STARTVAC®

Inactivated vaccine against E. coli, coliforms, S. aureus and coagulase-negative Staphylococci

less mastitis post-partum

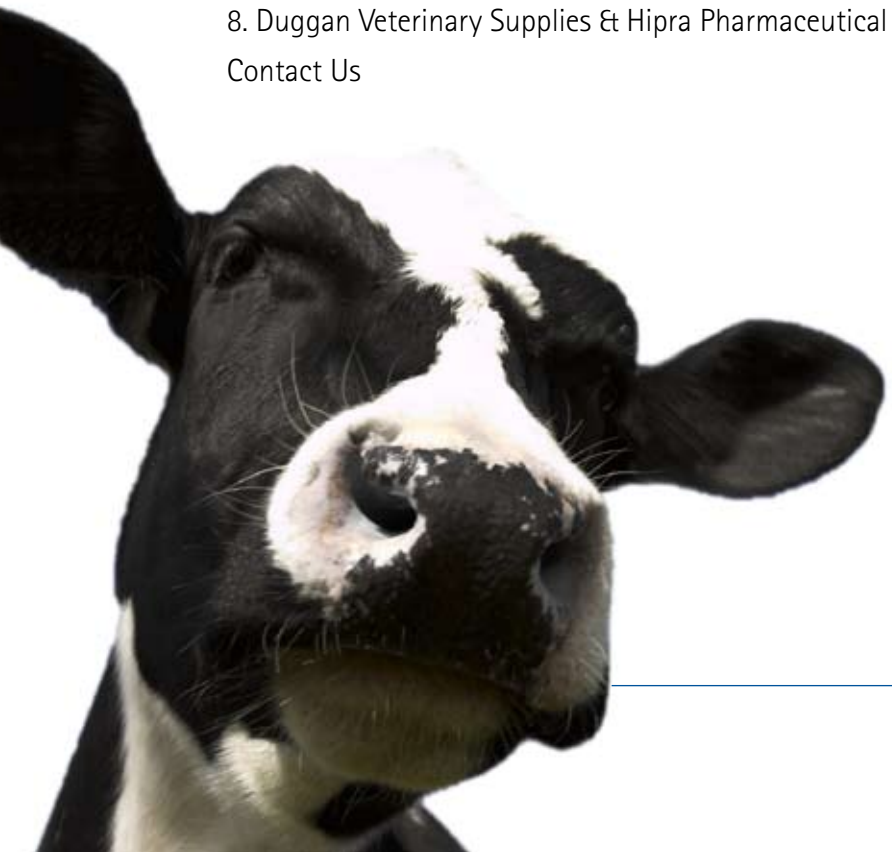
1st vaccine

against bovine mastitis post-partum

DugganVeterinary

CHOICE • EXPERTISE • RELIABILITY

Index



STARTVAC® Introduction

Startvac® is the first Vaccine approved by The European Medicines Agency (EMEA), against bovine mastitis to aid in reducing the incidence of mastitis in the herd. Following extensive research and trials, based on the three groups of most highly prevalent microorganisms on dairy cattle farms across Europe (coliforms, *Staphylococcus aureus* and coagulase-negative Staphylococci), HIPRA, a company dedicated to research, production and marketing of animal health products, has developed a new solution that contributes to improving the health of cows and, therefore, the quality and safety of their milk and its sub-products.

STARTVAC® is innovative in that, for the first time in the world, there is a vaccine that prevents the development of a layer, by the previously-mentioned bacteria, known as biofilm of slime. This biofilm, in addition to making the bacteria more pathogenic, protects against the action of antibiotics

Startvac® has the following therapeutic indications:

a) For herd immunisation of healthy cows and heifers, in dairy cattle herds with recurring mastitis problems,

b) to reduce the incidence of sub-clinical mastitis and

c) the incidence and the severity of the clinical signs of clinical mastitis caused by *Staphylococci Aureus,* coliforms and coagulase-negative staphylococci.

STARTVAC® has achieved, for the first time in a biological product of this type, authorization for marketing in over 30 EU countries. This represents a milestone for Animal Health professionals, who until now only had antibiotics available for controlling this disease.

Startvac® as a vaccine works by 'teaching' the immune system of the animal (the cow's natural defences) how to defend itself against a disease. Containing two forms of dead bacteria that normally cause mastitis (*Escherichia coli* and *Staphylococcus aureus*) Startvac® when administered to a cow, causes the animal's immune system to recognise the bacteria as 'foreign' and make antibodies against them. It teaches the immune system to be able to make the antibodies more quickly when it is exposed to the bacteria in the future. The antibodies will help to fight the bacteria, preventing mastitis occurring or reducing the severity of its symptoms.

STARTVAC® "Less Mastitis Post-Partum"

Startvac® reduces mastitis post partum, which in turn reduces the somatic cell count of dairy cows and this will aid the Irish dairy farmer in his efforts to secure improved bonuses due to the production of milk with improved lower cell count. Startvac® will play a valuable role in aiding farmers whose herds suffer from high somatic cell counts, reduce this problem and aid towards maximizing financial return from milk production.

For Startvac® to be effective , **it must be used in conjunction with a well maintained herd health plan if mastitis is to be dealt with successfully in the herd.** Immunisation has to be considered as one component in a complex mastitis control program that addresses all important udder health factors (e.g. milking technique, dry-off and breeding management, hygiene, nutrition, housing, bedding, cow comfort, air and water quality, health monitoring) and other management practices.

Technical Data Sheet

STARTVAC®

Inactivated vaccine against bovine mastitis

COMPOSITION PER ML

One dose (2 ml) contains: *E. coli* J5 inactivated >50 RED60*, *S. aureus* CP8 inactivated strain

SP140 expressing SAAC >50 RED80. *(Slime Associated Antigen Complex (SAAC)). * RED: Rabbit effective dose in 60% or 80% of animals (serology). Liquid paraffin: 18.2 mg. Benzyl alcohol: 20 mg.

INDICATIONS

For the immunization of dairy cows with recurring problems of mastitis, to reduce the incidence of sub-clinical mastitis and the incidence and severity of clinical signs of mastitis caused by *Staphylococcus aureus*, coagulase-negative staphylococci and coliforms. The complete schedule of immunization induces immunity from approximately 13 days after the first injection until about 78 days after the third injection (equivalent to 130 days after calving). **STARTVAC® acts differently depending on the microorganism;**

E. coli and coliforms: Startvac® acts by inhibiting the development of the cell wall, thereby preventing bacterial growth. STARTVAC® acts against the CORE antigen at a specific time in the growth of the wall, thus enhancing the recognition of the natural defences for the destruction of the bacteria.

S. aureus and CNS: STARTVAC® hinders the formation of SLIME. Slime or Biofilm is a layer of exopolysaccharides that surrounds the bacteria, enhancing their growth and resistance to antibiotics. STARTVAC® prevents the development of SLIME and also favours contact with neutrophils, enabling the destruction of bacteria. This highlights the importance of an immunization protocol and benefits of vaccination.

CONTRAINDICATIONS AND ADVERSE REACTIONS

- No contraindications applicable.
- Temporary slight to moderate local reactions may occur after a dose of the vaccine is administered.
- This may appear as: swelling (up to 5 cm2 on average), which vanish one or two weeks later at most. In some cases, pain may also appear at the inoculation site that spontaneously subsides by 4 days later at most.
- There may be a transient increase in body temperature of about +1 °C and up to +2°C in some cows in the first 24 hours after injection.
- Animals immunized with an overdose showed no adverse reactions other than those observed after administration of a single dose of the vaccine.
- If you notice any serious effects or any others not listed in this document, please report it to Duggan Veterinary Supplies.
- A minor increase in somatic cell count post administration for a one or two days.

WITHDRAWAL PERIOD

Zero days.

SPECIAL PRECAUTIONS FOR STORAGE

Keep out of reach and sight of children.

Store and transport refrigerated (+2 °C to +8 °C) and protected from light.

Do not freeze.

Do not use after the expiration date on the label.

Shelf life after opening the immediate packaging of the drug: 10 hours when kept between +15 °C and +25 °C.

Vaccine Administration

Startvac is administered intramuscularly. Administer one dose (2 ml) by deep intramuscular injection. The vaccination can administered via the neck area or the rump (gluteal muscle). The complete immunization programme should be repeated annually throughout the entire herd.

Protocols described below are set out to maximize the full potential of the vaccine. If vaccination dates are missed then the vaccine will not work to its full potential.

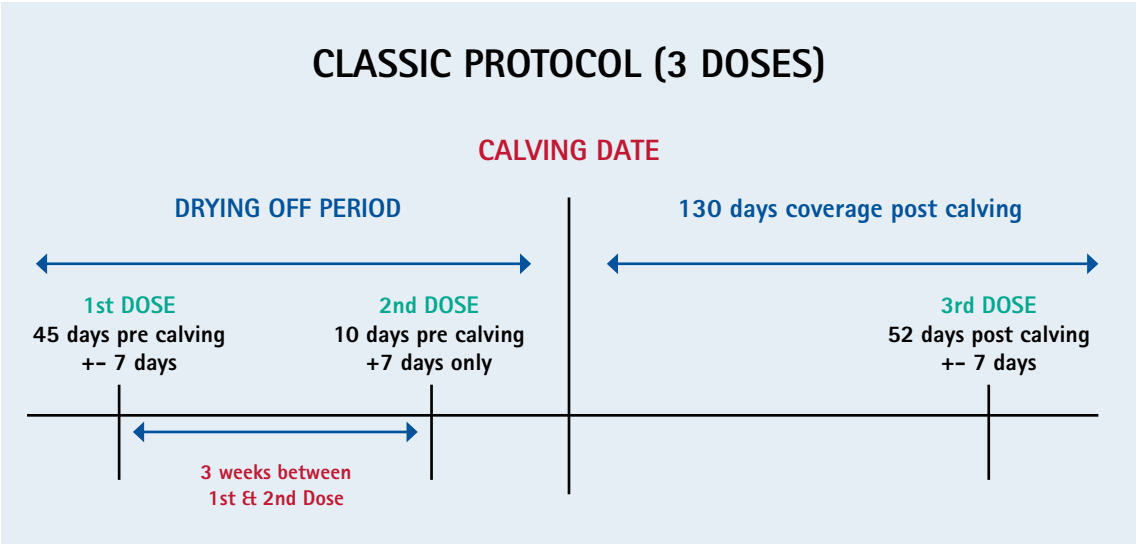
CLASSIC PROTOCOL (3 Dose)

Where the exact dates of covering the total period of gestation are confirmed, the classic protocol should be used. By using two applications before calving (45 and 10 days) and one application post calving (52 days) the objective of reducing mastitis is achieved at the time of greatest risk of infections and economic loss.

N.B There has to be 3 weeks between 1st and 2nd Dose.

45 days	+/- 7 days	(52 - 38 days)	Pre Calving	First dose
10 days	**+ 7 days only***	(17- 10 days)	Pre Calving	Second dose
52 days	+/- 7 days	(45 – 59 days)	Post Calving	Third dose

*In the case of an early calving, the 2nd dose can be administered 10 days post calving



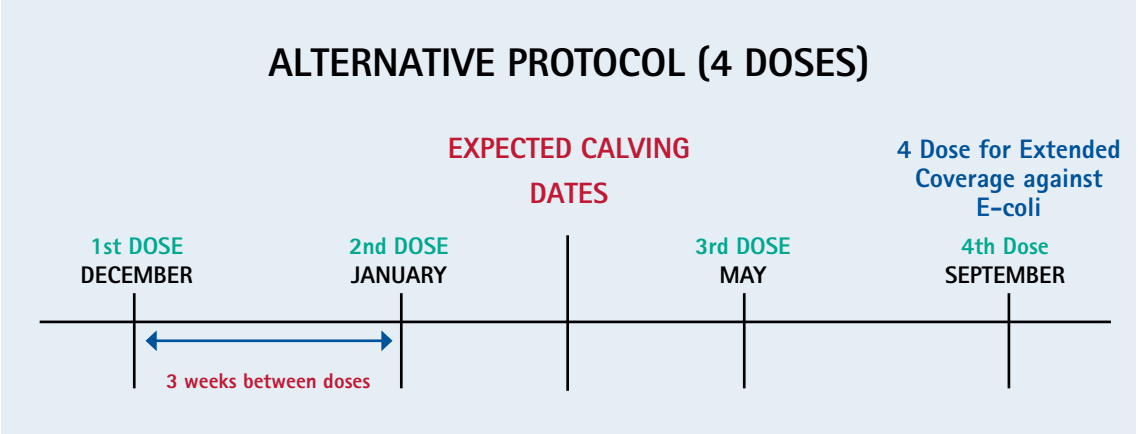
ALTERNATIVE PROTOCOL (4 Dose)

Where the gestation period of the herd is unknown a blanket 4 dose protocol (referred to as the Alternative Protocol) is to be administered in year one to ensure complete cover. In year 2, once the correct gestation dates have been recorded, the farmer can then revert back to the Classic Protocol and administer 3 doses per animal.

YEAR	FIRST DOSE	SECOND DOSE	THIRD DOSE	FOURTH DOSE
Year 1	**December**	**January**	**May**	**September**
Year 2	**45 ± 7 days**	**10 + 7 days**	**52 ± 7 days**	

N.B There has to be 3 weeks between 1st and 2nd Dose.

1. The entire herd should be vaccinated together in December (dose 1)
2. The entire herd should be vaccinated in January (dose 2), with no animal vaccinated within 3 weeks of 1st dose
3. After the 2nd dose its important to vaccinate every 4 months for hyperinmmunization (repeated injections of antigen leading to high levels of antibody). This will maximise the cows immunity against Coliforms / E-coli for a longer period of time.
4. The entire herd should be vaccinated in May (dose 3), regardless of when second dose was given
5. The entire herd should be vaccinated in September (dose 4)- this ensures continuous immunity against Coliforms / E-Coli
6. In year two, with correctly recorded data the Classic Protocol (3dose) can be adapted.



NOTE: * In year two, the classic protocol (3 doses) can be adopted if the farmer is aware of all calving dates. Otherwise a 4 dose protocol is to be used again. This results in the administration of an extra dose and an increased cost to the farmer.

NOTE: After administration of Startvac® the Somatic Cell Count of the vaccinated animal will rise slightly for 2/3 days before returning to its normal state. Startvac®, when administered, encourages the animals immune system to start fighting against infection thus causing the Somatic Cell Count to rise temporarily.

- Therefore if using the **Alternative Protocol (4 Dose)** it is important to be selective what day Startvac® is administered. Milk samples recorded after vaccination will show a temporary rise in Somatic Cell Countlevels.
- In the **Classic Protocol (3 dose)**, a select number of cows are vaccinated at one time and therefore the Bulk SCC reading will not be affected

EMEA Field and Laboratory Trials

EMEA (European Medicines Agency) REGISTRATION

On 18th of February 2009, Startvac® became the first vaccine against mastitis registered through the EMEA. The EMEA registration system is the most complete and thorough registration system in the world. Its safety and efficacy tests are priorities for the confirmation of the product, unlike other systems, such as the American FDA, which only gives importance to placebo tests for controlling product safety, but not its efficacy. At present Startvac® is registered in 30 EU countries.

EPAR EPEA SCIENTIFIC SCIENTFIC DISCUSSION

(This module reflects the scientific discussion for the approval of Startvac®)

INTRODUCTION

An application for the granting of a Community marketing authorisation of Startvac® has been submitted to the EMEA in accordance with Council Regulation (EEC)No. 726/2004 on 27 April 2007 by Laboratorios Hipra, S.A. STARTVAC® is presented in packs/containers of 3 ml (1 dose = 2 ml), 10 ml (5 doses) and 50 ml (25 doses). It contains inactivated *Escherichia (E.) coli* J5 and inactivated *Staphylococcus (S.) aureus* (CP8) and is indicated for herd immunisation of healthy cows and heifers, in dairy cattle herds with recurring mastitis problems, to reduce the incidence of sub-clinical mastitis and the incidence and the severity of the clinical signs of clinical mastitis caused by *Staphylococcus aureus,* coliforms and coagulase-negative staphylococci. The route of administration is intramuscular use. The target species is cattle (cows and heifers).

QUALITY ASSESSMENT

Composition

Startvac® is an immunological product containing whole cells of heat-inactivated *Escherichia coli* J5 strain and whole cells of a formaldehyde-inactivated *Staphylococcus aureus* (CP 8) strain. The vaccine is adjuvanted with liquid paraffin and contains benzyl alcohol as preservative. The quantitative composition has been defined below.

Active substances:

Escherichia coli J5 inactivated ..... > 50 RED60 *

Staphylococcus aureus (CP8) strain SP 140 inactivated, expressing Slime Associated Antigenic Complex (SAAC)..... > 50 RED80**

* RED60: Rabbit effective dose in 60 % of the animals (serology).

** RED80: Rabbit effective dose in 80 % of the animals (serology).

Adjuvant:

Liquid paraffin..... 18.2 mg

Excipients:

Benzyl alcohol.....20 mg

Liquid paraffin

Sorbitan monooleate

Polysorbate 80

Sodium alginate

Calcium chloride, dihydrate

Simeticone

Water for injections

CONTAINER

The colourless vials (3 ml/1 dose, 10 ml/5 doses and 50 ml/25 doses) are of type I glass according to European Pharmacopoeia *(Ph. Eur.)* 3.2.1. The vials are closed with grey bromobutyl stoppers. These stoppers are classified as Type I rubber stoppers and comply with *Ph.Eur.* Section 3.2.9.

DEVELOPMENT PHARMACEUTICS

Startvac® contains inactivated whole cells of two

bacterial strains incorporated in an oil-in-water emulsion in order to stimulate immunity. There is a comprehensive justification regarding the choice of the bacteria, adjuvant and preservative.

Staphylococcus aureus *(S. aureus)* is recognised as the main contagious pathogen in bovine mastitis. The strain included in the Startvac® vaccine is based on the presence of the Slime Associated Antigenic Complex (SAAC), which is an exopolysaccharide. This is an important virulence factor implicated in the adhesion of the bacteria to the epithelium of the mammary mucous. The induction of antislime antibodies will help the minor colonisation and subsequent multiplication of *S. aureus* in the glandular epithelium.

Escherichia coli *(E. coli)* are wide-spread in the dairy environment and considered as the most important cause of environmental mastitis. The strain *E. coli* J5 lacks the enzyme Uridin Diphosphate Galactose 4-Epimerase, which is responsible for binding the somatic antigen (O-chain polysaccharide) to the LPS molecule of the cell wall. Thus, the core antigen, which is common to many gram negative microorganisms, is better exposed to the outside of the bacterium and, therefore, better recognised by the immune system.

Liquid paraffin is chosen as adjuvant component. In spite of its mineral origin (non-biodegradable), the low percentage (oil-in-water-emulsion) used in this vaccine confers a good safety profile.

Benzyl alcohol is chosen as preservative. The efficacy of antimicrobial preservation is properly evaluated according to *Ph. Eur.* 5.1.3 and the Guideline for the Testing of Veterinary Medicinal Products, 1994: "Inclusion of antimicrobial preservatives in immunological medicinal products" (III/3469/92). The proposed in-use shelf life of 10 hours after first opening of the bottle is considered sufficiently substantiated by appropriate data (microbial safety as well as sterility and potency results, which were provided in a separate study).

Sodium alginate – calcium chloride was added to the vaccine composition to obtain a more viscous and stable emulsion.

METHOD OF MANUFACTURE

The manufacturing process corresponds to a classical procedure. Bacteria used in manufacture are handled in a seed-lot system. The strains are propagated in a scale-up system. In the case of *S. aureus*, the culture of the fermentor is inactivated and afterwards the antigens are concentrated by centrifugation.

The harvest of *E. coli* J5 is washed with PBS and then a concentration (centrifugation) is performed.

The inactivation procedures are adequately validated by appropriate inactivation kinetic studies.

The concentrated antigens are stored at +2 °C - +8 °C for a maximum period of 12 months until they are used for blending purposes. The bulks of active ingredients are blended with other components to an emulsion, filled in defined containers, labelled and packed to obtain the finished product. The maximum blending volume will be 300 litres.

The volume of antigens to be added in order to obtain the target concentration of 1 x 1010 microorganisms of each antigen per dose of 2 ml is calculated on the basis of concentration of total bacteria determined after the concentration step which follows the inactivation step. The method is considered properly validated for both antigens *E. coli* J5 and *S. aureus*. The consistency of the production is demonstrated on three pilot batches and one commercial batch.

CONTROL OF STARTING MATERIALS

Active substances;

The original strain of *Staphylococcus aureus* CP8 was obtained from the isolate collection

from DIAGNOS, the Diagnostic Centre of LABORATORIOS HIPRA, S.A. This strain was characterized as a phenotype producer of Slime (SP) by means of immunoelectrophoresis (IEP) and the Congo Red test. It was also determined by immunoelectrophoresis (IEP) as a strain belonging to Capsular Polysaccharide 8 (CP8).

The strain *Escherichia coli* J5 used in the production of Startvac® vaccine was isolated from old cultures *E. coli* O111:B4 by selection and subsequent cultivation of colonies "galactose-negative" (characterised by its lack of colour) and sensitive to galactose (characterised by its tendency to disintegrate after prolonged incubation).

Information relating to the vaccine strains *E. coli* J5 and *S. aureus* CP8, their origin, characterisation, passage history, preparation and storage conditions has been provided. Seed lot systems have been followed. Identity and purity of MSB and WSB have been confirmed by morphology, growth characteristic and biochemical analysis. Serotyping will be introduced as a routine control in any new *E. coli* working seed and S. aureus working seed.

Excipients;

Starting materials listed in a pharmacopoeia are sodium alginate, calcium chloride dehydrate, liquid paraffin, benzyl alcohol, Polysorbate 80, simeticone, sorbitan oleate, sodium hydroxide, glucose monohydrate, formaldehyde solution (35%), sodium chloride, potassium chloride, disodium phosphate dodecahydrate, gelatine (from porcine skin), sucrose, povidone, monosodium glutamate, water purified, water, highly purified and water for injections.

All starting materials are referred to *Ph. Eur.* with the exception of monosodium glutamate for which reference is made to the US Pharmacopoeia (USP). Defined specifications are provided in the Certificates of Analysis (CoA). The Certificates of Analysis comply with the related monograph and results match every specification.

Starting materials of biological origin not listed in a pharmacopoeia are Tryptic Soy Agar (TSA), Tryptic Soy Broth (TSB) and yeast extract. The following information about the TSA and TSB is included in the dossier: Certificate of analysis, raw material quality control sheet, copy of the catalogue of the supplier, technical sourced raw materials document, animal origin position statement, letter about the bovine milk component as well as information about their sterilisation. Yeast extract is derived from the soluble part of the yeast cells after autolysis. The corresponding certificate of analysis is provided. It is sterilised together with the culture medium once prepared.

The following in-house media are used: freeze-drying excipient, TSB-G medium, CB120 culture medium, PBS solution, sodium hydroxide solution and antifoam solution. The composition, preparation and sterilisation are adequately described. Sterility control is performed by direct inoculation. Shelf lives and storage conditions are defined.

Specific measures concerning the prevention of the transmission of animal spongiform encephalopathies

The following starting materials of animal origin used in the production of the final product comply with the current regulatory texts related to the TSE Note for Guidance (EMEA/410/01-Rev.2) and Directive 2001/82/EC: *Escherichia coli* J5, *Staphylococcus aureus* CP8, Seed Lots TSA and TSB .

Control tests during production

During manufacture the following *in-process* controls are carried out to assure the quality parameters.

***Staphylococcus aureus* CP8:**

Gram stain, viability/ purity, identity, count of viable bacteria, count of total bacteria, inactivation, pH, sterility and SAAC concentration.

***Escherichia coli* J5:**

Gram stain, viability/ purity, identity, count of viable bacteria, count of total bacteria, inactivation, Ph and sterility.Detailed information of the methods, their frequency, their function and their specifications are included in the dossier. Maximum pre-inactivation specifications (viable count according to the inactivation kinetic studies) are established.

The following methods are adequately validated:

- Concentration of viable bacteria

- Concentration of total bacteria
- SAAC concentration
- Test for complete inactivation.

Control tests on the finished product

The description of the methods used for the control of the finished product (appearance, viscosity, identification and quantification of the preservative, pH, volume control, residual formaldehyde, conditioning, sterility, determination of endotoxins, safety test, potency test: vaccination of rabbits and indirect ELISA for determination of *E. coli* J5 antibodies and *S. aureus* anti-slime antibodies) and the specifications are provided. The specifications proposed are appropriate to control the quality of the finished product. The results of the analysis of three consecutive pilot batches and one commercial batch were presented and comply with the required specifications.

The following methods are adequately validated:

- Identification and quantification of the preservative
- Residual formaldehyde
- Sterility test
- Determination of endotoxins
- Batch potency tests.

The handling of OOS results has been satisfactorily addressed. Furthermore, amendments regarding procedures for safety testing and vaccination of rabbits were made in accordance with the list of outstanding issues.

Stability

An antigen stability study has been provided. It has been properly demonstrated that antigen stocks stored for 12 months (2° C – 8° C) before blending are stable over the claimed shelf-life of the vaccine.

Samples (1 dose and 25 doses, respectively) from three consecutive pilot batches filled in glass bottles (colourless, Type I with rubber stoppers, Type I) were included in the stability studies. The parameters evaluated, their specifications and the methods were the same as established for the final product testing with the exception that volume and endotoxin content were not controlled. This was sufficiently justified.

The results support a shelf-life of 18 months post-manufacturing. This shelf-life is substantiated by appropriate data. It was further demonstrated that batches released with the minimum acceptable value of potency will be stable over the claimed 18 months storage period. The proposed in-use shelf-life of 10 hours after first opening of the bottle was considered sufficiently substantiated by appropriate data (microbial safety, sterility and potency results). New real time stability studies with one commercial batch (Batch no. 5Z5Y filled in 25-dose presentations and 1-dose presentations using the newly introduced 3 ml vial) have been initiated. The Applicant has undertaken the commitment to provide the results in regular intervals until the foreseen end of the stability study (Feb. 2010).

OVERALL CONCLUSION ON QUALITY

The product is manufactured in accordance with the principles of Good Manufacturing Practice at a licensed manufacturing site. Process validation data on the product have been presented in accordance with the relevant European guidelines.

The documentation meets the requirements of Directive 2001/82/EC and the current *Ph. Eur.* Monographs as well as the relevant guidelines.

The necessary data on the qualitative and quantitative composition have been provided.

The majority of starting materials used in production comply with pharmacopoeial monographs.

Biological starting materials used are in compliance with the current regulatory texts related to the TSE Note for Guidance (EMEA/410/01-Rev.2) and Directive 2001/82/EC.

The master and working seeds have been produced according to the Seed Lot System.

The tests performed *in-process* are described and the results of 3 consecutive runs, conforming to the specifications, are provided.

The tests performed on the final product are in compliance with the relevant requirements. The tests include validated potency tests (vaccination of rabbits and indirect ELISA for determination of *E. coli* J5 antibodies and *S. aureus* anti-slime antibodies), content of residual formaldehyde, sterility and determination of preservative (benzyl alcohol) as well as endotoxin measurement. Furthermore, appearance, viscosity, safety, extractable volume and pH measurement are established as final product tests. The controls during production and on the finished product guarantee compliance with the specified quality parameters.

Demonstration of the batch-to-batch consistency is based on the results of 3 batches (pilot) produced according to the method described in the dossier. Other supportive data (commercial batch) provided confirm the consistency of the production process.

Real time stability data on the finished product have been provided, demonstrating the stability of the product throughout its shelf-life (18 months) when stored under the approved conditions.

It has been properly demonstrated that antigen stocks stored for 12 months (2° C – 8° C) before blending are stable over the claimed shelf-life of the vaccine.

The in-use shelf-life of the broached vaccine (10 hours) is supported by data (microbial safety, sterility and potency).

SAFETY ASSESSMENT

Laboratory tests

Four laboratory studies were performed to assess the safety of the administration of a single, double and repeat single dose using batches of standard antigen content (antigen load is fixed to 1 x 1010 microorganisms for *S. aureus* and *E. coli* J5, respectively). The studies have been conducted according to GLP. The animals used were of the appropriate target species cattle and the most sensitive category of the target species for which the vaccine is intended for (heifers = primiparous cows, in the last trimester of pregnancy) has been included in the trials. Only heifers, verified serologically, were included.

One additional laboratory study was performed with two experimental vaccine batches which were not fully in compliance with the composition of the vaccine STARTVAC®.

Safety of the administration of one dose

Three studies were performed to investigate the safety of the administration of a single dose of the vaccine STARTVAC® to target animals.

Heifers at an age of 22 months onwards were immunised by the recommended intramuscular route 45 and 10 days before the expected parturition date (EPD) with a single dose of either the vaccine Startvac® or a placebo (without antigens).

The parameters local reactions, general clinical signs, rectal temperature, serological responses and evolution of pregnancy and new born calves were examined. No general reactions or adverse side effects were observed. In a few animals, a slight local swelling, scored as 1 (nodule < 2cm) was noticed 24 and/or 48 hours after 1st injection. No histopathological lesions were observed in the musculature of the immunised animals.

One additional laboratory study was performed with two experimental vaccine batches to investigate the safety of the administration of a single dose. The composition of these vaccine batches did not comply completely with the composition of the vaccine STARTVAC®. The concentration of *S. aureus* was 2 x 1010 total bacteria per dose instead of 1 x 1010. Heifers at an age of 22 months onwards were immunised by the recommended intramuscular route 45 and 10 days before the EPD with a single dose of either one of the two vaccine batches or a placebo (without antigens).

The parameters local reactions, general clinical signs, rectal temperature, serological responses and evolution of pregnancy and new born calves

were examined. No local reactions and no general reactions or adverse side effects were observed.

Safety of one administration of an overdose

Safety of the repeated administration of one dose

One study was performed to investigate the safety of the administration of an overdose and the safety of the repeated administration of one dose of the vaccine STARTVAC® in target animals. Heifers at an age of 22 months onwards were immunised by the recommended intramuscular route as follows: 1st injected with a double dose 45 days before the EPD, 2nd injection 35 days thereafter (corresponding to 10 days before the EPD) with a single dose and 3rd injection 28 days after the 2nd injection (corresponding to 18 days after the EPD).

The parameters local reactions, general signs, rectal temperature, serological responses and evolution of pregnancy and new born calves were examined.

No general clinical signs or adverse side effects in the gestating cows or their progeny were observed after all three injections. Slight to moderate local reactions were noticed after administration of the double dose characterised by swellings (up to 5 cm2) or nodules and local pain. The reactions disappeared within a few days.

Examination of reproductive performance

In four laboratory studies, the influence on gestation, calving and the progeny was investigated after immunisation of pregnant heifers during the last trimester of pregnancy. Heifers at an age of 22 months onwards were immunised by the recommended intramuscular route as follows: 45 and 10 days before the EPD with a single dose of either the vaccine Startvac® or a placebo (without antigens) or 1st injection with a double dose 45 days before the EPD and 2nd injection 35 days thereafter (corresponding to 10 days before the EPD) with a single dose. The evolution of pregnancy and new born calves was examined. No negative influence on gestation, calving and the progeny of the heifers was observed after immunisation

Examination of immunological functions

No specific studies have been carried out based on the justification that E. coli J15 and S. aureus have not the potential to adversely affect the immune system. STARTVAC® is a vaccine containing inactivated bacteria. Replication of vaccine bacteria in any cells involved in the immunised animals immune system is therefore not applicable and subsequently impairment of the immune system is not to be expected.

Special requirements for live vaccines

Not applicable as Startvac® is an inactivated vaccine.

Study of residues

No specific study on residues was performed. A withdrawal period of zero days was proposed and accepted.

The vaccine is inactivated and therefore the determination of residual organisms at the injection site is not applicable. The adjuvant and other components used for the formulation of STARTVAC® are included in the Regulation (EEC) No. 2377/90 and included in Annex II.

Interactions

No specific studies on interactions with other immunologicals or veterinary medicinal products were performed since no interaction is likely to occur.

Field studies

One multicentre field study was carried out in primiparous and multiparous cows using a batch of standard antigen content to assess the safety of the vaccine in dairy cows under real field conditions. The field trial was conducted according to GCP as a randomised double-blind, placebo controlled study.

Cows and heifers at an age of 22 months onwards were immunised in accordance with the proposed immunisation schedule by intramuscular route

either with the vaccine STARTVAC® or with a placebo: 1st injection 45 days before the expected parturition date, 2nd injection 35 days thereafter (corresponding to 10 days before the expected parturition date) and 3rd injection 62 days after the 2nd injection (corresponding to 52 days after the expected parturition date). The parameters local reactions, general clinical signs and adverse events, rectal temperature, effects on reproductive parameters and milk production (as secondary safety parameter) were examined.

Only individual animals showed general clinical signs scored as 1 or 2 at one or several observation time points. A significant increase of the rectal temperature in immunised cows was observed 4 hours after each injection. Single immunised animals showed an increase of up to 1.8° C, two immunized cows even more than 2° C. The rectal temperature became normal within the next 24 hours. Slight (< 2 cm) to moderate (2-5 cm) local swellings or nodules were observed after injections which normally disappeared within a few days. Local pain was recorded in single animals. Injections did not have any adverse effects on gestation, calving or the progeny of the immunised cows. Milk production was not affected.

User safety

Startvac® is an inactivated vaccine.

The raw materials used to prepare active ingredient and vaccine comply with the relevant Ph. Eur. monographs (where applicable) and are carefully controlled to prevent contamination with other infectious agents. The adjuvant comprises liquid paraffin. The excipients are sorbitan monooleate, Polysorbate 80, sodium alginate, calcium chloride dehydrate, benzyl alcohol, simeticone and water for injections.

All components are included in Regulation (EEC) No. 2377/90/ and included in Annex II.

Liquid paraffin is a mineral oil but its concentration in the vaccine is very low compared to other oily vaccines. It is known that, in case of accidental self-injection, an oily adjuvant might cause local tissue irritation and lesions to the person administering the vaccine. For that reason, the applicant has included in the SPC (section 4.5.) an advice to the user and to the physician in case of accidental injection/ self injection.

Benzyl alcohol is used as preservative with a quantity of 20 mg per dose in order to limit risks of product contamination after first use. It is used extensively as an antimicrobial preservative in a wide range of cosmetics and pharmaceutical formulations, including oral and parenteral preparations.

Therefore, the preservative is not expected to represent a hazard to the user.

Sorbitan monooleate and Polysorbate 80 (authorised food additive-E433) are emulsifiers which promote the dispersion of watery droplets of antigen throughout the oil. The use of these emulsifiers does not represent a toxicity hazard to the user.

Sodium alginate is a gelification polysaccharide extracted from giant brown seaweed that precipitates in presence of calcium chloride. Simeticone is a commonly used antifoam, water for injections is the dilution vehicle of the vaccine and also commonly used in medicinal products for parenteral administration. Their utilisation does not represent a risk.

The conclusion that no specific risk associated with the use of this vaccine is identified is supported.

Environmental risk assessment

A Phase I environmental risk assessment was conducted, including a hazard identification and assessment of the exposure to the hazard as well as the likelihood that the hazard may occur. As no hazard can occur, the likelihood of hazard is negligible and the consequences of the occurrence of any hazard can be considered as negligible. Therefore, the risk can be considered effectively zero.

Therefore, a Phase II study has not been considered necessary or adequate given the very low

environmental risk potential of the vaccine.

Overall conclusion on safety

The studies presented in the safety part were satisfactorily described. The Applicant conducted adequate laboratory studies and one field study to assess the safety of a single, double and repeat single dose after intramuscular administration using batches of standard antigen content in primiparous and multiparous cows during the last trimester of gestation. The vaccine may induce slight to moderate local reactions in the target animal, cows and heifers. These local reactions were characterised by slight (< 2 cm) to moderate (2-5 cm) local swellings or nodules and local pain. The reactions were transient; normally they disappeared within a few days. An increase in rectal temperature could be observed 4 hours after injection. The rectal temperature became normal within the next 24 hours. Other general clinical signs scored as 1 or 2 were only observed in a small number of animals. That means the vaccine will be well tolerated by primiparous and multiparous cows immunised in the last trimester of gestation.

No negative influence on gestation, calving and the progeny of the cows was observed after injection of pregnant cows during the last trimester of pregnancy. An assessment of ecotoxicity risks showed that the overall risk represented by the vaccine to the environment is effectively zero. No specific risk is expected for the user, the consumer and other animals.

EFFICACY ASSESSMENT

Introduction and General Requirements

Efficacy studies have been carried out in the target species, heifers (dairy cows in their first pregnancy) and pregnant cows (multiparous cows).

The selected challenge strain of *S. aureus* has been described as virulent strain in several published works and is assayed for virulence in own trials. The challenge strain is a slime producing strain which can even afford cross-protection immunity against coagulase-negative staphylococci (CNS).

The selected challenge strain of *E .coli* was assayed for virulence in own trials. The challenge strain possesses the *E. coli* core oligosaccharide-lipid A complex (common core antigen). It features chemical, structural as well as immunologic homology across species and genera of Gram-negative bacteria, which can even confer cross-protection immunity against coliforms.

Six laboratory studies and one field trial have been conducted:

- Studies for verifying the capacity of the test challenge strains to reproduce mastitis
- Studies assessing efficacy by challenge with *S. aureus* and *E. coli*
- Studies assessing duration and onset of immunity after basic immunisation and reimmunisation
- A field trial.

Laboratory trials

Two intramammary challenge studies were performed to study the pathogenicity of *S. aureus* and *E. coli* strains.

Three laboratory studies were performed to assess the efficacy of the vaccine STARTVAC® using batches of standard antigen content (antigen load is fixed to 1 x 1010 microorganisms for *S. aureus* and *E. coli* J5, respectively). The studies have been conducted according to GLP. The animals used were of the appropriate target species, cattle, and the most sensitive category of the target species for which the vaccine is intended for (heifers = primiparous cows, in the last trimester of pregnancy) has been included in the trials. One additional laboratory study was performed with two experimental vaccine batches which were not fully in compliance with the composition of the vaccine STARTVAC®.

As requested, the results of a previous study were provided to assess the immunogenicity of the slime associated antigen of a *S. aureus* bacterin. Heifers were immunised according to the immunization scheme (first and second injection) with STARTVAC® or PBS. Blood samples were taken from all heifers on day 0, 35, 49, 56, 77 and 98 respectively. It was shown that the experimental vaccine, manufactured with *S. aureus* slime antigen, is able to induce a high and persistent humoral immunity in heifers.

Establishment of a Challenge Model

The capability of challenge strains of *S. aureus* and *E. coli* was assessed to reproduce mastitis in cows in their first lactation cycle after inoculation of a suspension of these bacteria by the intramammary route to different quarters. In each case, two *S. aureus* and *E. coli* strains were used as challenge strains, one strain in two inoculation doses.

The parameters examined were general clinical signs including rectal temperature, histopathological analysis of mammary tissue, clinical signs of mastitis (quarter and milk appearance), bacterial count and somatic cell count in milk, milk production, serological evaluation (anti-slime antibodies in serum and milk, determination of anti-*S. aureus* antibodies in milk). It was shown that the chosen infection method – intramammary route – was adequate for this purpose for both strains. After intramammary challenge with *S.aureus*, a significant increase of the rectal temperature was detected in all animals 24 hours after challenge, an inflammatory reaction caused in quarters infected with *S. aureus* was observed, clinical signs of mastitis manifested in all quarters inoculated with the strains of *S. aureus* after challenge and a multiplication of both strains in the cistern of the mammary gland was observed. The correlation between the bacterial count in milk and clinical signs of mastitis was statistically significant and an increase in the number of somatic cells was detected in all inoculated quarters from 24 hours after challenge onwards. Daily milk production was reduced 1 and 2 days post-challenge, respectively.

The results obtained demonstrated the capacity of the test strains to cause sub-acute clinical mastitis in infected quarters. Although the results were satisfactory for both strains tested, the strain producing slime was chosen.

After intramammary challenge with *E. coli*, clinical signs of mastitis scored 2 and 3 were seen in the form of clots in the milk 24 and 48 hours post-inoculation in two of three quarters and in 3 of 6 quarters.

Maximum titres of the *E. coli* challenge strains were reached mainly within the first 24 hours

Post - challenge in all the infected quarters. An increase in the number of somatic cells in the milk of most of the infected animals was seen.

The results obtained demonstrated the capacity of the test strains to cause sub-acute or sub-clinical mastitis in infected quarters. Because of the score of the clinical signs of mastitis and the results of the count of cfu/ml, the *E. coli* strain was chosen.

Onset of protection

One laboratory study was performed with two experimental vaccine batches to investigate the efficacy of a bacterial strain of *S. aureus* compared to an intramammarian challenge in cows with a heterologous strain of *S. aureus* and to assess the degree of protection that a slime antigen associated with *S. aureus* confers. The composition of these vaccine batches did not fully comply with the composition of the vaccine STARTVAC®. The concentration of *S. aureus* was 2 x 1010 total bacteria per dose instead of 1 x 1010.

Heifers at an age of 22 months onwards were immunised by the recommended intramuscular route 45 and 10 days before the EPD with a single dose of either one of the two vaccine batches or a placebo (without antigens) and challenged at an average of 23 days post-parturition. Non-immun ised, nonchallenged animals were also included.

The parameters examined were: general clinical signs including rectal temperature, clinical signs of mastitis (quarter and milk appearance), milk production, bacterial count and somatic cell count in milk, serological evaluation (anti-slime and anti-*E. coli* J5 antibodies in serum and anti-slime, anti- *S. aureus* and anti-*E. coli* J5 antibodies in milk).

Immunisation with the two experimental vaccines induced a serological response of anti-slime antibodies in serum and also of anti-*E. coli* J5 antibodies in serum. On day 0 of the challenge, the average IRPC anti slime value in milk was higher in animals immunised with the higher concentration of SAAC.

After challenge, the maximum severity of clinical symptoms in the form of presence of lumps in milk and /or induration or local inflammation of quarters was seen 48 to 72 hours post-challenge in quarters inoculated with *S. aureus.* A multiplication of the challenge strain in the mammary gland was observed; the number of cfu per ml of milk progressively increased until 24 hours after challenge. Taking the entire post-challenge period as a whole, animals immunised with the higher concentration of SAAC had lower bacterial counts in milk than the others.

Nevertheless, it is difficult to draw a conclusion on the efficacy of these vaccine combinations from the results of the study. The argumentation that there is a connection between the higher value of IRPC anti-slime in serum and a lower bacterial count in milk on the day of challenge and a smaller severity of clinical symptoms of mastitis after challenge can be supported.

Vaccines with a greater amount of slime associated per dose displayed protection against a challenge, from the point of view that the immunised animals had lower bacterial counts than the control group during the post-challenge phase of the study.

Another study was carried out to demonstrate by intramammarian challenge that immunisation with STARTVAC® confers protection against virulent *S. aureus* in dairy cows.

Heifers at an age of 22 months onwards were immunised by the recommended intramuscular route 45 and 10 days before the EPD with a single dose of either one of the two vaccine batches or a placebo (without antigens) and challenged at an average of 23 days post-parturition. The parameters examined were: general clinical signs including rectal temperature, clinical signs of mastitis (quarter and milk appearance), milk production, bacterial count and somatic cell count in milk, serological evaluation (anti-slime antibodies in serum and milk).

The count of *S. aureus* in milk showed that temporarily less infected quarters were proven in the immunized animals (9 hours, day 1+2) and generally lower bacteria counts in the infected quarters of the vaccinates were found. The vaccine significantly reduces the multiplication of *S. aureus* in the first 48 hours after challenge. Thus, the conclusion can be supported that the trend to the elimination of the infection is more favourable in the group of cows that received the vaccine.

The number of somatic cells in the immunised group after the first few hours of the challenge increased.

The greater increase in somatic cells observed 9 hours post-challenge in the immunised cows can be attributed to the opsonisation of the bacteria by pre-existing specific antibodies (opsonins).

Immunisation induces a significant seroconversion of anti-slime antibodies in blood (humoral immunity) and milk (local immunity) with respect to the not immunised group. Since the humoral defence (formation of opsonins) is closely intertwined with the cellular defence and since both protection mechanisms aim at the elimination of pathogens, it can be assumed that the seroconversion in serum and milk accompanied by the negative correlation in a significant form with the count of *S. aureus* in milk 24 hours, 4, 7 and 21 days after the challenge can be an indicator of the protective effect of the vaccine against *S. aureus.* In order to establish a minimum value of anti-slime antibodies of *S. aureus* (IRPC anti-slime), indicative of protection, the anti-slime response in serum obtained in the immunised group on the day of challenge, was used to calculate a so-called minimum protective value.

A third study was performed to demonstrate by intramammarian challenge that immunisation with Startvac® confers protection against virulent *E. coli* in dairy cows.

Heifers at an age of 22 months onwards were immunised by the recommended intramuscular route 45 and 10 days before the EPD with a single dose of either one of the two vaccine batches or a placebo (without antigens) and challenged at an average of 23 days post-parturition. Non-

immunised, non challenged animals were also included. The parameters examined were: general clinical signs including rectal temperature, clinical signs of mastitis (quarter and milk appearance), milk production, bacterial count and somatic cell count in milk, serological evaluation (anti-*E. coli* J5 antibodies in serum and milk).

The count of *E. coli* of the challenged quarter was lower in the immunised group with respect to the non immunised group until day 8 post challenge, which may mean a reduction of the sub-clinical signs of *E. coli* mastitis. Cell numbers in the milk were increased in both groups after challenge; however, they then decreased more rapidly in the immunised animals. The greater severity of mastitis evaluated by the appearance of milk was observed in the non immunised group which may be due to the fact that the immunisation might reduce the clinical signs of *E. coli* mastitis.

The lower drop of milk production following challenge should be indicative of a reduction of the clinical signs of coliform mastitis in the immunised group. Also, a positive correlation between this reduced drop in milk production and the bacterial count of *E. coli* in milk has been demonstrated. In addition in the vaccinates, the milk quantity achieved 100 % of the pre-challenge level, while in the control group the milk production remained under the pre-challenge level throughout the post challenge period. These observations demonstrate the reduction of the clinical severity of an important bovine variable such as milk production by means of immunisation.

In the immunised group, a significant anti-*E.coli* J5 seroconversion in serum was observed on day 14 after the first dose. The differences with respect to the non immunised group remained significant until the day of challenge, except for the day of immunisation (day 0) and day 35 post-immunisation (day of administration of second dose).

Although the anti-*E. coli* J5 response in serum in the immunised group was significantly greater than in the non immunised group, no significant differences in the response in milk were detected between the two groups on the day of challenge (59 days post-immunisation)

In order to establish a minimum rate of anti-*Escherichia coli* J5 antibodies (IRPC anti-*E. coli* J5), indicative of protection, the anti-E. coli J5 response in serum obtained in the immunised group on the day of challenge, was used to calculate a so-called minimum protective value.

Onset and duration of Immunity

In the case of Startvac®, both immunisations were considered, i.e. the basic immunisation (1st injection: at 45 days before the EPD; 2nd injection: 35 days thereafter, corresponding to 10 days before the EPD) and the 3rd injection at day 97 (62 days after the 2nd injection, corresponding to 52 days after the expected parturition date) which is considered as a booster injection necessary to maintain the immunity, and being part of the regimen of immunization. This regimen of immunization must be carried out in each gestation period.

One study was carried out to investigate the onset and duration of the immunity of Startvac®.

Heifers at an age of 22 months onwards were immunised by the recommended intramuscular route as follows: 1st injection 45 days before the expected parturition date, 2nd injection 35 days thereafter and 3rd injection 62 days after the 2nd injection.

In order to establish the onset and duration of the immunity of the STARTVAC® vaccine, the serological response against *S. aureus* and *E. coli* obtained in the immunised group was compared with the serological response of the non immunised control group at different time intervals. Therefore, the antibody response against slime of *S. aureus* and against *E. coli* J5 in serum was determined.

These established minimum protective values were tried and used as a basis for definition of onset and duration of immunity, but it was not accepted

by the CVMP. Based on the results, obtained in the field study the following phrase was recommended: The full immunisation scheme induces immunity from approximately day 13 after the first injection until approximately day 78 after the third injection (equivalent to 130 days post-parturition).

Influence of Maternal Antibody on the Efficacy of the Vaccine

Not applicable as heifers and cows in the last trimester of gestation were immunised.

FIELD TRIALS - STARTVAC®

One multicentre field study has been carried out in primiparous and multiparous cows using a batch of standard antigen content to determine the efficacy of the vaccine in dairy cows under real field conditions. The field trial was conducted following GCP as a randomised double-blind, placebo controlled study. Farms with different conditions (type of milking, working procedures, park design, etc.), were included. The type of management employed in these farms (housing conditions, feeding, type of milking, parameterisation...) as well as the genetics of the animals used and the habitual mastitis problems found are common in the dairy farms around Europe. Cows at an age of 22 months onwards were immunised in accordance with the proposed immunization schedule by intramuscular route either with the vaccine Startvac® or with a placebo: 1st injection 45 days before the expected parturition date, 2nd injection 35 days thereafter (corresponding to 10 days before the expected parturition date) and 3rd injection 62 days after the 2nd injection (corresponding to 52 days after the expected parturition date).

The following parameters were examined and evaluated:

- incidence of clinical mastitis (appearance of new cases of mastitis) by means of evaluating the general clinical symptoms and local clinical symptoms;
- incidence of subclinical mastitis by means of the aseptic taking of milk per cow (from the
- 4 quarters) for microbiological analysis and somatic cell count and individual recording of the daily milk production in the totality of the animals included in the trial;
- severity of the symptoms - somatic cell counts, general clinical signs, clinical signs (milk and
- quarter appearance), dead cows due to mastitis or severe mastitis, mastitis treatments with
- pharmacological products;
- spontaneous cure rate (cured cases of mastitis per number of infected animals);
- Milk production, mastitis treatments and rate of antibodies in serum and milk samples.

The immunisation program as well as the dosage of 2 ml/animal and the administration route of the vaccine is efficacious in the reduction of the incidence of intramammary infection due to *S. aureus*, coliforms or coagulase-negative staphylococci, with clinical or subclinical manifestations in cows (multiparous) and heifers (primiparous) in the period of maximum incidence, i.e. post parturition. Immunisation also significantly reduces the severity of the symptoms, causes a significant increase in the spontaneous cure rate of the infected cows, significantly reduces the number of cows that need to be treated for mastitis and has positive effects on both the quantity and quality of milk production.

The rate of specific anti-slime antibodies of the Startvac® group was significantly higher compared to the placebo group until the end of the study (130 days post-parturition) and the rate of specific anti- E. coli J5 antibodies of the Startvac® group was significantly higher compared to the placebo group until approximately 90 days post-parturition.

The correlation between the serological response in serum (humoral) and milk (local) observed in the challenge laboratory trials and the two antigens were confirmed under field conditions.

The results obtained in the field trial demonstrate the efficacy of the Startvac® vaccine.

Overall conclusion on efficacy

Main findings in the laboratory or pre-clinical trials;

The vaccine significantly reduces ($p < 0.05$) the multiplication of *S. aureus* in the first 48 hours

after challenge. A significant increase ($p < 0.05$) is also observed in the number of somatic cells in the immunized group after the first few hours of the challenge, which would indicate a greater cellular response that contributes to an increase in phagocytosis and, therefore, to a reduction in infection.

Immunisation significantly reduces ($p < 0.05$) the drop in milk production caused by an intramammarian challenge with a virulent strain of *E. coli*. Also, a positive correlation between this reduced drop in milk production and the bacterial count of *E. coli* in milk has been demonstrated.

Main findings in the field trials; (see pg 21)

The immunisation program as well as the dosage of 2 ml/animal and the administration route of the vaccine are efficacious in the reduction of the incidence of intramammary infection due to *S. aureus*, coliforms or coagulase-negative staphylococci, with clinical or subclinical manifestations in cows (multiparous) and heifers (primiparous) in the period of maximum incidence, i.e. post-parturition. Immunisation also significantly reduces the severity of the symptoms of clinical or sub-clinical mastitis, causes a significant increase in the spontaneous cure rate in the infected cows, significantly reduces the number of cows that need to be treated for mastitis and has positive effects on both the quantity and quality of milk production.

The indication also includes the protection against the coliforms. The vaccine strain can confer crossprotection immunity against the coliforms attributable to the common core antigen. Also in consideration of the fact that the results of the field trial showed a significant reduction in intramammary infections caused by coliforms in the immunised heifers and cows in comparison to the placebo group, the conclusion that the vaccine also protects against coliforms is acceptable.

The indication also includes the protection against coagulase-negative staphylococci (CNS). The slime characteristic of the vaccine strain can afford cross-protection immunity against CNS species. In consideration of the fact that the results of the field trial showed a significant reduction in the incidence of mastitis attributed to CNS in immunised heifers and cows in comparison to the placebo group, the conclusion that the vaccine also protects against CNS is acceptable.

BENEFIT RISK ASSESSMENT

Startvac® is intended for use in healthy cows and heifers in dairy cattle herds with recurring mastitis problems in order:

- to reduce the incidence of intramammary infection caused by *S. aureus*, coliforms or coagulase-negative staphylococci, with clinical or subclinical manifestations (incidence means new cases of mastitis per number of healthy animals at risk during the observation period)
- to reduce the severity of the symptoms of the intramammary infection caused by *S. aureus*, coliforms or coagulase-negative staphylococci in the immunised group with respect to the control group based on analysis of Somatic Cell Counts (SCC), clinical signs, mastitis treatments and dead cows due to mastitis or severe mastitis.

Immunisation has positive effects on both the quantity and quality of milk production. Furthermore, a significant reduction of the number of mastitis treatments with antibiotics per cow was observed in the immunised group. Vaccination also increases the spontaneous cure in the immunised group (cure rate means the cases cured of mastitis per number of infected animals during the observation period).

The risk assessment is based on the estimated risks to target and non-target animals, users, consumers of animal derived food and to the environment.

Startvac® is an inactivated vaccine. The inactivation procedures and test on inactivation have been adequately validated. The intramuscular administration route does not allow release of the vaccine into the environment. The inactive antigens will be metabolised in the target animals and are therefore considered irrelevant concerning possible risks through residues. Thus, the proliferation, persistence and excretion of vaccine germs can be excluded. The adjuvant, the preservative and other constituents can be regarded as safe in the concentration used for the product.

Therefore, there is no risk regarding the transmission of live organisms to target and non-target animals, no shedding and no capacity of live product organisms to survive, establish and disseminate, and no pathogenicity to other organisms.

Based on a Phase I environmental risk assessment it can be concluded that the vaccine represents a negligible risk to the environment. The vaccine does not contain any ingredients that are likely to pose a risk for consumers of milk and meat.

Startvac® contains liquid paraffin, a mineral oil as adjuvant but the concentration is low. It is known that, in case of accidental self-injection, an oily adjuvant might cause local tissue irritation and lesions to the person administering the vaccine.

In the target animal, slight to moderate transient local reactions may occur after the administration of one dose of vaccine. They would mainly be: swelling (up to 5 cm2 on average), which disappears within 1 or 2 weeks at most. In some cases, there may also be pain at the inoculation site that spontaneously subsides in a maximum of 4 days. A mean transient increase in body temperature of about 1° C, in some cows up to 2° C, may occur in the first 24 hours after immunisation.

No negative influence on gestation, calving and the progeny of the cows was observed after immunization of pregnant cows during the last trimester pregnancy.

Based on the data presented immunisation with STARTVAC® reduces the incidence of sub-clinical mastitis and the incidence and severity of the clinical signs of clinical mastitis caused by *S. aureus*, coliforms and coagulase-negative staphylococci.

The risk of the use of the vaccine STARTVAC® for the immunised animal can be evaluated as minimal.

Only slight to moderate transient local reactions and a transient increase in body temperature in the first 24 hours after immunisation may occur.

For the user, special safety precautions (risk management measure) are mentioned in the product information as the vaccine contains mineral oil as adjuvant and it is known that, in case of an accidental self-injection, local tissue irritation and lesions to the person administering the vaccine can occur.

The benefits of the vaccine as stated above have been sufficiently substantiated. The risks identified for the target species, the user and the environment are considered acceptable. Therefore, the overall benefit-risk balance is considered as favourable.

Preventative measures other than immunization for mastitis control that should be applied, within a good management program, include: (a) a clean, stress-free environment (b) proper maintenance and operation of milking equipment; (c) good milking procedures including teat dipping; (d) a dry cow treatment program and culling chronic cows when necessary; and (e) a program for monitoring the health status of udders.

Based on the original and complementary data presented the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the quality, safety and efficacy of Startvac® were considered to be in accordance with the requirements of Directive 2001/82/EC, as amended, and that the benefit-risk balance was favourable.

Vaccine is given intramuscular. It is preferable that the injections be administered on alternate sides of the neck. Administer one dose (2 ml) by deep intramuscular injection into the neck muscles. The complete immunization programme should be repeated in each pregnancy. The vaccine can be administered by either farmer or veterinary surgeon.

Protocols described below are set out to maximize the full potential of the vaccine. If vaccination dates are missed then vaccine will not work to its full potential.

FIELD TRIALS – STARTVAC®

Results of field trials approved by the European Medicines Agency (EMEA):

1. Principal aims and results

	Variable	STARTVAC® Group	PLACEBO Group	Statistical significant differences between STARTVAC ® and PLACEBO (α =0.05)
Percentage of clinical and subclinical mastitis until 130 days	*S. aureus*	1.18%	10.34%	0.001
	E. coli	4.14%	17.82%	0.001
	CNS	16.57%	32.18%	0.001
Percentage of clinical mastitis until 130 days	*S. aureus*	0.00%	2.87%	0.032
	E. coli	1.78%	6.90%	0.02
	CNS	2.37%	6.90%	0.047
Percentage of subclinical mastitis until 130 days	S .*aureus*	1.18%	9.77%	0.001
	E. coli	2.37%	13.22%	0.001
	CNS	15.98%	39.89%	0.002
Spontaneous cure rate	Multiparous	44.19%	20.45%	< 0.05
	Primiparous	53.33%	50.00%	> 0.05
	Total	51.43%	32.18%	< 0.05

2. Secondary aims and results

Variable	STARTVAC® group	PLACEBO group	Statistical significant differences between STARTVAC® and PLACEBO (α =0.05)	Observations
Somatic cell count (mean SSC x 10^3)	328.2	548.6	SI (p<0.05)	Internationally recognized indicator for mastitis and milk quality
Milk aspect (>1)	11.42 %	19.79 %	SI (p<0.05)	Implies less economic losses due to lost quarters, discarded milk and replacement cows
Mammary gland aspect (>1)	14.44 %	24.03%	SI (p<0.05)	
Treatment with pharmacological products	34 treat.	93 treat.	SI (p<0.05)	Implies less economic losses due to treatments and reduces the risk of residues in milk
	22 cows	40 cows		
Death of cows due to mastitis	0	3	NO (p>0.05)	Low number of deaths, Death due to mastitis only occurred in the placebo group

Marketing & Promotional Material

There is a large variety of promotional material and technical aids available for use with Startvac®.

a) A Veterinary Technical Brochure on Startvac® outlining how the vaccine works when used to combat mastitis. This brochure also includes details on the field and laboratory trials conducted for the EMEA Registration.

b) A Farmers Information Booklet is available to all farmers to explain what exactly is Startvac®, Advantages of Startvac®, help with choosing a suitable vaccination protocol and also herd health plan recommendations.

c) A Startvac® Calendar Wheel as shown in figure 1, is supplied direct to the veterinary surgeon for distribution to each of his clients. This Startvac® Calendar Wheel is designed to allow the farmer calculate each date of vaccination for all the animals contained within the herd.

d) A Vaccination Table enables the farmer administer the vaccine accurately and efficiently to the herd (See Table 1). Each farmer should plan his vaccinations dates before starting to use the vaccine. As there is a seven day flexibility with administering the vaccine with the 1st and 3rd dose it allows for the herd to be split into groups. These groups are decided on depending on when cows are expected to calf during the year.

e) Startvac® California Milk Test (figure 2) is an aid for the farmer to detect subclinical mastitis in the herd.

f) Startvac® Video that describes the vaccine and how it works and the Vaccination Table are available for download on our website: www.dugganvet.ie/startvac.htm

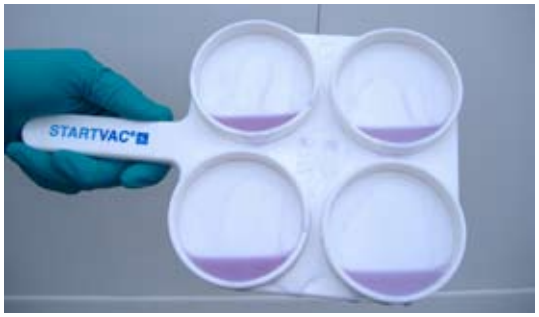
To obtain any of these items please contact you local Area Sales Manager or contact the Startvac Product Manager direct.

fig.1 **STARTVAC® CALENDAR WHEEL**

Is a useful tool to aid the farmer in monitoring vaccination dates within his herd. The rotating wheel gives accurate dates to allow the farmer plan all three vaccinations throughout the year.

See Table 1: Vaccination Dates

fig.2 **STARTVAC® CALIFORNIA MILK TEST**



Available for all Veterinary Surgeons who purchase Startvac®. (CMT) is a simple cow-side indicator of the somatic cell count of milk. It operates by disrupting the cell membrane of any cells present in the milk sample, allowing the DNA in those cells to react with the test reagent, forming a gel. It provides a useful technique for detecting subclinical mastitis.

STARTVAC® PRESENTATION

Startvac® is sold through 2ml vials (1 dose) in packs of 20 and also 10ml bottle (5 doses)

THE CLASSIC PROTOCOL: Sample Vaccination Table

	1st Dose 45 (+- 7 days) Pre Calving	2nd Dose 10 (+ 10days) Pre Calving	3rd Dose 52 (+-7days) Post Calving
Group 1	**January 1st**	**February 12th**	**March 26th**
101,102,103,104, 105			
106, 107, 108, 109, 110			
Group 2	**January 15th**	**February 26th**	**April 9th**
201, 202, 203, 204, 205,			
206, 207, 208, 209, 210			
211, 212, 213, 214, 215			
Group 3	**January 29th**	**March 12th**	**April 23rd**
301, 302, 303, 304, 305			
306, 307, 308, 309, 310			
311, 312, 313, 314, 315			
Group 4	**February 12th**	**March 26th**	**May 7th**
401, 402, 403, 404, 405			
406, 407, 408, 409, 410			
411, 412, 413, 414, 415			
Group 5	**February 26th**	**April 9th**	**May 21st**
501, 502, 503, 504, 505			
506, 507, 508, 509, 510			
511, 512, 513, 514, 515			
Group 6	**March 12th**	**April 23rd**	**June 4th**
601, 602, 603, 604, 605			
606, 607, 608, 609, 610			
Group 7	**March 26th**	**May 7th**	**June 18th**
701, 702, 703, 704, 705			
706, 707, 708, 709, 710			
Herd of 90 cows			

The Vaccination Table incorporates the Classic Protocol (3 Doses) and simplifies the vaccination process for the farmer. Each farmer should plan his vaccinations dates to obtain the maximum benefit from Startvac®. The Startvac® Calendar Wheel is suitable for the recording and planning of vaccination dates. As there is a seven day flexibility before and after administering the vaccine 1st and 3rd dose, it allows the herd to split into groups that can be vaccinated every two weeks. In the table above, the herd is split up into 7 groups (This may vary depending on size of the herd and length of calving period), the herd is then vaccinated every two weeks and by the time it comes to Group 4 (February 12th) there is an overlap with Group 1 (February 12th) second dose being administered at the same time as Group 4 first dose. When the vaccination dates overlap vaccination will be carried out more efficiently

Bio Film - what is it?

STARTVAC® hinders the formation of Biofilm. The Biofilm also known as Slime is a layer of exopolysaccharides that surrounds the bacteria, enhancing their growth and resistance to antibiotics. STARTVAC® prevents the development of Biofilm and also favours contact with neutrophils, enabling the destruction of bacteria. Biofilm can be defined broadly as a dynamic and well structured microbial community, attached to a solid surface and aggregated by an extracellular matrix.

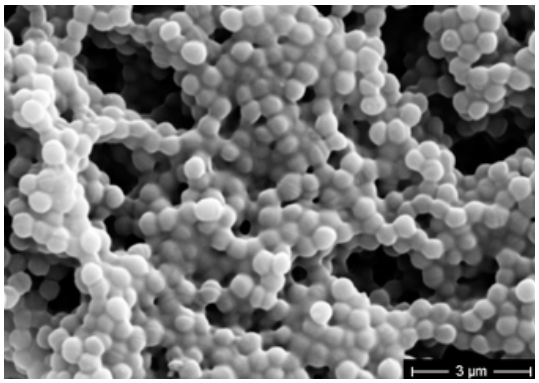


Diagram 1: Scanning Electron micrograph of Staphylococcus aureus biofilm grown invitro using the colony biofilm model. The biofilm of coccoid cells is blanked by extracellular polymer matrix (www.erc.montana.edu).

Biofilm resistance to antimicrobial agents may be due to difficulty in penetration of the antimicrobial agent through the extracellular matrix, to the decreased growth rate of biofilm cells (ß-lactam antibiotics are effective in Gram-positive cells that are actively dividing) or the existence of resistant phenotypes among a genetically heterogeneous population.

The contribution of biofilm to pathogenesis is attributed to its resistance to antibiotics and phagocytosis, thereby facilitating chronic infections. On the other hand, detachment of biofilm bacteria cells is a cause of septicaemia and new colonisations, while the production of endotoxins and exotoxins produce inflammation and tissue damage.

In bovine and ovine mastitis caused by staphylococci, bacterial cells attach to the epithelial cells of the mammary gland and grow into colonies surrounded by an extracellular matrix, thereby forming the biofilm. Because of its size, biofilm is not capable of being phagocytised by polymorphonuclear neutrophils or macrophages and, moreover, it confers resistance to antibiotics, thereby promoting the chronicity of infection.

The main defence mechanism of the mammary gland against infections is antibody-mediated opsonisation and subsequent phagocytosis by polymorphonuclear neutrophils. However, we must not exclude that the biofilm-specific antibodies also act in a direct manner in protection, binding to cells and preventing bacterial adherence to epithelium and intercellular interaction that leads to the formation of biofilm.

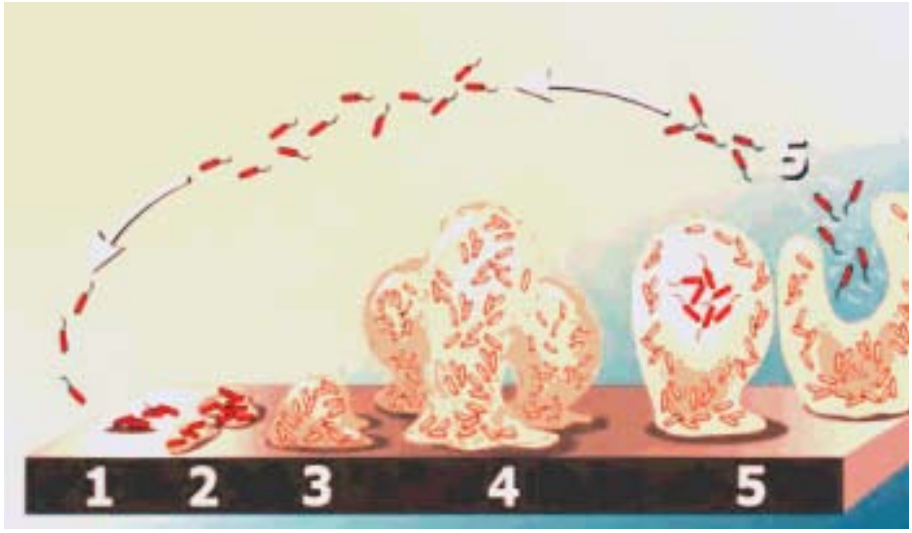


Diagram 2: General model of biofilm development. Graphic by Peg Dirckx and David Davies © 2003 Center for Biofilm Engineering Montana State University.

Frequently Asked Questions

What is Startvac®?

Startvac® is a vaccine for cows that contains inactivated (killed) bacteria called Escherichia coli and Staphylococcus aureus. Startvac® is an emulsion for injection that is available in a 2ml vial and a 10ml bottle.

What is Startvac® used for?

Startvac® is used to strengthen the immunity of whole herds of otherwise healthy dairy cows in herds that are known to have problems due to mastitis (inflammation of the udder due to infection). The strengthened immunity reduces the number of cows affected and the severity of clinical signs. Startvac® is given to all healthy cows in a herd, during and after pregnancy. It is given using preferably the Classic Protocol (3 dose) or otherwise the Alternative Protocol (4 dose)

How does Startvac® work?

Startvac® is a vaccine. Vaccines work by 'teaching' the immune system (the body's natural defences) how to defend itself against a disease. Startvac® contains killed forms of two bacteria that normally cause mastitis (Escherichia coli and Staphylococcus aureus). When it is given to a cow, the animal's immune system recognises the bacteria as 'foreign' and makes antibodies against them. In the future, the immune system will be able to make the antibodies more quickly when it is exposed to the bacteria again. The antibodies will help to fight the bacteria, preventing mastitis occurring or reducing the severity of its symptoms. The vaccine also contains an 'adjuvant' (liquid paraffin) to stimulate a better response.

How has Startvac® been studied?

The company has carried out a number of studies, including one main study that looked at the effectiveness of Startvac® in dairy cows under field conditions. The study compared cows that were given Startvac® with those that were given placebo (a dummy vaccine) and looked at the number of cows with mastitis, the severity of mastitis symptoms, and milk production.

What benefit has Startvac® shown during the studies?

The studies showed that Startvac® reduced the number of cows with mastitis due to Staphylococcus aureus and related bacteria and it reduced the severity of the symptoms in the cows that had mastitis. Vaccination with Startvac® also led to an increased number of cows being cured of the infection, a reduction in the number of cows that needed treatment for mastitis, and an increase in the quantity and quality of milk production. Startvac® injections did not have any harmful effects on pregnancy or giving birth, or on the cows' calves.

What is the risk associated with Startvac®?

The vaccine may cause temporary swelling and pain at the site of injection. It may also cause a temporary increase in body temperature.

What are the precautions for the person who gives the medicine or comes into contact with the animal?

Startvac® contains liquid paraffin (a type of mineral oil). Accidental injection or self-injection could cause severe pain and swelling, particularly if the vaccine is injected into a joint or finger. In rare cases, this could result in the loss of the affected finger if prompt medical attention is not given. If you are accidentally injected with this product, seek medical advice promptly, even if only a very small amount is injected, and take the Package Leaflet with you. If pain persists for more than 12 hours after medical examination, seek medical advice again.

What is the time to allow before the animal can be slaughtered and the meat used for human consumption (withdrawal period)?

The withdrawal period is zero days. The animal can be slaughtered for food at any time after injection.

What is the time to allow before milk can be taken from the animal for human consumption?

Milk can be taken at any time after injection.

Why has Startvac® been approved?

The Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the benefits of Startvac® exceed the risks for herd immunisation of healthy cows

and heifers, in dairy cattle herds with recurring mastitis problems, to reduce the incidence of sub-clinical mastitis and the incidence and severity of the clinical signs of clinical mastitis caused by Staphylococcus aureus, coliforms and coagulase-negative staphylococci, and recommended that Startvac® be given a marketing authorisation.

Does the Somatic Cell Count rise at vaccination?

Startvac® challenges the animal's immune system, as do all vaccines, therefore causing the SCC to rise slightly for a few days before returning to normal. This rise is only temporary and usually only seen in some animals.

Is there any Side effects affecting the quality of milk resulting from the use of Startvac®?

There are no side effects with fat and protein levels. Milk contents (i.e. fat and protein levels) are not affected in any way by the vaccine. The only adjustment is with respect of the reduction of the Somatic Cell Count.

Is there continuous lowering of SCC?

If the origin is caused by Staph and coliforms bacterial origin the answer is yes, but if the Somatic Cell Count origin is multi-variable (strepto, milking parlour and poor hygiene and milking technique) then the answer may be no. The vaccine is part of an overall measure to reducing SCC and must not be seen as a single resolution to reducing SCC. There is a threshold where the animal is coming to the end of lactation at which stage the SCC can not be further reduced.

How effective is Startvac® against mastitis should there be an infection present?

(Not the worst but yes a more frequent one) The vaccine is best suited where infections are already present on the farm. Hence, if the infection is present on the farm it is likely that all animals have certain exposure to the pathogens, by vaccinating all animals you increase their immunity to these pathogens and decrease their chances of severe infection when they are run down and immunity is depressed at the stressful time of calving and during lactation. Although the initial immunization with the vaccine may cause a short increase in the Somatic Cell Count the long term effects warrant its use.

Should SCC fall below 100 is the cow come at risk from more severe infection?

One of the characteristics of this vaccine is that reduces the SCC but simultaneously reduces also the severity of other infections caused by Staph and Coliforms. These effects are best seen when the vaccine is used in combination with a proper herd health protocol to reducing both contagious and environmental mastitis treats on the farm.

Is there a residue or taint as with some drugs?

No. As this is a vaccine, there is no residual tainting or necessity to withhold milk, as is the case with antibiotic therapy.

Does the vaccine inhibit yoghurt or cheese cultures?

No, the vaccine only works on the named bacteria creating a stronger immune system to fight the bacteria associated with mastitis, it does not have any effect on bacteria associated with yoghurt and cheese production, rather the contrary, reducing the mastitis pathogens that are unwanted.

If a vaccine date is missed what are the consequences?

As the Classic Protocol has been devised to target the periods when immunity is best suited to its use, a missed vaccine may decrease the duration of the immunity and increase the likelihood of the animals exposure to mastitis, however an adaption may be made to the protocol in response to a missed vaccination, to re adjust the immunity levels of the animal. (See the Alternative Protocol).

Is Startvac® a Prescription Only Medicine (POM)?

Yes

Any further questions can be directed towards Donnacha Duggan, Startvac® Product Manager, Ireland.

Tel: (086) 601 7749 or Email donnacha@dugganvet.ie

Duggan Veterinary

CHOICE ▪ EXPERTISE ▪ RELIABILITY

Duggan Veterinary Supplies was founded in 1987 with the aim of providing a premium service to the Irish veterinary market. Situated in Holycross, Co Tipperary, Duggan Veterinary has proven to be a market leader when it comes to providing new and innovative solutions and ideas to the Irish Veterinary industry.

Uncompromising in our effort to provide a fast effective and cost efficient service, Duggan Veterinary is recognised throughout Irish veterinary and has established itself as a market leader when it comes to providing a complete service to the Irish veterinary industry. The Duggan Veterinary product catalogue includes a wide range of anti microbial, biological products, consumables and instruments to later for all aspects of veterinary practice.

Hipra Pharmaceutical

HIPRA

HIPRA is a European veterinary pharmaceutical company working on the development, production and commercialisation of animal health products, especially biological and pharmacological products. Hipra has many subsidaries based world wide and have sales representation on every continent.

With more than 50 years of experience in the veterinary pharmaceutical industry Hipra consists of personnel who share values of commitment, enthusiasm and team work. Hipra remain leaders of industry and are currently immersed in an ambitious international development process involving new and innovative biological products. A successful collaboration with Duggan Veterinary in Ireland now ensures that the Hipra catalogue of products has been made available to the Irish veterinary industry.

PRODUCT OVERVIEW

COMPOSITION PER DOSE (2 ML): Inactivated *Escherichia coli* (J5) 50 RED_{60}*; Inactivated *Staphylococcus aureus* (CP8) SP 140 strain expressing SAAC** 50 RED_{80}***. Adjuvant. * RED_{60}: Rabbit effective dose in 60% of the animals (serology). **SAAC: Slime Associated Antigenic Complex. ***RED80: Rabbit effective dose in 80% of the animals (serology).**INDICATIONS:** Cows and Heifers: To prevent Mastitis. For herd immunisation of healthy cows and heifers, in dairy cattle herds with recurring mastitis problems, to reduce the incidence of sub-clinical mastitis and the incidence and the severity of the clinical signs of clinical mastitis caused by Staphylococcus aureus, coliforms and coagulase-negative staphylococci. The full immunisation scheme induces immunity from approximately day 13 after the first injection until approximately day 78 after the third injection (equivalent to 130 days post-parturition). **SIDE EFFECTS:** Slight to moderate transient local reactions may occur after the administration of the vaccine, which disappears within 1 or 2 weeks at most. **ADMINISTRATION ROUTE:** Intramuscular, into the neck muscles. The injections should be preferably administered on the alternate sides of the neck. It is advisable to administer the vaccine at a temperature between +15 and +25 ºC. Shake before use.**DOSAGE:** Cows and Heifers: 2 ml/animal. Generally, the following vaccination programme is recommended: First injection: at 45 days before the expected parturition date. Second injection: 35 days thereafter (corresponding to 10 days before the expected parturition date). Third injection: 62 days after the second injection (equivalent to 52 days post-parturition). The full immunisation programme should be repeated with each gestation. The whole herd should be immunised. Immunisation has to be considered as one component in a complex mastitis control program that addresses all important udder health factors (e.g. milking technique, dry-off and breeding management, hygiene, nutrition, bedding, cow confort, air and water quality, health monitoring) and other management practices. It can be used during pregnancy and lactation.**WITHDRAWAL PERIOD:** 0 days. **SPECIAL PRECAUTIONS:** Store at +2 to +8 ºC, avoiding freezing. Protect from light. **PACKAGING:** Pack of 20 vials of 1 ds. **EU**/2/08/092/003) / 5 ds vial. (**EU**/2/08/092/004) 25 ds bottle. (**EU**/2/08/092/006). Under veterinary prescription.

STARTVAC®

1. **Reduction of the incidence of clinical and sub-clinical intramammary infections up to day 130 post-partum**
2. **Reduction in the severity of symptomatology of clinical cases (both in appearance of milk and quarters)**
3. **Reduction in somatic cell counts (SCC)**

DugganVeterinary
CHOICE • EXPERTISE • RELIABILITY

Holycross, Thurles, Co. Tipperary.

Tel: 0504 43169

Fax: 0504 43147

Email: startvac@dugganvet.ie

Website: www.dugganvet.ie/startvac.htm

Donnacha Duggan

Startvac® Product Manager

Mob: 086 6017749

Donal Duggan

South Ireland Area Representative

Mob: 086 2662402

Ronnie Grace

East Ireland Area Representative

Mob: 086 2621519

Raymond Young

West / North Ireland Area Representative

Mob: 086 8192400