during the first days of life as a supplement to colostrum from the dam.

SCIENTIFIC DISCUSSION

1. INTRODUCTION

The product, the subject of an application from Biokema, is Locatim, a substitute or supplement where there may be a deficiency of protective properties of colostrum for calves. It is recommended for use in new-born calves in the first 12 hours of life to reduce mortality caused by enterotoxicosis associated with *E. coli* F5 (K99) adhesin during the first days of life and as a supplement to colostrum from the dam.

A similar product, was registered in Belgium, Germany, Luxembourg, The Netherlands and Switzerland, having been derived from different antigenic components not containing any recombinant constituents. On the basis of scientific advice, provided by the CVMP in December 1996 (EMEA/CVMP/260/96), the product was considered eligible for authorisation through the centralised procedure as one of the substances used in the manufacture of this product is derived from a genetically engineered source.

Scientific Advice had also previously been given in response to questions on the exclusion of contamination with extraneous agents, the potential risk of transmission of BSE from the donor cows and the conduct of a challenge test based on that described in the European Pharmacopoeia monograph Neonatal Ruminant Colibacillosis Inactivated Vaccine.

2. OVERVIEW OF PART II OF THE DOSSIER: ANALYTICAL ASPECTS

2.1 QUALITATIVE AND QUANTITATIVE PARTICULARS OF THE CONSTITUENTS

The composition of the constituents of the product are specified below:

	Names of ingredients	Quantity per ml
Active ingredients	Anti-*E. coli* F5 specific IgGs	$\geq$2.8 $\log_{10}$
Constituents of the excipients	Propionate buffer	

Container:

The containers used for the product are 60 ml single dose pharmaceutical type III amber glass bottles closed with a polypropylene screw cap with a polyethylene seal. The closure has a tamper evident ring in order to indicate whether a possible opening of the container has occurred.

The bottles are in compliance with the European Pharmacopoeia requirements for type III glass containers suitable for preparations not for parenteral use. The bottles are steam sterilised at 134^{O}C for 10 minutes.

The Applicant was requested to present additional data on the screw cap and seal and a certification of conformity of the glass containers with the requirements of the European Pharmacopoeia for type III glass containers. The presented information was considered sufficient and moreover, the quality of the type III glass was considered acceptable, as the product is not intended for parenteral use.

The polyethylene seal closure is in compliance with the IR spectrum for identification Test A of the European Pharmacopoeia requirements for preparations for parenteral use and the stoppers are irradiated with a dose of not less than 25 kGy. A Biokema certificate of analysis stating conformance for a batch is presented in the dossier.

Since the safety and stability of the product have been addressed satisfactorily, the Committee accepted that no further information regarding the extent to which the seals were in conformity with

the requirements of the European Pharmacopoeia were required since the concerned product is for oral use only.

Product Development Studies:

The oral administration of the product provides an antibody supply in the lumen of the intestine of calves where the Immunglobulin G gives humoral protection. Immunglobulin G is directed against F5 (K99) pilus antigen which is one of the major adherence factors on enterotoxigenic *E. coli*, associated with neonatal diarrhoea. The source of the Immunglonulin G in the product is colostrum from cows immunised with an *E. coli* vaccine.

The formulation is based on the European Pharmacopoeia monograph 'Immunosera for Veterinary Use'; based on colostrum instead of blood products. An inactivating agent is used in order to inactivate potential extraneous agents, with a validated process. The product is filled aseptically into the wide necked tamper-evident, single dose containers which allow easy use, a precise dose, and good conservation of the product with no need for a preservative. No overage is necessary due to the good stability of the finished product.

The requirements of the monograph 'Immunosera for Veterinary Use' provide guidance for the production of such a colostrum-product. However, as the European Pharmacopoeia does not contain a monograph regarding colostral antisera or *E. coli* antisera, the Applicant was not obliged to address all the requirements in the monograph 'Immunosera for Veterinary Use'.

2.2 METHOD OF PREPARATION

Flow charts of the steps of manufacture have been provided in the dossier.

Description of the main steps of manufacture

The main steps of manufacture are as follows:
1. The containers of frozen colostrum are weighed.
2. The serum is thawed.
3. The colostrum is blended.
4. Lactoserum is clarified by several steps of filtration.
5. The material is concentrated.
6. Immungloblin G concentration is adjusted.
7. The material is sterilised and filled into final containers.

The manufacturing method was well described in the dossier. However, a number of areas were identified in the original dossier, where more information was required to provide assurance on batch to batch consistency. Variations in the times and temperatures required were also identified as factors which might result in variations in the quality of the product in terms of the active ingredients and the potential for the multiplication of bacteria. The Committee requested that the Applicant provide further information. The responses summarised below are considered acceptable:

- The frozen colostrum is stored for a minimum of one year prior to thawing.
- The reproducibility of the conformity of this number on 3 consecutive blends is confirmed. The integrity of each container of colostrum selected for preparation of a blend is visually checked before thawing. The released containers are then chosen according to their entrance control date (a first in / first out system is used) which ensures that the first and second milkings from each cow is pooled.
- The temperature of the skimmed colostrum is consistent from batch to batch and considered acceptable.
- The sterilization step includes a filtration on line and a chemical sterilization.
- The Applicant has made no claim on general immunostimulating effect for the product. The active site of Immunoglobulin G is the Fab region, which is important for bacterial antigen

neutralization. The integrity of this active site is checked for every batch and throughout the stability studies by the microagglutination test. The Applicant has indicated that there is no intention to extend the claims of the product to an immunostimulating effect involving the Fc receptor of an immunocompetent cell.

The Validation Data were provided which contain results of tests carried out on three consecutive batches. One batch was deliberately diluted approximately 10% and one batch was deliberately concentrated by 10%.

2.2.1 Reproducibility of the finished product

For each of the batches, the volumes of colostrum and lactoserum at the different stages of production and the resultant protein and Immunglobulin G concentration in the finished product have been provided.

2.2.2 Homogeneity of the finished product during filling

The Immunglobulin G concentration has been measured separately in 5 containers taken at intervals during filling of each batch and the results and the statistical analysis of these are presented in the dossier. From these results, the Applicant concludes that the overall manufacturing and filling process is reproducible and the product is homogeneous.

2.3 CONTROL OF STARTING MATERIALS

Listed in a Pharmacopoeia

Details of starting materials are provided in the dossier as follows:

a) For some of the starting materials a statement of compliance with the relevant European Pharmacopoeia monograph and a Biokema Certificate of Analysis for a batch with a statement of conformance were given.

b) For the other starting materials a statement of compliance with the British Pharmacopoeia monograph and a copy of the relevant monograph and a Biokema Certificate of Analysis for a batch with a statement of conformance were given.

Not Listed in a Pharmacopoeia

Biological Origin

1. Colostrum

1.1. Donor animals

Colostrum is collected from dairy cows of herds under permanent disease surveillance in 2 Cantons of Switzerland. The cows must have been born after 1 January 1991, which is 1 month after the introduction of the ban on the feeding of meat and bone meal to ruminants. All cows are fed on vegetable fodder and must never have been fed meat and bone meal. Herds of origin must be free from Brucellosis, Tuberculosis, Paratuberculosis, Enzootic bovine leukosis, Infectious bovine rhinotracheitis and have had no cases of Bovine Spongiform Encephalopathy (BSE). Donor cows are not the progeny of cows in herds with cases of BSE nor have these been sourced from a herd where BSE has occurred or originated in a country with a high incidence of BSE. Donor cows must be healthy and free from mastitis. The occurrence of disease in donor cows must be reported to Biokema

and all treatments must be recorded on specific forms of which a copy is included in the dossier. The cows are routinely vaccinated against rota- and coronaviruses.

However, to complete the assessment of the product and to minimise the risk of contamination of the product with extraneous agents and maximise batch to batch consistency, a certain number of requirements for donor animals are specified in the Volume VII guideline: 'Specific requirements for the production and control of immunosera and colostrum substitutes' (The Rules Governing Medicinal Products in the European Union). In addition the Applicant has addressed the following points:

- The Applicant has provided satisfactory information on the upper age limit set for the use of cows as donors. As the collection network includes only cows born after January 1991, there is no donor cow older than 7 years. However, the Applicant has fixed a limit at 10 years of age for cows included in the colostrum collection network.

- The Applicant provided information on the arrangements for quarantining of foreign cows and checking the health of any Swiss animals entering the herds, which was subject of the GMP inspection. No herds with foreign cows have been included in the collection network since September 1996. Swiss cows can be sold and transferred only after examination and certification of health by a veterinarian. The herd of origin must be free of any disease on OIE lists A and B and any notifiable diseases. Any minor disease or treatment must be recorded on the official sheet. An official herd inspector distinct from the farmer gives transfer authorization. The arrangements described in the dossier ensure that, at the time of introduction to the source herds, these new cows will be in conformance with the health requirements of donor cows in the herds.

- The Applicant provided an official document explaining the Veterinary Services of Switzerland. There are a number of federal Swiss laws which provide the framework for disease control which address notifiable diseases, the functions of veterinarians, registration and identification of farm animals, processing of animal waste, regulation of the registration, production, import and trade in sera and vaccines. Cantons may impose additional regulations to take account of local conditions. Many large animal veterinary practitioners are part-time governmental or cantonal officials. The Federal Veterinary Office operates the controls on imports of animals and animal products. They aim to maintain Switzerland's freedom from all OIE List A diseases and many List B diseases. Switzerland has been officially recognised as free from:

  Foot and Mouth disease
  Vesicular Stomatitis
  Rinderpest
  Lumpy Skin disease
  Rift Valley Fever
  Bluetongue

  In addition, annual reports of the Swiss Veterinary Services have reported a very low incidence of:

  Brucellosis
  EBL
  IBR-IPV
  Tuberculosis
  Rabies

  In Switzerland it is obligatory to report any OIE List A and B diseases and this information is published twice per month. Donor cows are in the Cantons of Vaud and Fribourg. The cows are under continual sanitary control by veterinarians. The veterinarians and the farmer sign an agreement to respect the quality assurance parameters and health status required by the Applicant. This agreement requires source colostrum from healthy cows only, free of mastitis and signs of all other diseases or events which may affect the quality of the colostrum. All OIE List A and B diseases and BVD must be declared immediately and the herds must be free from Brucellosis, EBL, IBR-IPV, Tuberculosis, Paratuberculosis and BSE. The cows are all identified and

registered. The details of the donor cow are included in the bar-coded label of the container containing the colostrum. Nevertheless if no official control of BVD infection takes place, the product may contain antibodies directed against BVD/MD pestivirus.

- The Applicant has provided information on bovine extraneous agents as given in the Volume VII guideline: 'Table of extraneous agents to be tested for in relation to the general and species-specific guidelines on production and control of mammalian veterinary vaccines' (The Rules Governing Medicinal Products in the European Union). Many of these agents are not present in the colostrum because they are not present in Switzerland and/or are not excreted in milk. For some of the agents, there are references to published data on their susceptibility to inactivation. The Applicant provided evidence of published data on the effectiveness of inactivating agent in high protein concentrations. The main points of the Applicant's risk assessment for each agent has been summarised in a table in the expert report of the dossier.

  As requested by the CVMP, the Applicant has provided a study to ensure the effectiveness of the inactivation step against bovine viral diarrhoea virus, bovine parvovirus and bovine adenovirus, undertaken at the Central Veterinary Laboratory (CVL, Weybridge). The high level of immunoglobulin present in the product appears to have a masking effect on all the viruses in the positive control. The Applicant argues that dilution (10x) of the product prior to spiking would probably eliminate the masking effect, but this would not demonstrate the efficacy of the inactivating step as used during the manufacturing process. A repetition of this experiment using bovine viral diarrhoea virus done at the Merial Virology QC Laboratory at Lyon (F) has confirmed the same masking effect on the positive control.

  However, the safety level regarding viral contamination can be considered as satisfactory if a viral non-specific purity test and a specific purity test for BVD and IBR is undertaken on a batch basis. The following are relevant points:

  1) the maximum limit of protein in the product has been reduced to 12%,
  2) the literature demonstrates the effect of the inactivating agent in serums and immunoglobulin preparations,
  3) the inability to demonstrate the viral inactivation by the current *in vitro* technical expertise: an *in-vivo* experiment in this context has been confirmed at the Moredun Institute in the UK.

1.2. BSE Risk Assessment for donor animals

The Applicant has provided a copy of the Swiss Veterinary Federal Office BSE fact sheet, dated November 1996. BSE was first identified in Switzerland in 1990. Measures to control the disease have been taken by the Swiss Veterinary Federal Office since 1990 and include the requirement for notification of cases of BSE, slaughter and histological examination of suspected BSE cases, compensation payment to farmers, incineration of carcasses of BSE cases, tattooing of progeny of BSE cases as well as all other cattle in the herd, and a ban on feeding of meat and bone meal to ruminants. Switzerland has banned the importation of live cattle and certain products from the UK since June 1990. In addition since 1996, the use of animal carcasses in animal feed has been banned and the importation of cattle sheep and goats from any country where there is no ban on the feeding of mammalian protein to ruminants has been forbidden.

In 1996 EU experts conducted that Switzerland was a country with low incidence of BSE. They further concluded that the disease was not endemic and the control measures in place at that time were suitable, well executed and effective.

The WHO information sheet, dated April 1996, states that transmission of BSE through milk is unlikely and that no transmission of TSEs in other species had ever been observed through milk.

The Applicant included official information from the Swiss Veterinary Federal Office on the numbers of BSE cases in Switzerland between 1990 and 1996. A peak of 68 cases has been observed in 1995 and the number of BSE cases has decreased to 45 in 1996 of which only 10 cases have been diagnosed in the second half of the year 1996. The Swiss Veterinary Federal Office claims that the bone meal

ban and related actions have contributed to the decrease of numbers of BSE cases and that the actions of slaughter of high risk animals will further decrease the incidence of BSE.

The Swiss veterinary authorities have undertaken most steps considered necessary, and recommended by European experts, to eradicate BSE. However, based on an analysis of the incidence of reported BSE cases in different countries, concern has been expressed by some experts that the slaughter of all animals in the herd once a BSE case has been identified is a disincentive to report.

The feeding of ruminant protein to non-ruminants can be of a concern. However, a large number of measures have been operating in Switzerland since 1990 in order to minimize the risk of transmission of diseases (in particular TSEs) and cross-contamination in the case of feeding ruminant protein to non-ruminants. Official procedures to control of potential cross-contamination with non-ruminant feed in the mills have been undertaken since 1991 and show that less than 1% of cross-contamination with animal proteins has occurred. The level of cross-contamination of the positive samples was < 0.3%. Any bone meal contaminated with potential low-level animal protein is always treated by the validated TSE inactivating procedure.

The incidence of scrapie is very low in Switzerland (5 cases since 1990). Furthermore, the sheep/cattle ratio is low in Switzerland (11.7%) and the monitoring system for scrapie is identical to that of BSE. Together with the other measures relating to TSEs in Switzerland, it is considered that measures in place to control scrapie provide sufficient assurance for the product.

1.3. Additional measures for a BSE free area for colostrum collection

The Applicant's additional measures for a BSE free area for colostrum collection to reduce the risk of contamination of the product with BSE include the following:

- Traceability of the donor cow through the bar code identification system
- Exclusion of herds with BSE cases, progeny or herd mates of cases (through Swiss tattoo identification system for herd mates or progeny of cases)
- Use of cows born only after the feed ban
- Quarantine of colostrum for 1 year before use
- Transparency of information on cases referred from the veterinary services
- Internal quality assurance

These additional measures proposed by the Applicant are considered in relation to the requirements of the revised 'Note for Guidance for minimising the risk of transmitting animal spongiform encephalopathy agents via veterinary medicinal products' (EMEA/CVMP/145/97-FINAL), in particular with the requirements indicated in section 3.1.2 - materials sourced from countries with low numbers of BSE cases. Two important factors regarding the risk of transmitting BSE referenced in the guideline, are applicable to the product namely:

a) The oral route of administration is a less efficient route of BSE transmission than parenteral routes,
b) Milk and its derivatives are unlikely to present a risk of contamination with BSE (Category IV)

The Applicant has also confirmed that if and when Commission Decision 97/534 comes into force in the EU, the same measures will be applied in Switzerland, which the Applicant intends to comply with. As a result:

- all specified risk material prohibited for any use and incinerated
- every bovine, ovine, or caprine aged over 6 months inspected regarding TSEs before slaughtering
- obligatory declaration of unexpected death of every bovine, caprine, ovine, and porcine to veterinary survey services.

The colostrum is held in quarantine for 1 year from collection to further processing, which gives some assurance regarding the transmission of BSE. However, for a maximum protection against BSE, the colostrum would need to be held until the donor cow would have been slaughtered, its brain examined and the animal shown not to have been infected with BSE. As this procedure is impractical, the 1 year quarantine of colostrum is considered a reasonable compromise to exclude transmission of BSE.

1.4. Immunisation of donor cows

The antigen used is an *E. coli* vaccine, manufactured by Merial. This vaccine was first authorised in France in 1981 and has since been authorised in a number of other EU Member States. Applications for Marketing Authorisation for the *E. coli* vaccine have been made in 4 further Member States. It contains *E. coli* antigens of the attachment factors F5, Y and 31 A.

The donor cows are immunised with a double dose of the *E. coli* vaccine from the seventh month of pregnancy followed by a single dose 3 weeks later and at least 2 weeks before calving. A single dose booster is given annually 3-5 weeks before calving providing it is not more than a year since the previous primary vaccination otherwise a primary vaccination course is required. The Applicant states that the immunising schedule has been validated by the immune response data obtained with three batches and the historical data for the similar product, which is already authorised in four member states of the EU. The similar product was briefly described in the dossier of this application in order to support data on safety and efficacy. However, following the introduction of Locatim to the market, the Applicant will stop manufacturing the similar product and Locatim will replace it in the market place. The vaccines used for immunising donor cows for the similar product are an *E. coli* vaccine and a rota- coronavirus vaccine. The vaccines used for immunizing donor cows for Locatim are another *E. coli* vaccine and the same rota- coronavirus vaccine.

All the procedures associated with the collection, identification, storage and transport of colostrum, including health monitoring and control of the herds are identical for all colostrum used in the manufacture of both the similar product and Locatim. However different farms are used to supply colostrum for each product and no farm or veterinarian is used to supply colostrum for both products, thus ensuring total separation and integrity of the colostrum used for each product. Full details of the origin of every sample of colostrum are recorded on the bar coded labels attached to each sample, whether the sample is to be used in the manufacture of the similar product or Locatim, thus ensuring full identification and traceability throughout the manufacturing process. The final containers for each product have different coloured labels corresponding to that of the immunising vaccine.

According to the Volume VII guideline: 'Specific requirements for the production and control of immunosera and colostrum substitutes' (The Rules Governing Medicinal Products in the European Union) the immunising antigens should, if possible, have a marketing authorization in one of the EU Member States, which is the case for both the *E. coli* vaccine and the rota- coronavirus vaccine. The *E. coli* vaccine does not have a marketing authorization in Switzerland, but the Applicant has received a special authorization from the Swiss regulatory authorities to use it for immunisation for production of colostral immunoglobulins. The rota- coronavirus vaccine has a marketing authorization in Switzerland.

The Applicant has furthermore provided details of all vaccines used in donor cows. These include details of the active ingredients used and whether they are live or inactivated vaccines. For products not authorised in the EU, information has been provided on the substances of animal origin used in manufacture and the sources of these including the country of origin of the donor animals. The vaccines used in donor cows are registered in Switzerland. However, the following 7 vaccines are not registered in the EU:

1. Inactivated *C. chauvoei* and *C. septicum* toxoid vaccine I: produced on broths containing bovine meat meal and liver of animals from BSE-free countries,
2. Inactivated *C. chauvoei* and *C. septicum* toxoid vaccine II: produced on broths containing bovine meat meal and liver of animals from BSE-free countries,

3. Inactivated Leptospira vaccine: produced on fully synthetic broths containing no substances of animal origin,
4. Inactivated Moraxella bovis vaccine: contains substances of animals from BSE-free countries,
5. Inactivated Papillomavirus vaccine I: extracted from bovine skin from animals of Swiss origin,
6. Inactivated tetanic toxoid vaccine: produced on broths containing bovine meat meal of animals from BSE-free countries,
7. Inactivated Papillomavirus vaccine II: extracted from bovine skin warts from animals of Swiss origin.

For one of these 7 vaccines the Applicant gives assurances that no substances of animal origin are used. For 5 of these 7 vaccines bovine material is used. The Applicant gives assurances that only vaccines containing bovine material of class 4 BSE risk group will be used for donor cows, except the two inactivated *C. chauvoei* and *C. septicum* toxoid vaccines which contain bovine liver, class 3 BSE risk group, but from animals sourced from BSE free countries.

Therefore the vaccines used in the donor cows are and will continue to be:

1) either registered in the EU or
2) free from substances of animal origin or
3) containing only bovine material of BSE risk class 4 and originating from BSE-free countries or countries with a low incidence of BSE or bovine material of BSE risk class 3 and originating from BSE free countries in compliance with the revised Note for Guidance for minimising the risk of transmitting animal spongiform encephalopathy agents via veterinary medicinal products.

The updated dossier provides information on the vaccines, which may be used in the herds. It includes live vaccines for rotavirus, coronavirus and BVD that are also authorised in Member States of the EU and not be given to donor cows, or cows in contact with them, within the last 3 weeks before calving.

1.5. Collection of colostrum

The colostrum from the first 2 milkings are pooled and collected and after each milking the colostrum is transferred to a polypropylene 10-litre container for single use. The colostrum is immediately frozen after milking (within 1 hour) by the farmer and stored at -20^{O}C until use. Each container has a bar code label, which includes details of herd identification, donor cow tag number, 'birth' date and the milking dates. The frozen containers containing colostrum are transferred from the farms to collection centres (-20°C freezers) which are under supervision of the Applicant. The containers are collected from the centres every week by the Applicant and after registration and controls, outlined below, are transferred to the storage zone and stored at -20°C.

1.6. Control of colostrum

According to the Swiss Federal law on Quality Assurance of milk collection, every dairy cow has to be tested every month for mastitis. The recording of cell count must be below 150'000/ml of milk. If the cell count exceeds 150'000/ml, a Schalm test must be done. If the result is ++ or +++ positive, the milk collection is stopped.

The monthly monitoring, as well as all veterinary treatments, are recorded on an official form, which is kept for two years (Contrôle mensuel de la santé du pis). In addition, most of the veterinarians have introduced a supplementary monitoring programme measuring the cell counts for every cow during the lactation period. Cell counts are done by official milk control laboratories. Both the farmer and the veterinarian are contractually obliged to suspend colostrum collection and inform the Applicant of any sign of mastitis.

The European Pharmacopoeia monograph Immunosera for Veterinary Use requires a minimal withdrawal period of 8 days after a penicillin treatment of donor cows. With the use of intramammary antibiotics during the dry period, this requirement is of course fulfilled and not appropriate in this

particular case (the normal dry period in Switzerland is 10 weeks). Farmers and veterinarians are required to commit themselves to use only intramammary antibiotics registered in Switzerland for which the MRLs have been set in accordance with annexes I, II and III of Council Regulation (EEC) No 2377/90. Every medicinal treatment must be recorded and the Applicant has access to this information for two years. In the Swiss federal law on Quality Assurance of milk collection, it is prescribed that every medicinal treatment, whether under veterinary prescription or directly carried out by the farmer has to be recorded and the withdrawal period calculated and respected (Carte d'étable pour bétail laitier). Further control of colostrum includes:

- Bar code data and containers (the details from the bar codes are included in the batch record)
- Checking the BSE status in the herd of donor cows (there must be no BSE in herds of donor cows)
- Checking of containers (must be undamaged)
- Weighing of containers
- Visual control of the colostrum (the colostrum must be white to yellow and be free from blood)
- Checking the vaccination protocol form

The Applicant rejects colostrum which does not fulfill these criteria. The Applicant controls the continued absence of BSE in the herds of donor cows 1 year post collection of the colostrum and prior to its further processing, in accordance with official published information.

In addition the Applicant ensures that every donor cow is under permanent veterinary control in the dairy farm. After the collection, veterinarians and farmers are still required to declare any disease. Moreover, the Applicant exercises its own cross-check by screening the official information reports of the Swiss Veterinary Services, for incidence of the following diseases in the collection area:

Leucosis,
Tuberculosis,
Paratuberculosis,
Brucellosis,
IBR/IPV,
Salmonellosis,
Coxiellosis,
BSE and
OIE lists A and B diseases

If any case of these diseases occur in the region, the farm and cow identification are requested. According to the aetiology of the disease and the delay of declaration after the milking, the identified colostrum provided by the particular donor cow or by all the donor cows in a herd is isolated and destroyed, the destruction recorded. In the particular case of BSE, the absence of BSE in all the herds is screened for at least 1 year after the collection of colostrum and again on the day of manufacture.

In addition the Applicant provided the Official Swiss statistics showing that Switzerland is free from:

Brucellosis
Mycobacterium bovis
Bovine leucosis
IBR-IPV

No reports of these diseases in the Cantons Vaud or Fribourg (the cantons where the farms with the donor cows are located) have been declared since at least 1993. Furthermore, there is a very low incidence, in Vaud and Fribourg of the following diseases:

Mycobacterium paratuberculosis
Coxiella burnetti

BSE
Salmonella

All these infections are under continuous survey and subject to obligatory declaration to both the health authorities and to Applicant, and detailed information is published twice a month. In particular, none of the donor cows from herds from which colostrum is collected have suffered from these diseases since at least 1994. If any herd with donor cows is affected by one of these diseases, the Applicant can easily identify and discard the collected colostrum via the bar-coded label system.

BVD virus is subject of continuous survey and to obligatory declaration to the Applicant. However, it is proposed that validated extraneous agent and specific pestivirus tests will be done in routine tests on the finished product allowing the identification of any BVD virus contaminated batches to be discarded.

2.4 CONTROL AT INTERMEDIATE STAGES OF THE MANUFACTURING PROCESS

The flow chart of production is repeated in the dossier and indicates the controls undertaken including tests carried out. The description in the dossier includes references to temperature controls and the results obtained in tests on three consecutive batches. In addition, the Applicant has included references to the check on the volume of fill of the bottles (61 $\pm$ 1ml) and appearance at labelling (clear and particle free).

There is no in-process monitoring or control on the amount of F5-specific Immuglobulin G. However, both methods used for the determination of anti-*E.coli* F5 (K99) specific immunoglobulins G are based on factor 2 dilutions. Consequently, the results can not be precise enough to be considered for the dilution step. On the other hand, the quantification of immunoglobulins G by high performance size-exclusion liquid chromatography is precise and the level of IgGs can be correlated with the specific antibody.

The microbial contamination is controlled and reproducible and it is therefore unnecessary to monitor this parameter as a routine test.

2.5 CONTROL OF THE FINISHED PRODUCT

1. Test for Total protein

The test is carried out using a sulphuric acid digestion (Kjeldahl) method. The method used is that specified by the European Pharmacopoeia in the monograph Immunosera for veterinary use; the limits have been set to 8.0-12.0 % w/v.

2. Immunglobulin G concentration

The quantitative determination of IgGs is carried out using High Performance Size-exclusion Liquid Chromatography. UV-spectroscopy is then used for detection of the substances of interest and quantification is performed by reference to measurement of the results of a standard. The validation of this test is presented in the dossier. The parameters studied are specificity, linearity, precision and repeatability. A calibration of the column and UV spectrum has been also performed.

Purity of the sample was shown not to affect the final result.

3. Test for F5 (K99) specific Immunglobulin Gs by Microagglutination

A standard F5 purified antigen is used. An antigen control and a negative serum control are included on each microagglutination plate. Complete agglutination is taken as the end point. The validation of

this test is presented in the dossier. The parameters studied are specificity, linearity and precision. The F5 (K99) antigen used is prepared from the reference strain *E. Coli C* 38-72 serotype 0101: K', K99. To ensure a similar quantity of the F5 antigen preparations each batch produced is tested 6 times against the current standard before release for routine test.

4. Test for bacterial and fungal sterility

The test is conducted in accordance with the requirements of the European Pharmacopoeia. It is explained that the test may be conducted using the method of membrane filtration or by direct inoculation. The validation data to show the lack of interference of Locatim with the sensitivity of the test. The European Pharmacopoeia states that the method of membrane filtration should be used wherever possible and the Applicant has agreed this to.

5. Absence of bovine viruses

The IPB3 cells are grown in medium containing 5% foetal calf serum without anti-pestivirus antibodies. A 5-ml sample of Locatim is tested by inoculation of 1.25 ml onto each of 4x75 cm^2 flasks. Negative control cell cultures are maintained. The cultures are observed for a week and passaged. Freeze-thaw cycles are carried out. It would appear that the cultures are observed for a total of 14 days. Screening has been carried out on a sample flask and a reference flask as follows: Haemagglutinating viruses in the supernatant, haemadsorbant viruses on the monolayer. One other sample flask and one reference flask is also screened for abnormal appearance and inclusion or cytomorphological abnormalities by MGG colouring. Further information has been provided by the Applicant regarding the details of the times of passaging the cells and the check tests that are done at the various stages.

The non-specific viral purity test according to the protocol described in the dossier has been implemented in order to determine the sensitivity to detect bovine adenoviruses, bovine parvovirus and bovine viral diarrhoea virus. Concerning the detection of rota and coronaviruses, the product contains 2.7 $\log_{10}$ and 3.8 $\log_{10}$ of these specific antibodies respectively, it is technically impossible to reveal the presence of these two viruses in the product. It was claimed that the inactivation of rota and coronavirus by the addition of inactivating agent has been demonstrated in the dossier. Two specific detection tests (immunofluorescence) will be included on a routine basis for batch release. This will be done for BVD and IBR/IPV. The evaluation of the sensitivity of these two tests and all the data requested for the specific and non-specific viral purity test were provided at the oral explanation. The CVMP requested assurances from the Applicant on the effectiveness of the inactivation and that batch testing can adequately demonstrate the absence of antibodies against Infectious Bovine Rhinotracheitis (IBR) virus. Furthermore, confirmation was required as to the validation of the quality control tests for extraneous agents and that contamination of the product by BVD virus did not occur.

The CVMP concluded that the inactivation as tested in an *in vivo* BVD challenge model and in an *in vitro* bacterial inactivation model on viruses and bacteria provides assurances that the inactivation process used in Locatim manufacturing is effective. The Applicant demonstrated that sufficient different measures were in force to ensure that contamination of the product by BVD virus or other extraneous agents does not occur. The CVMP concluded that the sourcing of colostrum in a IBR/IPV free country in conjunction with additional IBR/IPV antibody testing gives sufficient assurances of the absence of IBR/IPV antibodies in the product.

To guarantee the absence of BVD antibodies in a product manufactured from a pool of colostrum is impracticable. Furthermore, BVD serological diagnosis is rarely undertaken in calves younger than 4-6 months of age, in order to allow a sufficient clearance of potentially specific Immunglobulin Gs originating from the dam. Regarding IBR/IPV, Switzerland is considered free of this disease, and no IBR/IPV vaccine is allowed.

The applicant's claim that the inactivation of rota and coronavirus has been demonstrated is only partially true as the data in the dossier was from an inactivation validation test conducted on viruses in

tissue culture fluid, not virus in the product. The applicant may, however, be correct in their statement that in the presence of specific antibodies it may not be possible to detect these viruses in vitro.

In addition to the survey of the absence of viral contamination in sourcing of colostrum and in the routine inactivating process, the applicant has implemented routine non-specific and specific quality control tests for the following extraneous agents: BVD, IBR/IPV, Bovine Adenoviruses and Bovine Parvoviruses. The applicant agreed that all these tests would be done on a routine basis for batch release. The validation studies intended to evaluate the sensitivity of these tests regarding these viruses have been undertaken by the applicant and show that the different techniques used for the detection of these viruses are sensitive to detect at least 1.0 $CCID_{50}$/ml of each virus.

6. Test for absence of mycoplasmas

The method described for testing the absence of mycoplasma is in accordance with the requirements of the European Pharmacopoeia. The first three batches were tested as a validation test. According to the 'Specific Requirements for the Production and Control of Immunosera and Colostrum Substitutes' and given that the product is an oral preparation, it is unnecessary to test for the absence of mycoplasmas.

7. Test for absence of mammalian cells

The sediment obtained from centrifugation of 40 ml of product is smeared on a glass slide and stained with May-Grunwald then Giemsa stain and examined microscopically. No cells must be seen.

8. Safety test

The safety test has been carried out satisfactorily for 3 batches with calves not older than 4 hours; 500 ml of colostrum having been administered at the same time. For routine testing calves not older than 12 hours of age will be given 120 ml of the test batch of the product in 500 ml of milk. Rectal temperatures are monitored before and 4 hours after dosing and general and local reactions are monitored for 14 days. No increase in body temperature greater than $1.5^{O}C$ and no significant adverse local or systemic reactions are accepted. In accordance with the requirements of the European Pharmacopoeia monograph Neonatal Ruminant Colibacillosis, the possible maximum temperature increase allowed has been set to 1.5 °C.

Local or general reactions in the list below are considered as abnormal in the routine safety test of the product:

- temperature increase over 1.5°C
- vomiting
- unexplained death
- anaphylactic shock
- severe diarrhoea or bowel syndrome of unknown origin
- severe depression and apathy
- delayed type hypersensitivity reactions
- other clinical symptoms are considered as non-significant or not considered as adverse reactions

The results are satisfactory and described in the dossier.

The milk used for dilution is the colostrum of the dam. The recommendations for use have been amended to indicate that the product may be given as presented or mixed with milk or milk replacer in calves up to 12 hours of age which may or may not have obtained colostrum prior to dosing.

9. Results for three batches

The certificates with detailed results are presented in the dossier. The results are given for batches 6227, 6241 and 6248. Batch 6227 has been diluted by 10% for use in efficacy studies and 6241 has been concentrated by 10% for use in safety tests.

The results presented indicate batch consistency. The effect of the deliberate dilution and concentration is clear enough from the results of the Immunglobulin G concentrations and this indicates the effectiveness of the current in-process control testing and dilution step on the basis of the result of this test.

10. Other comments on the control tests

Immunglobulin G concentration

The protein concentration always varies from 8-12% w/v which are the set limits. Furthermore, the method used for quantifying the IgG concentration is high-performance size-exclusion liquid chromatography. This technique allows separating the different proteins as described in the dossier. Thus, the purity of the sample does not affect the final result. The method chosen for measuring the final titer of anti - *E.coli* F5 (K99) specific IgGs is the method of microagglutination.

The F5 (K99) antigen used is prepared from the referenced strain *E. coli*, number C38-72, serotype O101:K^-,$K99^+$. The detailed description of the method of preparation and control is given in the dossier. To ensure a similar quality of the F5 (K99) antigen preparations, the method of preparation is strictly respected and each batch produced is tested 6 times against the current standard before release for routine test.

Microagglutination

The negative standard used for this test is a foetal calf serum (embryonic fluid), bovine sterile Sigma ref. E1761. The absence of IgG is checked by high-performance size-exclusion liquid chromatography. The detailed description is given in the dossier. Only the preparation of the solution is different: 1.0 ml is diluted to 10.0 ml with the eluent and filtered. The negative standard is aliquoted and stored at -18°C ± 2°C.

The positive standard is a lactoserum from hyperimmunized cows. It is analysed three times according to the microagglutination test before use as positive standard. The three results must meet the specifications of 3.1 log10 ± 0.3 $\log_{10}$, which correspond to the linearity range. The positive standard is aliquoted and stored at 5°C ± 3°C. The shelf life of the positive standard is set to 24 months.

2.6 STABILITY

Results are presented for three batches (6227, 6241 and 6248). Parameters studied are appearance, pH measurement, IgG concentration and specific anti-*E. coli* F5 IgGs measured by both the ELISA and microagglutination methods. Total proteins were measured initially only. Samples are stored at 5 ^{O}C $\pm$ 3 ^{O}C and at ambient temperature (defined as 25 ^{O}C). Results are available for 25 months storage for 2 batches and 24 months for the third.

The results show that there is no decrease in the values obtained for any of the parameters mentioned except for a slight decrease in total IgG concentration in all the samples stored at ambient temperature. In support of the requested 24-month shelf life for this product, the Applicant has submitted results from stability testing of 3 batches of a similar product. There has been no marked decrease in the values obtained for the parameters measured (including total protein, IgG concentration and specific anti-F5 IgG) over storage periods from 44 to 53 months.

Council Directive 81/852/EEC requires real-time stability data to be submitted for the product to be marketed. The fact that batch 6227 has been deliberately diluted from normal concentration and 6241

has been concentrated compared to the normal batch is considered as not ideal. However, any effect of these changes in manufacturing procedure on their stability will be undetectable.

On the basis of the data submitted a shelf life of 21 months was granted at the time of the original Opinion on 9 December 1998.

In accordance with Article 4 of Commission Regulation (EC) No 542/95 as amended, the company submitted to the EMEA on 17 June 1999 an application requesting an extension of the shelf-life to 30 months. Stability data were submitted for 3 batches, one of which was stored for 33 months, the 2 other batches were stored for 34 months under the same storage conditions as in the stability trials of the original dossier. The parameters studied were: appearance, pH, IgG concentration and specific anti-*E.coli* K99 IgGs (measured by both ELISA and microagglutination methods). The test methods and specifications limits accepted during the registration procedure were applied. A slight decrease was observed in the IgG concentration. No changes were observed for the other parameters. The extension of the shelf-life to 30 months was approved.

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

3.1 SAFETY

The product is intended to provide passive immunity to new-born calves by administration to calves up to 12 hours of age against infections with *E. coli* containing F5 adhesin. The bovine immunoglobulins are homologous with those of the calf and thus there is no risk of adverse reactions due to non-homologous immunoglobulins. The safety of the product has been examined in a field study using pre-established protocols.

3.2 LABORATORY TESTS

Safety of the Administration of One Dose

This has not been specifically addressed as no adverse effect is expected. Reliance is placed on the results of the safety of an overdose.

Safety of One Administration of an Overdose

The overdose study has been carried out in accordance with the principles of Good Laboratory Practice and has been audited. Since the study has not been carried out in an EU Member State it will not be possible to have a certificate from a GLP Compliance Unit. The Applicant's statements and the format of the report indicate sufficient compliance with the principles of GLP.

The study has been undertaken with 11 fit and healthy calves on different local farms in the Vaud canton of Switzerland. They received their normal dam's colostrum and a double dose (120ml) of Locatim. The age of the calves has been within a maximum of 12 hours from birth when the product has been administered. The batch used was 6241, which had been concentrated by approximately 10% to contain maximum IgG concentration.

Calves were monitored at times 0 hours, 4 hours, 8 hours, 24 hours, 48 hours and day 7 and 14 after the administration of the product. Parameters measured and scored were rectal temperature, consistency of faeces, demeanour and respiratory rate. The Applicant has provided two references in support of their statements regarding normal rectal temperatures and respiratory rates in calves.

The results obtained are described in the dossier. One calf developed aspiration pneumonia and has been euthanased after 48 hours. Three calves have had an increased respiration rate at one of the first three observation periods, six calves had some diarrhoea on observation day 7 and/or 14. A seventh calf had diarrhoea at the 4-hour observation period. It is stated that this farm normally suffered from 'neonatal diarrhoea' and other calves on the site are similarly affected. There are no recorded increases

in rectal temperature except on day 7, in two of the calves with diarrhoea. The 10 calves observed until day 14 remained in good general condition throughout.

The Applicant concludes that the study demonstrates that an overdose is well tolerated by newborn calves under field conditions. The SPC and product literature have been modified accordingly recommending use of product within the first 12 hours. Furthermore, an additional field trial has been carried out in order to compare the safety evaluation of an uptake of the product within the first 4 hours of life as well as within the next 8 hours.

The 'most sensitive target species' could be expected to be a calf which has not received any other colostrum and a test has been carried out in some calves in this category. There is no claim for the product as substitute or a supplement to normal colostrum but only its eventual deficient protective property, it is an aid in the control of neonatal diarrhoea in calves. In the normal practice, feeding calves with colostrum is the usual procedure and the recommendation that the product be given alone or with milk or milk replacer takes this into account, which has been addressed in the SPC and product literature.

All the calves are monitored for 14 days. In this case the observation has started after the first 48 hours after administration of the product. Nevertheless, observations concerning the animals' behaviour, appetite and general health have been done daily with ten calves without any signs of abnormality.

The safety test has been carried out with the maximum amount of IgG described in the dossier. Although the specification of the product allows a maximum value of 79 mg/ml of IgG, the batch tested for safety has been measured at 77 mg/ml of IgG. This represents a difference of 2,5 %, which is within the normal range for an HPLC assay. The upper limit of protein content is set at 12% instead of 17%. The protein concentration of the batch used for the safety study is consistent with this value.

Safety of the Repeated Administration of One Dose

No study of repeated administration of one dose has been undertaken as the product intended to be administered only once in the animal's life. A suitable warning in the SPC and product literature to the effect that the calf should only be dosed once has been made as follows:

> *"In the absence of information specifically demonstrating the safety of more than one dose, it is recommended that calves should only be dosed once."*

Examination of Reproductive Performance

No study on reproductive performance has been undertaken as the product is to be administered to new-born calves which will not be used for reproduction for approximately two years. A warning or statement has been introduced to the SPC and product literature that the product is not to be administered to pregnant animals.

Examination of Immunological Functions

No study has been undertaken as the product is composed of colostral immunoglobulins and proteins, similar to normal colostrum, and since it is to be mixed with colostrum or milk it is unlikely to affect the immune system of the calf any differently from normal colostrum.

Special Requirements for Live Vaccines

Not applicable

Study of Residues

Although the active ingredients need not be considered further regarding residues, the excipients used for the product are listed in Annex II to Council Regulation (EEC) No 2377/93.

Interactions

Given the natural biological origin of the product, no further study has been undertaken. The following statement is included in the interaction section of the SPC and product literature:

"No studies have been undertaken."

3.3 FIELD STUDIES:

Although the overdose safety test was conducted with animals on farms, the Applicant explains that safety field trials are considered as unnecessary because of their previous experience from the use of a similar product. No confirmed cases of adverse reactions after sale of approximately 500,000 doses of this product over a period of 8 years in 7 European countries have been reported. However, the statement does not adequately address this issue but, taking account of the nature of the product, the lack of safety data from large scale field trials is not a cause for concern due to the satisfactory data generated from appropriate, well controlled clinical trials. In addition the Applicant has provided a protocol and the report of the field trial to assess the safety. This trial has been conducted in order to address the issues regarding the double dose safety evaluation. In summary the protocol demonstrate that:

1. a single dose administration of the product within the first 4 hours (as well as within the next 8 hours of life) does not induce any adverse effects;
2. the volume of normal colostrum given together or separately does not interfere or mask a potential adverse reaction;
3. there is no statistical significance between treated and untreated groups regarding adverse clinical signs, which include, temperature increase, abnormal general health, modified appetite and weight gain.

3.4 ECOTOXICITY

The active substance, any material not adsorbed and excreted and any unused or waste product which is disposed of does not present any more hazard to the environment than natural bovine colostrum. It has therefore been concluded that there is no potential dangerous exposure to the environment of the product. The excipients are buffers.

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

The product is intended for use in new-born calves to provide additional protection against mortality associated with *E. coli* F5 infections (enterotoxicosis) during the first days of life and as a supplement to colostrum from the dam.

Two publications are quoted in support of the rationale for the use of the product and its likely efficacy. The first study reviews the literature describing and demonstrating the importance of ingestion of colostrum and the absorption of immunoglobulins for protection of calves from morbidity and mortality due to neonatal enteric and septicaemic infections. Some authors have emphasised the importance of organism specific antibodies, especially against *E. coli* K antigens for protecting calves. Others considered that there is a non-specific antibody of importance, which is present in colostrum.

The second study points out that there is a clear relationship between high calf serum IgG levels (from absorbed colostrum) and absence of disease in the calves but this relationship is much more pronounced when the calves are in an environment with low prevalence of disease. The level and pathogenicity of the challenge, other stressors, the presence of antibodies specifically against the pathogen and the presence of specific IgM and IgA in the gut for enteric conditions are also considered to be important.

One reference supported the ability of the *E. coli* vaccine to provide passive immunity to the offspring of the vaccinated dams. This *E. coli* vaccine is used to immunise the donor cows for Locatim. Three challenge studies have been undertaken with the immunising vaccine. Good protection against F5 bearing challenge organisms is reported and there is severe disease and deaths in most of the controls. The results of antibody responses in the cows to the vaccinations and the resultant antibody levels in the calves is discussed and compared with the apparently lower levels found in controls. Results from field trials are described in the dossier and a table with a summary of the results is presented. The beneficial effects are obtained against neonatal diarrhoea and there are lower rates of isolations of F5 positive *E. coli* in the calves from the vaccinated cows compared to the controls.

In addition, the Applicant has provided data on the efficacy of a product consisting of concentrated IgG from colostrum, against neonatal colibacillosis. This product has been used in 41 calves on 8 farms with a history of neonatal colibacillosis. The causes of the diseases seem to be a range of organisms and none are stated to be due to *E. coli* with F5, but the product contains IgGs to a range of *E. coli* antigens and rota- and coronaviruses. Twenty-three of the calves have been given the product only, the rest have been given a range of other treatments as well. Good survival rates were obtained. In conclusion the product has shown an obvious clinical activity, the importance of an oral antibody supply for intestinal passive immunity and presented favourable future indications for effective protection against infectious enteritis.

The administration of Locatim cannot according to the applicant provide absolute protection and it is intended as an aid in control e.g. delaying the onset of clinical symptoms, diminishing the severity of the symptoms and increasing survival time, giving more opportunity for supportive therapy and enabling the farmer to limit economic loss.

4.1 LABORATORY STUDIES

One challenge study has been conducted with Locatim. The study has been carried out in accordance with the principles of Good Laboratory Practice and an audit statement by the Quality Assurance unit of the Applicant. The study has been undertaken with 10 treated and 5 control colostrum-deprived calves shown to be IgG negative. The product was administered when the calves were up to 4 hours old. The calves have received 1200 ml of UHT milk on arrival in the SPF Unit and treated calves received 60 ml of Locatim (batch 6227) mixed in it and all calves accepted this voluntarily. Thereafter the calves received up to 2000ml of UHT milk daily. Refused milk has been recorded.

The calves have been challenged orally, immediately after sodium bicarbonate to neutralise abomasal acidity, when they have reached 11 and 13 hours of age. The challenge is a dose of 152 ml containing between 5.3 and 8.4 x 10^{10}cfu of *E. coli* F5 -B41 (Serotype 0101:K-K:K99). The calves have been monitored on a regular basis for up to at least 5 days post challenge. For the first 42 hours, clinical examination has been carried out every 6 hours. Thereafter, the calves have been examined twice per day. Parameters scored were rectal temperature, consistency of faeces, demeanour and respiratory rate. The details of the scoring system were given in the dossier. Severely affected calves and calves with scores of 6 or more have been euthanised and given the maximum possible score of 8 for the rest of the study. Faecal material has been collected for estimation of faecal dry matter and confirmation of the presence of F5. Blood samples have been collected for measurement of pcv and sodium and potassium content.

The scoring system was based on severity of diarrhoea and depression. Increased rectal temperatures have usually been seen in calves with severe diarrhoea. Calves with severe diarrhoea have not always had raised temperatures. Increased respiratory rates have been observed as calves started to become depressed or distressed. All calves including survivors have showed signs of disease although these were limited in severity and or duration for the five calves, which survived. No significant difference has been found between the clinical scores of the two groups except for survival times, which is significantly longer in the treated group compared to the control group. All calves excreted *E. coli* expressing F5 in their faeces. The onset is a little later in the treated compared to the controls. From 18 hours after treatment all calves excreted *E. coli* expressing F5 in their faeces throughout the observation period. The percentage faecal dry matter (%DM) provides further evidence of the delay in onset of disease in the controls. There is a rapid decrease in the group mean %DM by 6 hours in the controls while the same low level does not occur in the treated group until 24 hours.

Plasma sodium levels dropped to their lowest level more quickly in the controls than in the treated group. In the case of plasma potassium levels, the levels for the control calves were higher than the treated calves.

Treatment increased survival time. 50% of treated calves survived compared to 0% of the controls. The onset of diarrhoea was delayed and so the dehydration. It is considered that the challenge had been too severe. Compared to a field infection, the calves had been injected a highly virulent strain and a much higher challenge dose compared to a field infection, the calves were colostrum deprived and were treated with sodium bicarbonate before challenge. The calves received no supportive therapy when clinical signs started. The delay in onset of clinical signs and increased survival time would allow more time for treatment to be given.

The study had been carried out correctly and reflects the worst case scenario with the calves not having been given any other colostrum. The product was given in milk and it would appear that this should be part of the recommendations for use. The study is conducted broadly in line with the method described in the potency test of the European Pharmacopoeia monograph.

There is no claim made for duration of protective effect of the product but as most neonatal infections with *E.coli* occur during the first hours of life, the product has been demonstrated as efficacious. The duration of the monitoring post-infection, as most of the other parameters of the trial, had been conducted along the lines of the potency test of the Ph. Eur. monograph for Neonatal Ruminant Colibacillosis. In this monograph the potency test requires at least a 3 days monitoring period (72 h) after challenge.

In this study the monitoring period lasted after 114 h or 5 days following the challenge, which seems a convenient and sufficient period to detect any infection consequent to a severe *E.coli* challenge. The fact that all the control animals died within the 66 hours after the challenge confirms this. The Ph. Eur. monograph for Neonatal Ruminant Colibacillosis is being considered for revision, in particular the challenge-method. However, the CVMP expressed concern that the only evidence regarding the efficacy was contained in one challenge study which was considered as a very extreme model, and where the control group was totally colostrum deprived, and no calf received any supportive treatment. Therefore, the applicant was asked to elaborate on the findings that treatment with the

product did not affect clinical signs significantly, but only reduced mortality, as a consequence of the heavy challenge, the results of these trials showed that the mortality in the control calves was 100% while in the treated calves it was 50%. The justification given was that although no significant differences were found in respect of clinical score, the treatment resulted in a statistically significant increase in the survival time. Furthermore, the delayed onset of diarrhoea and subsequent dehydration seen in these trials was reinforced by all experimental parameters monitored (PCV, %DM, plasma Na and plasma K).

4.2 FIELD STUDIES

The Applicant originally considered that efficacy had been demonstrated in the controlled challenge study. This was justified by the fact that the disease was of a sporadic nature and the clinical picture was variable depending on the strain of *E. coli* present and other factors. As a result, it was considered that it was likely that a field trial would add little to the evidence of efficacy obtained from the challenge study.

A field trial was however carried out, involving 164 calves. The results of the trial confirm the safety of the product when used in accordance with the proposed directions for use of the product. An attempt was also made to evaluate the efficacy of the product when used in accordance with the proposed directions for use. However, given the multifactorial nature of neonatal diarrhoea in calves and the considerable normal variation in the severity of the disease depending on a variety of factors including, but not limited to, the nature and virulence of the organism(s) to which the calves are exposed and the general standards of hygiene and husbandry on the farm, it is impossible to ensure that under the conditions of a field trial, sufficiently appropriate and consistent conditions exist to enable a valid evaluation of the efficacy of the product.

This was the reason given originally for focusing the efficacy revaluation of the product on a controlled laboratory trial, carried out at the Moredun Institute in an isolation unit and using an artificially severe challenge with *E.coli* F5 (K99). The observations made on the efficacy of the product during the field trial, whilst not statistically significant, were nevertheless consistent with the results obtained in the challenge study and give support for the proposed indications for the product.

The CVMP raised concerns that the field trial provided to support the safety file did not demonstrate statistically significant beneficial effects as evidenced by the conclusion that there is no statistical significance between treated and untreated groups. The Applicant justified the use of these trial results in support of the proposed claim in view of the multifactorial nature of neonatal diarrhoea in calves and the variation of the severity of the disease depending on a variety of factors. These include the nature and virulence of the organism(s) to which the calves are exposed and the general standards of hygiene and husbandry on the farm, so that it is difficult to ensure that under field conditions, sufficiently appropriate and consistent conditions exists to enable a valid evaluation of the efficacy of the product. Consequently, the CVMP considered the efficacy data for the product could be supported by controlled laboratory trial, in an isolation unit and using an artificially severe challenge with *E.coli* F5 (K99).

Implied claims

In the original dossier there were references in the SPC and product literature to the fact that the product also contained antibodies to other *E. coli* antigens and rota- and coronaviruses. It was considered by the CVMP that this was an implied claim and although such antibodies would, no doubt, be present; since no tests were carried out to verify the presence of these antibodies, the references to these have been removed.

The presence of maternally derived antibodies can interfere with the active immunisation of young animals against certain infectious agents. The product contains a number of antibodies in addition to those to *E.coli* F5 (K99) as a result of both vaccination of the donor cows and their natural exposure to infectious organisms in their environment. The vaccine used to induce antibodies to *E.coli* F5 (K99)

also contains antigens to Y, 31A, 078. Furthermore all the donor cows used to supply colostrum for the product are required to be vaccinated against rota and corona viruses as part of a normal prophylactic programme, using a vaccine registered within the EU.

Reference to the other specific antibodies is made in the SPC and product literature in order to be taken into account by the veterinarian when planning active immunisation or other prevention and/or control measures for calves which have received the product. The reference appears under the headings Incompatibilities, Special Warnings. As this is a warning concerning the possible presence of such specific antibodies, the committee concluded that it was not required to introduce a qualitative test to prove it.

There is no claim for systemic passive immunization and it is not in the Applicant's intention to make such a claim. However, it is well documented in the literature that the natural uptake of colostrum within the first hours of life induces both a local and a systemic passive immunization. Most of the total amount of immunoglobulins transferred by the dam will be ingested during early feeding in the first hours after birth under natural conditions. After that, the concentration of immunoglobulins in the colostrum is drastically decreased. Protection against bacterial and viral diarrhoea, as described in the literature, is due to a combination of local presence of IgG and transfer of serum IgG into the intestinal lumen. For the product the efficacy data demonstrated a significant increase of survival time in the treated group which received the product. It can therefore be concluded that even with the prevalence of systemic passive immunization, the product is still efficacious.

Compatibility

The Annex (Part 4) to Council Directive 81/852/EEC requires the Applicant to address the compatibility of products recommended for administration together or at the same time. Since no such recommendation is given the Applicant has not addressed this. The entry for this in the SPC and product literature states that there is no incompatibility known.

The method of collection of colostrum from donor cows and the production process will result in antibodies to agents other than *E. coli* F5 in the product. It is considered, therefore, that the statement under Incompatibilities in the SPC and product literature include as a warning that use of the product may result in antibodies in the serum of the calves that could interfere with early vaccination against bovine diseases. The following statement is included in the SPC and product literature:

> *"The product is produced from colostrum collected from cows kept under field conditions. Consequently, in addition to antibodies to E. coli F5 (K99) it also contains antibodies to other organisms, as a result of vaccination and/or exposure of the donor cows to organisms in their environment. This should be borne in mind when planning vaccination programmes for calves, which receive Locatim."*

Real data for the efficacy of the product is from the one challenge study. The information from the published literature and the expert report for the *E. coli* vaccine can be described as supporting data including the advice that the administration of colostrum to calves is only one aspect of prevention of disease.

From the data available from the challenge studies, a limited though useful effect was observed. The Applicant has clarified in the SPC and product literature the benefits users may expect from the product. The indications now read as follows: 'Reduction of mortality caused by enterotoxicosis associated with *E. coli* F5 (K99) adhesin during the first days of life as a supplement to colostrum from the dam.'

5. RISK-BENEFIT ASSESSMENT AND CONCLUSION

Based on the original and complementary data presented, the Committee for Veterinary Medicinal Products concluded that the quality, the safety and the efficacy of the product are considered to be in

accordance with the requirements of Council Directive 81/852/EEC and supports the revised claims of the Applicant.

The Applicant provided further data on the following:

- validation of the inactivation process by an *in vivo* study
- confirmation of stability of 21 months
- the absence of IBR antibodies in batch testing
- validation of quality control tests on the finished product for extraneous agents

The product is not intended as a colostrum substitute, but reduces mortality caused by enterotoxicosis associated with *E. coli* F5 (K99) adhesin during the first days of life of calves. It is intended to be given in addition to natural colostrum from the dam, in order to supplement its eventually deficient protective properties. A number of studies have shown that the administration to calves of colostrum from cows vaccinated against *E.coli* F5 (K99) offers a significant level of protection against challenge with *E.coli* F5 (K99). The product is derived from immune colostrum from vaccinated dams. The role of the product is to ensure that the calf receives a protective complementary dose of specific *E.coli* F5 (K99) antibodies. The observations seen in the efficacy challenge study, confirmed by the field trial, show that treatment with the product increases survival time leaving more opportunity to institute supportive therapy and support the claim made for the product.

On 9 December 1998 the Committee agreed by a majority of 19 to 8 votes that the product could be recommended for the granting of a Community marketing authorisation. Eight members of the Committee considered these data to be inconclusive, because in their opinion there was a lack of proven efficacy in the clinical documentation. Therefore, these CVMP members expressed divergent positions.