Biogénesis Bagó Foot and Mouth Disease Vaccine

TECHNICAL REPORT

The evolution of animal health

Biogénesis Bagó

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INTRODUCTION

Leading-edge technology with international quality standards in the control and eradication of Foot and Mouth Disease

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Biogénesis Bagó is a leading global company, with significant scientific and commercial achievements built on more than 80 years of experience. Its main objective is to provide effective solutions to major animal diseases that impact animal health and herd productivity around the world.
For decades, it has focused in the research and development of veterinary products with the highest quality standards thus becoming a referent company in FMD vaccine manufacturing and distribution worldwide. Biogénesis Bagó is globally committed to providing technological tools for the prevention, control and eradication of FMD.

United States
Department of Agriculture

UNITED STATES VETERINARY BIOLOGICAL PRODUCT PERMIT

SALE AND DISTRIBUTION

NO. 428A

Not Applicable

Transboundary Animal Biologics, Inc.
3515 University Blvd
Ames, IA 50010

Biogenesis-Bago, S.A.
Ruta Panamericana Km 38.5
Garin, Prov de Buenos Aires 1619
Argentina

Any Port

The producer/permittee agrees to submit to periodic reinspections of the production facility under terms to be specified in a Cooperative Agreement between the parties. The permittee agrees to be responsible for all costs associated with these inspections.

See attached restriction(s).

JUL 16 2020

Distribution and Sale Permit of FMD Vaccine granted by the United States Department of Agriculture for Biogénesis Bagó.

For decades, the company has worked with animal health, research and scientific organizations making significant investments in infrastructure, research and development in order to help eradicate Foot and Mouth Disease in Latin America and worldwide. During different FMD outbreaks and emergencies, Biogénesis Bagó provided the necessary vaccines, playing a leading role in the fight against the disease.

In 1997, when Taiwan faced a devastating FMD outbreak, Biogénesis Bagó immediately provided FMD vaccines, that were the first FMD vaccine approved by the Animal Health Authorities of that country. Biogénesis Bagó contributed with a large amount of vaccine doses which were administered during emergencies and thereafter in regular vaccination campaigns.

In 2000, Biogénesis Bagó was selected to create the first South American Bank of Antigens and Vaccines for the prevention of FMD. This bank responded efficiently in emergencies that appeared in Argentina and Uruguay in 2001 and 2002.

The international acknowledgement for its technological capability, product quality and production capacity allowed Biogénesis Bagó to be awarded a supplying contract in 2006, through an international tender of the Antigen and Vaccine Bank of North America, as part of the FMD emergency preparedness plan in United States, Mexico and Canada.
Biogénesis Bagó also obtained a license for their FMD vaccine in Canada in October 2010 as part of the contingency plan in a country free of the disease.
Furthermore, in July 2011 it became the first company to obtain a “Permit for Sale and

Distribution" of their FMD Vaccine "Bioaftogen" in the United States of America.
Today, Biogénesis Bagó is the only FMD vaccine manufacturer that holds free sale and distribution authorizations in every country of Latin America in which FMD vaccination programs are implemented, and the use of its is approved case of emergencies in Canada, Mexico and USA. Moreover, Biogénesis Bagó supplies antigens and vaccines to the North American FMD Vaccine Bank and exports vaccines to Asian countries.

BIOGÉNESIS BAGÓ'S ACHIEVEMENTS AND CONTRIBUTIONS TO THE CONTROL OF FOOT AND MOUTH DISEASE

1952
First license for FMD Vaccine in Argentina.

1989
First production of FMD Vaccine in the Garín manufacturing site, Buenos Aires, Argentina.

1996
OIE Biosafety Level 4 Certificate granted by the National Health and Agrifood Quality Service (SENASA).

1997
Vaccine supplier for Taiwan emergency.

2000
Award of the contract for the first Argentine and Regional FMD Antigen and Vaccine Bank.

2001
Biogénesis Bagó supplied the Argentine Emergency Vaccination Campaign with 120 million doses per year.

2003
Foundation of the Inter-Institutional Network of Research and Development of FMD (RIIDFA), integrated by SENASA, INTA, CONICET and Biogénesis Bagó.

2006
Biogénesis Bagó was awarded the contract as supplier for the North American FMD Vaccine Bank (NAFMDVB).

2008
Biogénesis Bagó FMD Vaccine licensed in Brazil.
Increase in production capacity up to 200 million doses per year of polyvalent vaccines.

2010
Biogénesis Bagó FMD Vaccine licensed in Canada.

2011
The first company to obtain the Permit for Sale and Distribution of FMD Vaccine in the United States of America.

2013
Start Up of the Joint Venture in China – Yangling: JINHAI Biotechnology.

2016
First export emergency vaccine to South Korea.
License of Aftogen Oleo in South Korea.

2017
Aftogen Oleo vaccine licensed in Vietnam.

2018
Supplier for Emergency in Vietnam and licensing of Bioaftogen in South Korea.

2019
Vaccine Bank for Taiwan. Licence for regular use in South Korea of Bioaftogen.

2020
The company was awarded the contract as supplier for the National Animal Vaccine and Veterinary Countermeasures Bank (NAVVCB) from USA. Bioaftogen vaccine licensed in Vietnam. Updated of Permit for Sale and Distribution of FMD Vaccine in the United States of America. License of Bioaftogen ID (dose volume 0,5 mL) in South Korea.

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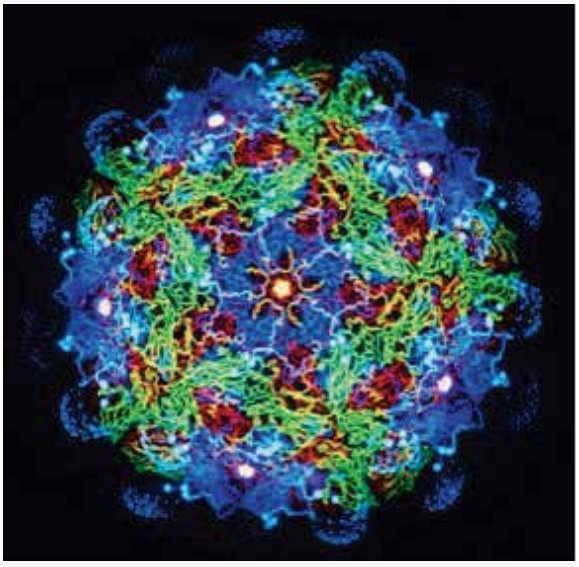
FOOT AND MOUTH DISEASE

FMD is a highly contagious illness that affects cloven-hoofed animals, mainly cattle, bubalines, pigs, sheep and goats. Susceptible species also include llamas, antelopes, deer, wild boars and others. The disease, clinically described for the first time in Italy in 1546 and in 1897, was identified as the first viral agent to affect animals.

The disease is clinically characterized with an initial fever, followed by vesicles or blisters on the skin and mucous membranes, generally visible on the mouth, nose, nipples and interdigital spaces.



Tridimensional image of FMD virus

The virus is directly transmitted through saliva, milk and semen or indirectly through fomites as medical devices (needles), contaminated clothes, vehicles, wild animals and aerosols. Despite the low mortality rate, the economic losses are significant due to the impact in the animal growth, weight loss lowering productivity of the infected animals, and the costs of implementing control measures.

Additionally, the main economic impact of FMD is the restriction of international trade of products of animal origin (meat, milk, genetic material, etc.) from countries where the disease is present.

The causal agent of FMD belongs to the Picornaviridae family and is one of the smallest animal viruses. The 28 nm viral particle has a non-enveloped icosahedral capsid with a single-stranded positive RNA molecule in its interior that acts as a messenger. It is highly sensitive to high or low pH, sunlight and high temperatures.
One of the most distinctive characteristics of the FMD virus is its antigenic variability. Like other RNA viruses, it has a high capability to mutate.
Globally, it is classified into seven different groups or serotypes, O, A, C, Asia 1, SAT (South African Territories) 1, 2 and 3. Within each serotype there are several strains, and in some cases with low antigenic relatedness among them. Infection or vaccination against one serotype does not provide protection against another serotype.
This variability has great significance to control the disease since it demands constant epidemiological controls and vaccines with

antigenic components that provide protection against circulating strain/s in the field.

Regarding cross protection among strains of the same serotype, it is well known the relevance of using proper vaccine strains with high immunogenicity and broad antigenic coverage against circulating viruses. A successful example is O1 Campos FMD virus strain included in high quality vaccines which has been shown to have satisfactory antigenic coverage to strains emerged in South America and Asia (see Chapter VI "Vaccine strain selection and vaccine matching"). The role of FMD reference laboratories in monitoring and characterization of circulating strains through vaccine matching tests is vital to provide information on selection of vaccine strains with acceptable cross protection to decision makers.
A key factor to be considered is the cross protection of specific vaccines since vaccine quality is paramount to warranty the best cross protection.

CHAPTER I

Biogénesis Bagó Manufacturing Technology and Expertise to Produce FMD Vaccine

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Biogénesis Bagó FMD Vaccine is the result of over 30 uninterrupted years working in research and development together with reference laboratories and institutions to fight against the disease.

The product obtained under a controlled quality system and a highly standardized production process guarantees in the vaccinated animals an optimum level of protection, using a safe product with adequate syringeability and due to its purity in terms of non- structural proteins (NSP) there is no interference with the sero-epidemiological surveys.

The production site is located in Garin, in the province of Buenos Aires, Argentina and is amongst the most important of the world due to its production capacity, technology, human resources qualification, and capabilities.

For the production and control of the FMD Vaccine, Biogénesis Bagó follows the World Organization for Animal Health (OIE) and other international guidelines, as well as regulatory requirements of the countries where the product is commercialized.

This production method allows the process to take place in a set of closed circuit bioreactors, with a high level of security and under compliance with GMP.

BHK21 (Baby Hamster Kidney cell line) cell line culture is the substrate of the production method. To ensure product quality and affirm its commitment to the environment, Biogénesis Bagó complies with the strictest international quality standards of GMP, ISO 9001 and ISO 14001.

1.1 Cell culture

For virus production, Biogénesis Bagó uses cell culture of the BHK21 cell line suspension. The cells used are highly susceptible to infection with the FMD virus and allow the scaling up to large scale suspension cultures.
Appropriate aliquots of BHK 21 cell line are stored over liquid nitrogen in a standardised cell bank system. This system ensures uniformity and allows implementing controls that guarantee the absence of adventitious agents. Cell cultures of BHK21 cells require bovine serum previously irradiated and filtered in order to ensure the absence of any bacterial, viral or fungal impurities.

Biogénesis Bagó only uses bovine serum from countries with negligible Bovine Spongiform Encephalopathy (BSE) risk status recognized by OIE. The testing of serum free media in antigen production gave promising results in antigen yield, safety and efficacy, which will allow the vaccine produced to gain the market shortly.

1.2 Virus strains and production seeds

The virus strains to be included in FMD vaccine are chosen based on their high antigenic coverage and their antigenic relatedness with the strains circulating in the field. They are adopted as vaccine strains after antigenic, genomic and cross immunity studies, according to the country of destination.
The virus is adapted to the BHK21-suspension cultures to create the Master Seed Bank for each vaccine strain.

All Master Seed Banks used for the production of the vaccine are subject to several controls, including purity, identity, infectious titer, absence of specific pathogens and adventitious viral, bacterial or fungal agents and mycoplasmas. They are kept at low temperatures to maintain their characteristics and are used for the uniform industrial production of antigens.
In the event it is required to adapt a field emergent strain to vaccine strain, Biogénesis Bagó has the capability and expertise to immediately adapt the new strain to cell culture and incorporate it in the production system.

1.3 Antigen production

The steps of antigen production whilst handling live virus, should be carried out in a high-containment facility. The production plant must comply with biosafety rules, requiring appropriate facilities that prevent escape of active virus, trained personnel, valid procedures and a periodically audited system regulated by entities from different countries.
The infection process of BHK21 suspension cultures consists of adding the virus seed and incubating for 12 to 24 hours until the optimum concentration is achieved. The virus is clarified, to remove proteins and cell debris. Subsequently, the antigen is chemically inactivated to eliminate infectivity, but maintaining its immunogenic properties. Binary ethyleneimine (BEI) as a first order inactivant is used; this procedure assures complete virus inactivation without altering its antigenic structure.
The process is monitored by inactivation kinetics to screen for virus residual activity by inoculation onto highly susceptible cells.
Moreover, antigenic controls are performed during the process to verify the antigen integrity of the virus particles.
Inactivated antigens are purified and concentrated in order to obtain virus particles free from any residues of the production process, especially NSP of the FMD virus.

Concentration of the antigen is required for further formulation, according to the antigen payload and potency required.
Concentration and posterior storage at low temperatures is the basis of Antigen Banks, allowing rapid availability of the antigen and formulation of vaccines for urgent supply in the event of FMD outbreaks.

1.4 Formulation and packaging

Biogénesis Bagó has developed an original formula for its FMD vaccine, a single water-in-oil emulsion, in which the antigens are emulsified in the oil phase composed of mineral oil and emulsifiers. These components are safe for the injected animal and for human consuming foodstuff

derived from these animals. Throughout the years, Biogénesis Bagó has great experience in handling adjuvants for the FMD vaccine (single, double and mixed emulsions and aqueous formulations). This knowledge allows developing tailored vaccines according to each market requirement.
The Biogénesis Bagó FMD vaccine is composed of single antigen or different antigen combinations, in various dose volume and different packaging nature in order to comply with specific demands of each country.

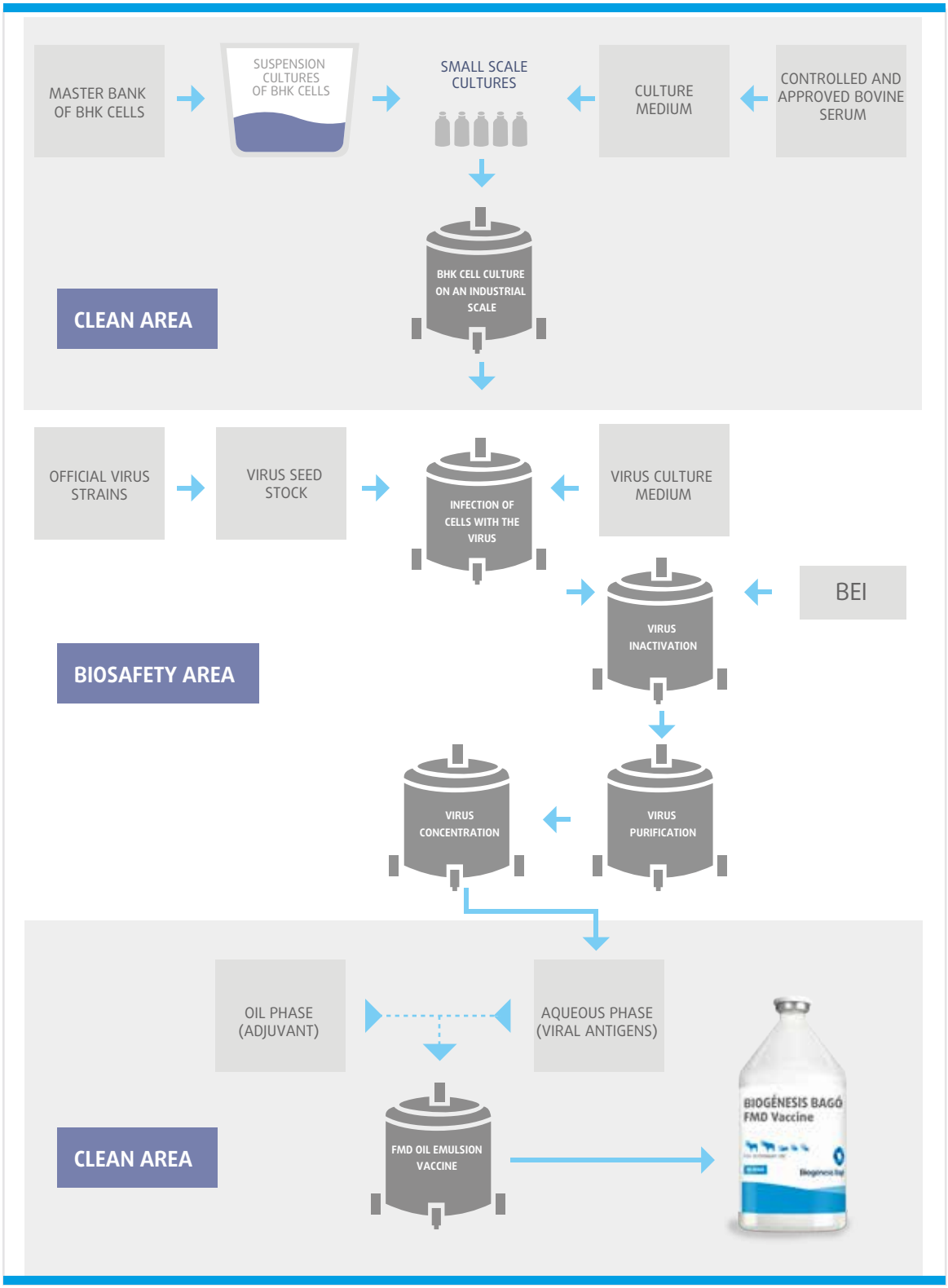
1.5 Quality controls

Every step of the manufacturing process of Biogénesis Bagó FMD Vaccine should undergo strict quality controls. The results of these controls are verified to comply with the technical specifications of each step of the process. These must be satisfactory in order to proceed and to obtain approval of each vaccine batch.

For internal controls, it is worth emphasizing the use of innovative analytical technology to monitor the different steps of the process, for example characterizing the oil emulsion with laser diffraction analysis (MIE-scattering) that guarantees homogeneity of the product. Similarly, monitoring quantity and quality of the antigens during the different steps is accomplished through the FMD virus typing test with monoclonal antibodies using ELISA (enzyme-linked immunosorbent assay) and determining the antigen payload through sucrose gradients and HPLC (High Performance Liquid Chromatography) and the coupling of an inline dynamic light scattering (DLS) detector to the HPLC equipment affords a simultaneous, direct assessment of viral particle size of the FMD virus antigen. This combined quantitative and qualitative approach provides a robust control and patent reference for the quality parameters of the antigen in both intermediate manufacturing materials and final vaccine products. Besides in-company quality controls, each vaccine batch is controlled by the Argentine Animal Health Authorities (SENASA - National Health and Agrifood Quality Service) according to current legislation and the OIE terrestrial manual standard. Additional controls are performed by Veterinary Services of the country of destination

Foreign and local authorities implement permanent audits to evaluate the different processes in agreement with the established standards that regulate procedures, quality and biosafety.

1.6 FLOWCHART | MANUFACTURING PROCESS OF BIOGÉNESIS BAGÓ FMD VACCINE




2000 mL
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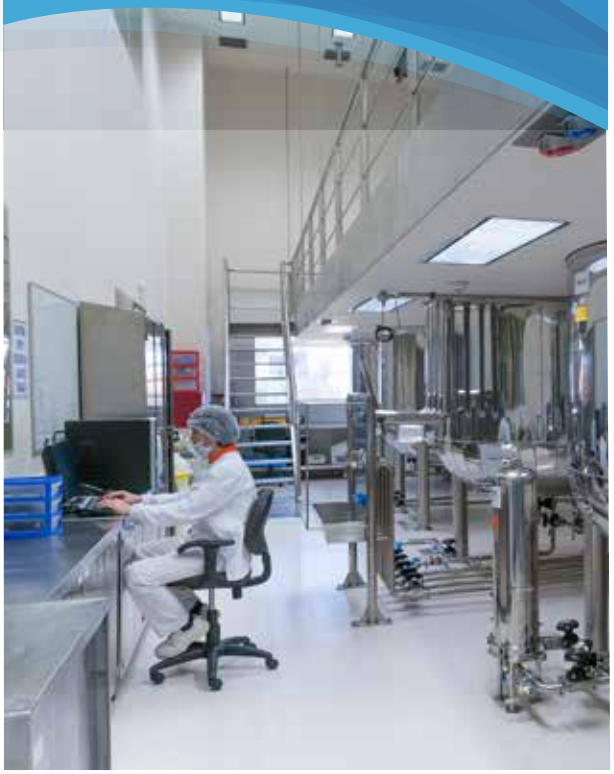
CHAPTER II

Product Profile



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Biogénesis Bagó FMD vaccine is an inactivated vaccine, formulated with an oily adjuvant and manufactured including different combinations of FMD virus strains, able to protect susceptible animal population against the disease and following international quality standards.

The FMD virus strains included in each formulation comply with requirements of Animal Health Authorities of the countries in which the vaccine is licensed.

2.1 Antigen composition and formulation

The antigen composition on the commercial product meets the requirements of each country of destination. The FMD virus is replicated in suspension cultures of BHK21 cells, inactivated by binary ethyleneimine (BEI) and finally, purified and concentrated. The antigen suspension is emulsified in an exclusive oily formulation developed by Biogénesis Bagó that provides low viscosity, good balance between early immune response and duration of the immunity, and safety for use in all susceptible species.

2.2 Product information

Biogénesis Bagó FMD Vaccine is a product ready to use, presented typically in polypropylene bottles closed with elastomer closures and aluminium seals like Flip-Off® with plastic seals or Hermeta®. The series code, manufacturing and expiration date are printed on each bottle by an unalterable system. A variety of vaccines are available, monovalent, bivalent and polyvalent, in bottle sizes of 10, 25, 50, 60, 100 and 125 dose of 2 mL.

Biogénesis Bagó FMD Vaccine has a shelf life of at least twenty-four months.

Biogénesis Bagó FMD vaccine should be kept between 2°C and 8°C, and away from light. It must not be frozen. To guarantee the cold chain, vaccines are conditioned and transported under specific conditions of controlled temperatures.

Biogénesis Bagó FMD Vaccine is recommended for cattle, pigs, sheep, goats, bubaline and any other species susceptible to the disease. For intramuscular administration, vaccine should be given in the upper neck, and for subcutaneous administration, the recommended area is behind the shoulder. It is also suggested to vaccinate two dose 3 or 4 weeks apart, and then every six months. The vaccination

scheme can be modified according to the programs designed by each National Animal Health Authority. The product must be prescribed, applied in aseptic manner, and controlled by a Veterinarian

2.3. The usual term is Precautions and warnings

To obtain the vaccine's best results and most adequate immune response the following is recommended:

- Control seal integrity of vaccine bottles.
- Verify expiration date.
- Maintain the cold chain until vaccine application.
- Do not heat the vaccine before application
- Use new bottles and avoid storing leftovers
- Shake the bottle gently before filling the syringe.

2.4. Animal care

- Animals should be rested before vaccination.
- Vaccinate animals in good health and nutritional status.
- The injection site needs to be clean and dry.
- Do not enclose animals for long periods of time, especially during the hottest hours of the day.
- Avoid performing any invasive procedures in cattle, such as castrations during vaccination period.
- Do not dip or spray livestock on vaccination day.
- It is advisable to restrain properly the animals in order to avoid kicking; Animals should be handled with calm.

2.5. Materials

- Use properly cleaned and disinfected tools.
- Use syringes in good conditions and with fixed doses.
- Previously control that the dose volume fixed is correct.
- Purge the syringe after each load.
- Only use a clean needle to draw out vaccine from the bottle.
- Change the needles every 10-12 animals and whenever the tip is deteriorated. Never use a blunt needle
- Dispose of used needles (and blades) in a proper sharps bin.
- Return full sharps bins to the veterinarian for safe disposal.
- Do not share needles with another stockperson.
- Needle size: see on the tables the specific needle size recommendations.
- At the end of the vaccination, carefully disinfect the tools and materials used.

2.6. Accidental Self Injection

Inform manager (or assistant manager) immediately. Seek medical attention

2.7. Local and general reactions

The vaccine may be applied to animals of all ages, even to pregnant or lactating females, or newborns. Once vaccines have been administered, it is recommended to observe the animals for at least 60 minutes to detect any adverse reactions.

Due to the oily adjuvant, small local inflammatory reactions are normal and assure an effective immune response. These reactions can be more or less visible; depending on how deep the application was or what age category animal was vaccinated. These reactions, when they occur, dissapear without treatment in 4-8 weeks after vaccination.

Rarely, in animals an injectable drug may trigger a sporadic allergic or anaphylactic reaction. In these cases, administer adrenaline and corticoids according to the practising veterinarian criteria.

PIGS (INTRAMUSCULAR ROUTE)		
	NEEDLE GAUGE	**NEEDLE LENGTH**
Piglets	19 or 17G (1.2 or 1.5 mm)	12 mm
	19 or 17G (1.2 or 1.5 mm)	15 mm
Adults	16G (1.6 mm) or 15G (1.8 mm)	20 to 35 mm

CATTLE AND BUBALINE			
ROUTE	**AGE CATEGORY**	**NEEDLE GAUGE**	**NEEDLE LENGTH**
Intramuscular	Calf	15G (1.8 mm)	15 mm
	Adult	14G (2.0 mm)	20 mm
Subcutaneous	Calf or Adult	15G (1.8 mm) or 17G (1.5 mm)	12 mm to 15 mm

SHEEP AND GOATS			
ROUTE	**AGE CATEGORY**	**NEEDLE GAUGE**	**NEEDLE LENGTH**
Intramuscular	Lamb/ Goat kid	20-19G (0.81-0.91 mm)	12.7-15.87 mm
	Adult	14G (2.0 mm)	20 mm

CHAPTER III

Antigen and Vaccine Banks

The Antigen and Vaccine Banks (AVB) consist of reserves of formulated vaccines that are ready-to-use and reserves of concentrated antigens at ultra-low temperatures ready to be formulated into high potency vaccines. In some cases, they have the operating capacity to produce vaccines on a large scale when facing an outbreak dissemination. These measures represent an efficient strategic tool for the contingency plans in areas or countries free of FMD, with or without vaccination.

Since 1967, when the first AVB was created in Denmark, many countries have decided to have their own banks or become part of an alliance that administrates a bank of antigens and vaccines.

Biogénesis Bagó has extensive experience in the production and long-term storage of concentrated antigens and manufacturing of emergency vaccines against FMD. Since 2000, the company was awarded the AVB in Argentina and had supplied the emergency vaccines for the epidemic in 2000-2001 in South America, which contributed to the rapid control of the disease.

Furthermore, it has the competitive advantage that all raw materials of animal origin for the production of antigens and vaccines are from Argentina, a country where the risk for bovine spongiform encephalopathy (BSE) is negligible. Since 2006 Biogénesis Bagó provides these products for the North American FMD Vaccine Bank (NAFMDVB), which includes the United States, Canada and Mexico. Additionally, in 2010 and 2011 Biogénesis Bagó was the first vaccine producer worldwide to obtain a sales and commercialization permit for FMD in Canada and the United States of America, respectively.
Furthermore, in 2019 the company was awarded the contract fot the Taiwan´s Vaccine Bank and in 2020, became supplier of the National Animal Vaccine and Veterinary Countermeasures Bank (NAVVCB) from USA.
The AVB components (concentrated antigens and vaccines manufactured with concentrated antigens) undergo strict quality controls during manufacturing process as well as follow-up stability controls during storage, such as antigen quantification and integrity analysis with sucrose gradients or HPLC, DLS and MIE-scattering and field trials in ruminants and pigs.

The strains kept in the AVB are monitored by their antigenic coverage against FMD viruses causing outbreaks in the American continent and against relevant emerging FMDV worldwide. Biogénesis Bagó, as part of the Inter-Institutional Network of Research and Development of FMD (RIIDFA), closely participated with the institution members, in cross-protection assessment between vaccine and FMDV field strains (See Chapter VI. Vaccine strain selection and vaccine matching). The SENASA laboratory, an OIE reference laboratory for FMD, has the capabilities to monitor the antigenic and genomic characteristics of emerging strains through cross virus neutralisation tests and sequencing of the coding region of the capsid proteins. These results together with antigenic yield, antigen stability and epidemiological data from FMD reference centers provide decisive information that will determine the most appropriate vaccine strains to be kept in the AVB.


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CHAPTER IV

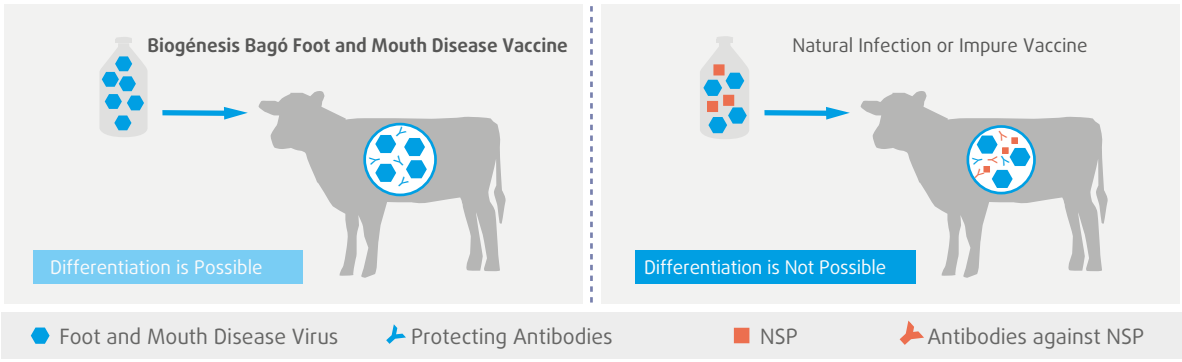
Key Features of Biogénesis Bagó FMD Vaccine

4.1 Production capacity

Biogénesis Bagó FMD vaccine production plant is amongst the most important in the world due to its high production capacity and its quality and biosafety standards. The production level reaches 300 million doses of oil emulsion vaccine per year. From this facility Biogénesis Bagó supplies 11 countries in different continents.

4.2 Antigen purification

During the production process of Biogénesis Bagó FMD vaccine there are several steps of purification to remove cell impurities and the viral NSP. This purification process is critical to ensure there is no interference when differentiating the vaccinated animals from the infected animals during epidemiological surveillance. These tests are based on identifying NSP antibodies that are produced after infection or vaccination with non-purified vaccines. The purified Biogénesis Bagó FMD vaccine does not induce such antibodies, thus, no interference with the detection of infection or viral circulation is generated, which is the basis to establish and monitor the sanitary FMD status of destined countries and regions.



Absence of Non Structural Proteins

With the Foot and Mouth Disease Vaccine, Biogénesis Bagó provides the possibility of differentiating infected animals from vaccinated animals in order to demonstrate an area or country free of virus circulation. Non purified vaccines do not allow this differentiation.

4.3 Quality and traceability

Biogénesis Bagó is at the forefront in terms of the quality and safety of its products. The company has a certified quality management system that guarantees an excellent production process, as well as product traceability throughout each manufacturing step: product design, research and development, production, commercialization and technical service.

The ISO 9001 certification, granted by the TÜV CERT of Germany was reached by 2000. The Quality System also complies with Good Manufacturing Practices (GMP) requirements.

GMP certification has been granted by the National Health and Agrifood Quality Service (SENASA) since 2006. The system guarantees that controls to raw materials, intermediate products and bulk products are carried out following the established methods and that the necessary process controls and validations are performed. Also it guarantees that finished products are conditioned and inspected pursuant to defined processes and that there is a correct supply and use of raw and packing materials.

The FMDV antigen manufacturing process facility has been certified by SENASA as complying with the OIE Biosafety Level 4 requirements.

Additionally, Biogénesis Bagó Quality Management System is periodically audited by Regulatory Authorities from different countries.

Biogénesis Bagó is not only committed to the quality of its products but also to the environment. Its Environmental Management System has been certified according to ISO 14001 standards, by TÜV Rheinland in 2011.

The strict international quality standards applied to the production of Biogénesis Bagó FMD Vaccine guarantees consistent and reproducible vaccine batches. All production methods assure total traceability of raw materials, procedures and internal controls of the manufactured batches.

Biogénesis Bagó believes that the excellence of their products will provide animal health solutions to veterinarians and farmers who are using them to meet the global demand for food with the quality and quantity required.

Certifications of the process and the production facility of the FMD vaccine

REQUIREMENT | STANDARDS	AUTHORITY CERTIFICATION BODY	YEAR
OIE Biosafety | Level 4	SENASA	**1996**
ISO 9001	TÜV Rheinland	**2000**
Good Manufacturing Practices (GMP)	SENASA	**2006**
ISO 14001	TÜV Rheinland	**2011**

CHAPTER V

Relevant features

The differential keys of Biogenesis Bagó FMD vaccine are the combination of relevant features such as safety, early immune response and protection with long lasting immunity. In addition, Biogénesis Bagó potent vaccines achieve a degree of purity, in terms of NSP content, which allows for an accurate interpretation of the results of sero-epidemiological surveillance, needed to support evidence to confirm the animal disease status of FMD free zones.

Biogénesis Bagó has a thorough and wide experience in field experiments to monitor vaccine performance in different conditions. To accomplish this, we have highly skilled veterinarians trained in good clinical practices and in running trials to study safety, potency, purity and the efficacy of the vaccine, doing trials which involve virus challenge in different species, such as cattle, pigs, goats and sheep.

5.1 Efficacy

Efficacy is tested in vaccinated cattle and pigs to demonstrate protection against virus challenge (Protection against generalized foot infection-PGP- or 50% protective dose -PD50-). Additionally, batch to batch testing is performed by serological tests that predict protection level by determining specific post vaccination antibody response.

The challenge tests are performed in biosafety facilities OIE BSL 4 supervised by health authorities following OIE guidelines. Briefly, cattle or pigs free from specific antibodies are challenged at 4 weeks post vaccination or as determined by needle inoculation of FMD virus. Naïve cattle or pigs are used in each trial as a control group. The animals are observed for at least seven days post inoculation. Unprotected animals show lesions at sites other than inoculation site. The potency test is performed according to each country regulations.

Bioaftogen vaccine showed an efficacy of more than 6 PD50 either in cattle or pigs against homologous challenge.

Alternative protocols are used to study early protection or evaluate virus transmission.

Batch to batch control potency is tested by vaccinating target species free from FMDV antibodies and determining the antibodies induced against each viral strain present in the formulation through tests correlated to protection against challenge such as Expected Percentage of Protection (EPP) in the target animal determined by Liquid phase blocking sandwich ELISA (LPBE) or virus neutralization test (VNT).

5.1.1. Clinical trials in cattle

Early protection

Induction of early protection is essential to prevent dissemination of the disease between susceptible animals. One dose of the polyvalent Biogénesis Bagó FMD Vaccine in cattle induced protective immunity against virus challenge as early as 7 days post vaccination (dpv).

EARLY PROTECTION AGAINST VIRUS CHALLENGE AT 7 AND 14 DAYS POST VACCINATION IN CATTLE

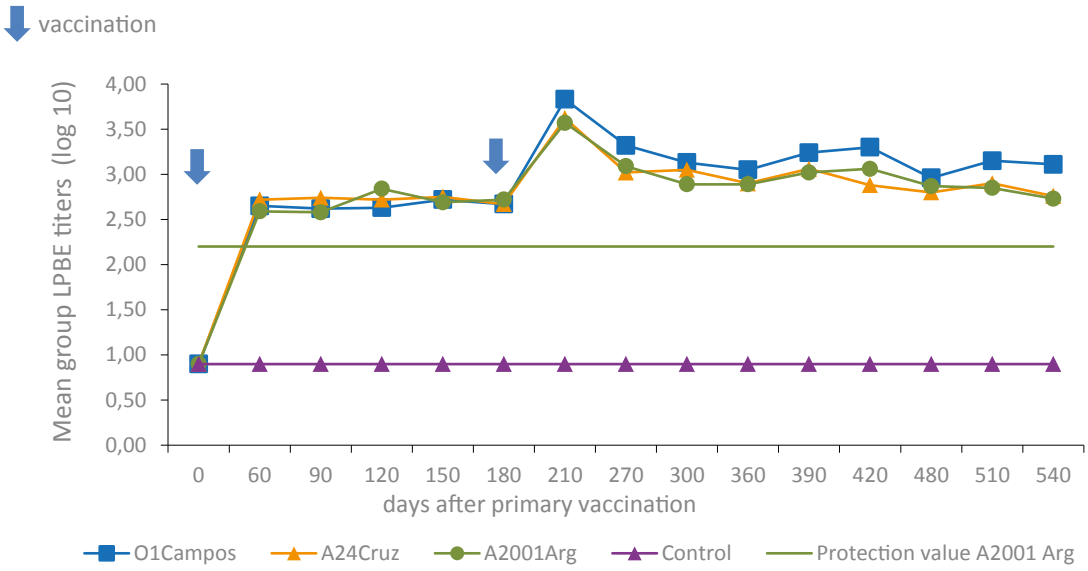
Vaccine	Challenge virus	Challenge method	Vaccination in days prior challenge (N)	Protection
Polyvalent	O1 Campos	Exposed by contact with infected pigs	7 days (5)	80 %*
Polyvalent	A Arg 2001	Intranasal	7 days (5) 14 days (5)	100 %**
Monovalent A24 Cruzeiro	A24 Cruzeiro	Intradermo lingual	7 days (5)	100 %

Non vaccinated cattle became FMD clinically infected in all three trials
References: * Quattrocchi et al. /Vaccine 32 (2014) 2167–2172 ** Duffy et al. / Vaccine: X 5 (2020) 100063

Duration of immunity

After a single administration of FMD Vaccine in cattle, antibody response is developed from eight dpv. A peak of antibodies is reached at 30 to 60 days, remaining at high levels up to 6 months after vaccination. Protection levels remained high for at least a year after the second dose.

DURATION OF IMMUNITY IN CATTLE POST VACCINATION AND REVACCINATION
POLYVALENT OIL VACCINE

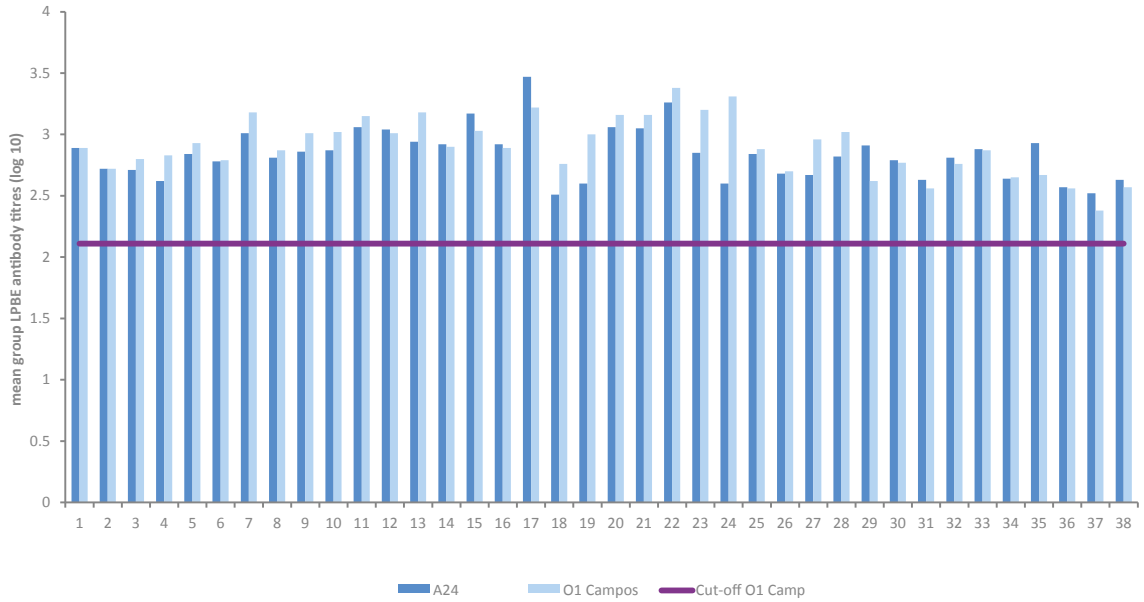


Mean group antibody responses of cattle after vaccination and revaccination with polyvalent oil vaccine as measured by LPBE-Vaccinations: 2 mL/dose by intramuscular route. Cattle: Hereford breed, aged 18-36 months, originating in North Patagonia Region (FMD free zone without vaccination) and free of FMDV antibodies prior vaccination. Arrow indicates vaccinations (at day 0, and 6 months after primary vaccination). Vaccinated group N=17. Non vaccinated group N=2.

Potency performance of Bioaftogen vaccine in cattle

Potency tests of industrial vaccine batches of Bioaftogen vaccine are regularly carried out in 18-36 month-old cattle free from specific antibodies to FMDV. Serological determination on serum samples taken at 60 dpv is examined by LPBE against each vaccine strain component. Mean group antibody titers of batches were above protection value associated with 75 % of protection.

POTENCY TEST IN CATLLE. VACCINE BATCHES OFFICIALLY TESTED.
PERIOD 2016-2020

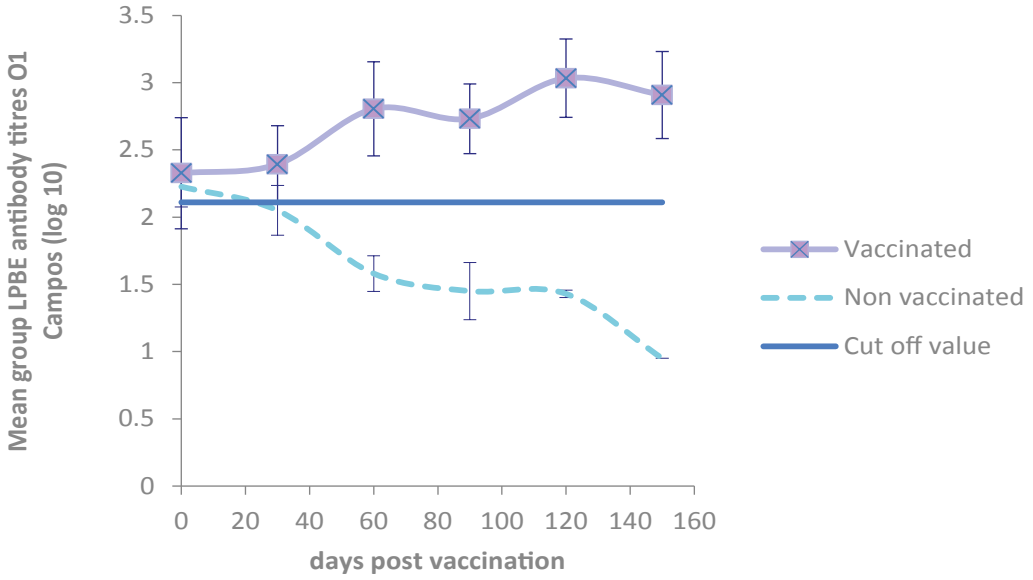


Results of the potency control tests, expressed as mean group LPBE antibody titers, performed on series of foot and mouth disease vaccine submitted in the period 2016–2020 to National Health and Agrifood Quality Service (SENASA).

Induction of immunity in calves in the presence of colostral antibodies

Vaccinating calves born from multi vaccinated cows with Biogénesis Bagó FMD vaccine showed high levels of protective antibodies up to 150 dpv. Calves were vaccinated at the age of 60 to 90 days old.

IMMUNE RESPONSE AFTER VACCINATION IN THE PRESENCE OF MATERNALLY DERIVED ANTIBODIES



Mean group antibody responses of calves to vaccination with polyvalent vaccine in the presence of maternal antibodies as measured by LPBE. Vaccination: 2 mL/dose by intramuscular route. Calves from 60 to 90 days old, born to multivaccinated cows were used. Vaccinated group N=22. Non vaccinated group N=5.

At earlier ages (younger than 1-3 months), certain degree of suppression of immune response after vaccination due to high levels maternal derived antibodies may be detected. However, vaccination at this age is recommended to confer adequate herd protection in this age category.

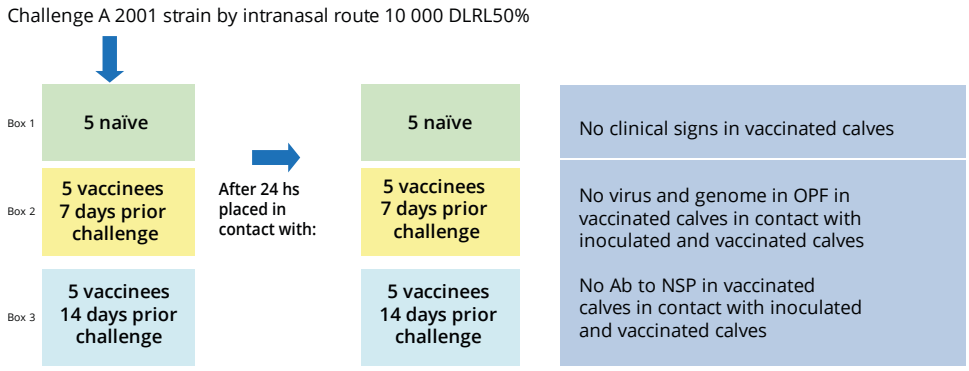
Reduction of virus transmission in cattle vaccinated one or two weeks before challenge

The effect of vaccination using polyvalent Bioaftogen vaccine on the transmission of FMDV in cattle was studied. It was demonstrated that vaccination 7 and 14 days prior to challenge induced full clinical protection against virus inoculation. Additionally, the vaccinated calves' contact-exposed to vaccinated and inoculated calves did not become infected. Thus, no virus transmission occurred from vaccinated and subsequently infected calves to cohabitating vaccinated calves.

These data provide useful knowledge for the design of future control strategies.

REDUCTION OF FMDV TRANSMISSION IN CATTLE VACCINATED 1 OR 2 WEEKS BEFORE CHALLENGE

Cattle: Calves Holando breed 6 months old / **Vaccine:** Bioaftogen / **Vaccine dose:** 2 ml intramuscular route



No virus transmission occurred from vaccinated and subsequently infected calves to cohabitating vaccinated calves (R = 0)

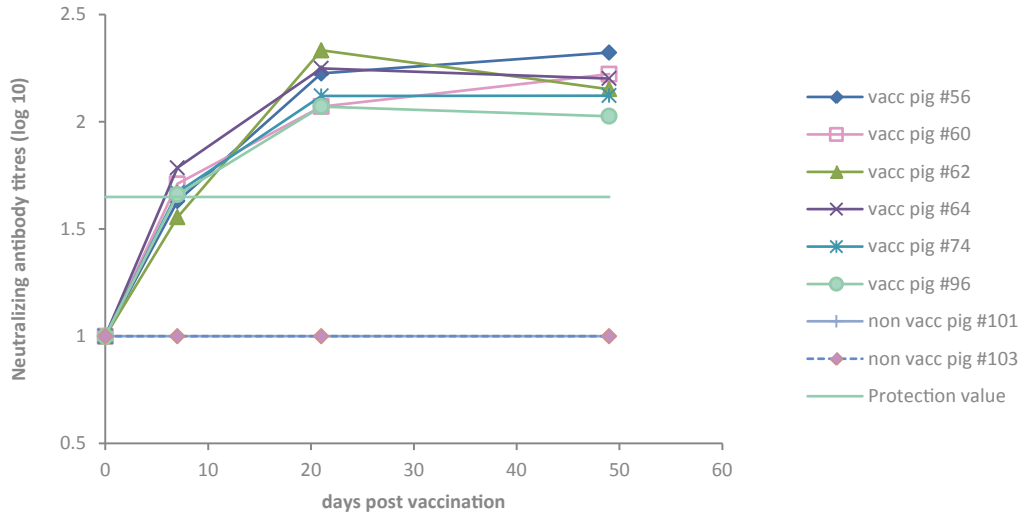
Source: Reduction of foot-and-mouth disease virus transmission in cattle vaccinated one or two weeks before challenge using a commercial polyvalent vaccine. S Duffy et al. Vaccine: X 5 (2020) 100063

5.1.2. Clinical trial in pigs

Onset of immunity in pigs

Eleven 2 month-old Duroc Jersey pigs free from specific antibodies were each vaccinated with 2 mL Biogénesis Bagó FMD Vaccine containing O1 Campos FMDV vaccine strain. Neutralizing antibodies were detected as early as 7 days post vaccination. High neutralizing antibody titers were shown at 21 and 49 days post vaccination.

ONSET OF IMMUNITY IN PIGS



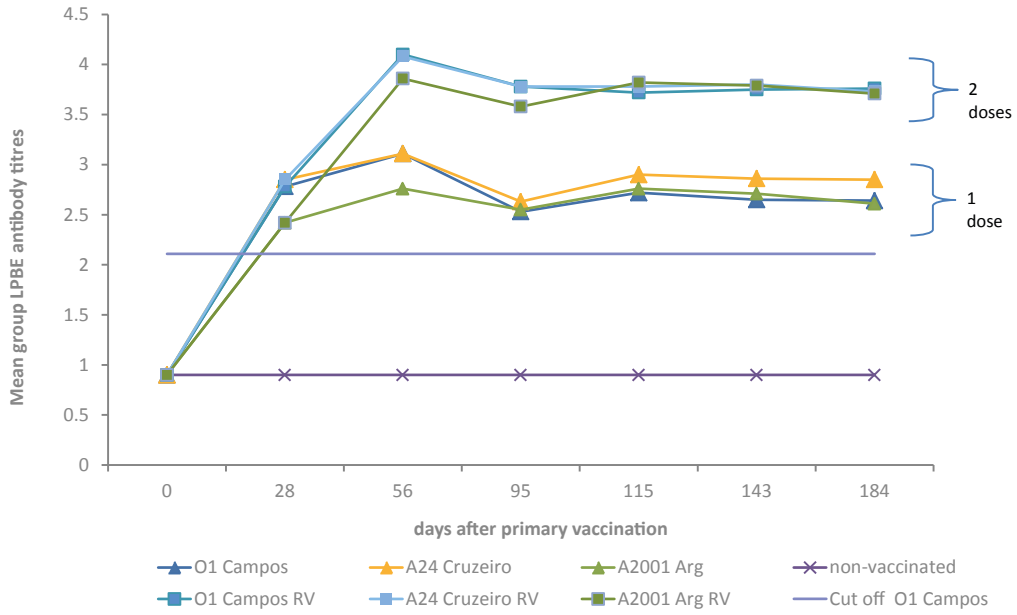
Individual antibody responses of pigs after vaccination as measured by virus neutralization test against O1 Campos FMDV. Vaccination: 2 mL/dose by intramuscular route. Pigs: free from FMDV antibodies prior to vaccination, aged 2 months, Duroc Jersey breed. Vaccinated group N=6. Non vaccinated group N=2.

Duration of immunity in pigs

After one dose of Bioaftogen vaccine in pigs, specific antibodies were detected by LPBE and persisted in high levels up to 6 months post vaccination.

The administration of a second dose in pigs induced higher levels of specific antibodies that persisted for at least for 6 months after primary vaccination.

DURATION OF IMMUNITY IN PIGS. ONE OR TWO DOSES
POLYVALENT VACCINE

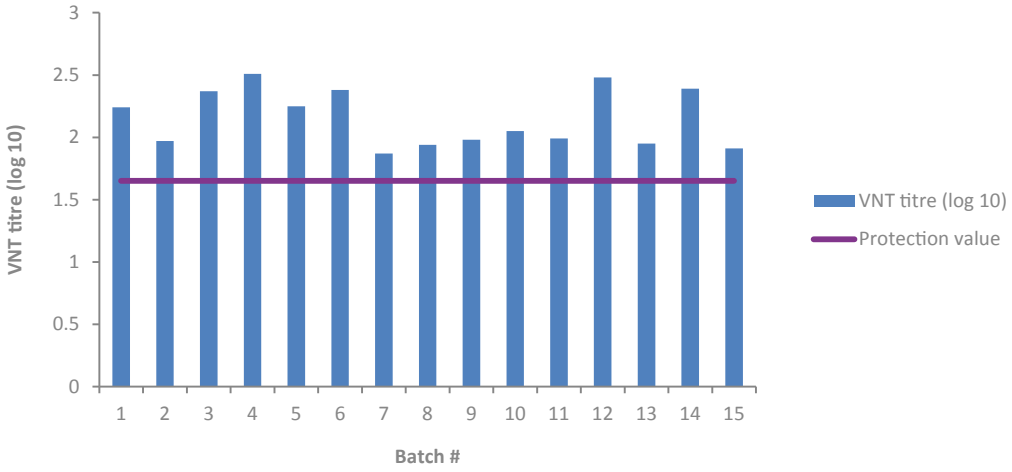


Mean group antibody responses of pigs after vaccination and revaccination of polyvalent vaccine as measured by LPBE against O1 Campos , A24 Cruzeiro and A2001 Argentina FMDV strains (log 10). Vaccinations: 2 mL by intramuscular route, at day 0 and 28 days after primary vaccination. Pigs: free from FMDV antibodies prior to vaccination, aged 2 months, Hybrid breed. Vaccinated group N=5. Non vaccinated group N=2 .
RV= revaccinated group

Potency performance of Bioaftogen vaccine in pigs

After one dose of Bioaftogen vaccine in pigs, Potency tests of industrial vaccine batches of Bioaftogen vaccine are regularly carried out in 2 month-old pigs free from specific antibodies to FMDV. Serological determination on serum samples taken at 4 weeks post vaccination is examined by VNT against each vaccine strain component. Mean group antibody titers of batches were above protection value associated with 75 % of protection.

OFFICIAL POTENCY CONTROL OF VACCINE BATCHES
IMMUNE RESPONSE IN PIGS AFTER VACCINATION



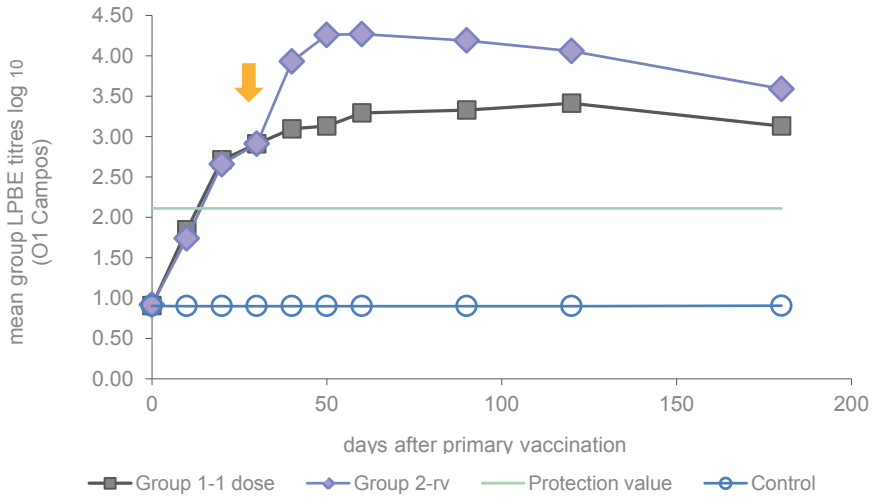
Results of the potency control tests of industrial monovalent O1 Campos vaccine batches expressed as mean group neutralizing antibody titers. Potency test are regularly carried out in groups of 10-14 two-month-old pigs free from specific antibodies to FMDV. Pigs are vaccinated by the intramuscular route with 2 mL/dose. Serological determination on serum samples taken at 21 days post vaccination is examined by virus neutralization test (VNT) against O1 Campos strain. The horizontal line indicates protection cut-off value (log 10=1.65)

5.1.3. Clinical trials in sheep and goats

Onset and duration of immunity

After a single dose of polyvalent Biogénesis Bagó FMD Vaccine, sheep developed antibody titers as early as 10 days post vaccination which persisted in high levels for at least 6 months.
The administration of a second dose 10 to 30 days after primary vaccination induced high levels of specific antibodies that persisted for at least 6 months.

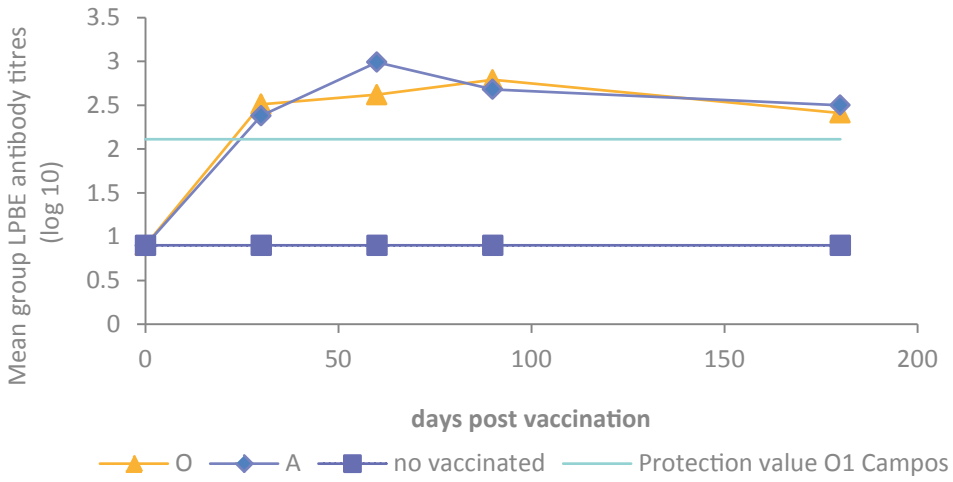
BIOGENESIS BAGO POLYVALENT VACCINE:
IMMUNE RESPONSE IN SHEEP



Mean group LPBE antibody titers (log 10) following vaccination (Group 1-1 dose) and revaccination at 30 days (Group 2-rv) after primary vaccination with polyvalent oil vaccine.
Arrow indicates revaccination of group 2. Open circles indicate non vaccinated control group.

Goats vaccinated with polyvalent vaccine developed specific antibodies that last at least for 6 months in high levels.

DURATION OF IMMUNITY IN GOATS.
POLYVALENT OIL VACCINE

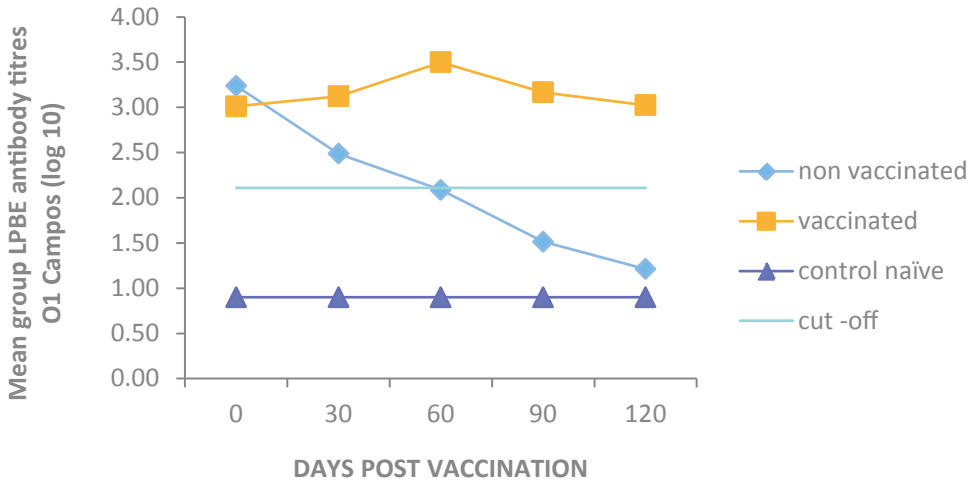


Ten goats were vaccinated by IM route with polyvalent vaccine. Five remained non vaccinated.
Mean group LPBE antibody titers (log 10) following vaccination.
Animals: Two month-old-goats free from FMDV antibodies

Induction of immunity in lambs in the presence of colostral antibodies

Lambs 30-90 days old with maternally derived antibodies respond to FMD oil vaccine and produce high levels of antibodies that persist at least for 4 months after vaccination.

IMMUNE RESPONSE AFTER VACCINATION IN LAMBS
IN THE PRESENCE OF MATERNAL DERIVED ANTIBODIES

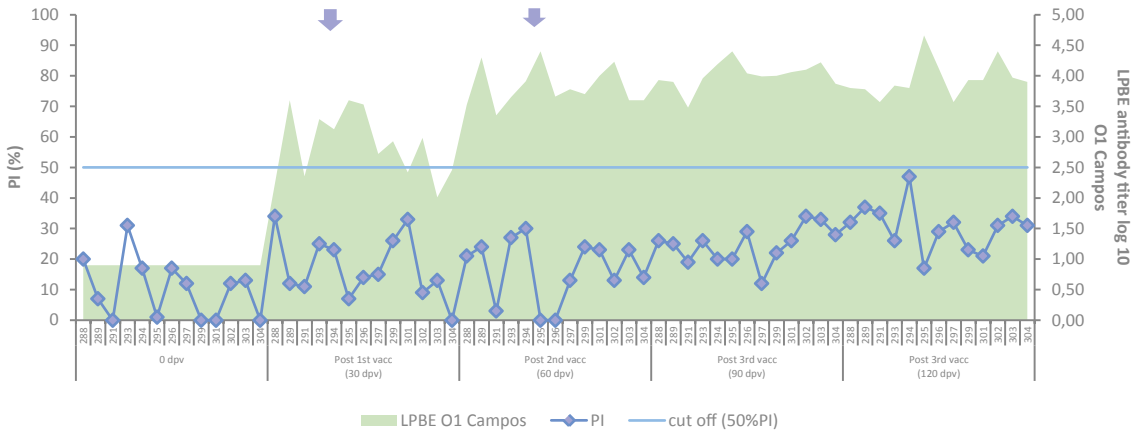


Mean group antibody response of lambs to vaccination with polyvalent vaccine in the presence of maternal derived antibodies as measured by LPBE. Vaccination: 1mL/dose by intramuscular route. Corriedale 35 day-old lambs born to vaccinated ewes were used. Vaccinated group N=28. Non vaccinated group: N=13. Naïve lambs born to non vaccinated ewes N=4. Cut-off indicates correlation to 75 % protection.

5.2. Antigenic purity

The antigens used to formulate the vaccine should include purification steps to remove NSP and other impurities; consequently vaccines allow differentiation of vaccinated from infected animals and therefore avoiding interferences with the interpretation of sero-epidemiological surveys. The antigens that are not sufficiently purified induce in vaccinated animals antibodies against NSPs of the virus, mimicking a post infection immune response which interfere with the surveillance directed to assess viral activity and consequently, threatening the sanitary status of a country or a zone. The experiments to determine vaccine purity, in terms of NSPs, consist of demonstrating the lack of detection of antibodies after repeated vaccination using a vaccine with high antigenic payload.

PURIFIED VACCINE: NO INDUCTION TO ANTIBODIES
TO NON STRUCTURAL PROTEINS



Individual antibody response to structural proteins (area in green) against O1 Campos strain determined by LPBE. Individual antibody response to non structural proteins determined by PrioCHECK FMDV NS antibody ELISA kit, following repeated vaccination in cattle with a polyvalent vaccine containing 4 vaccine strains and administered with double dose (4 mL).
dpv: days post primary vaccination

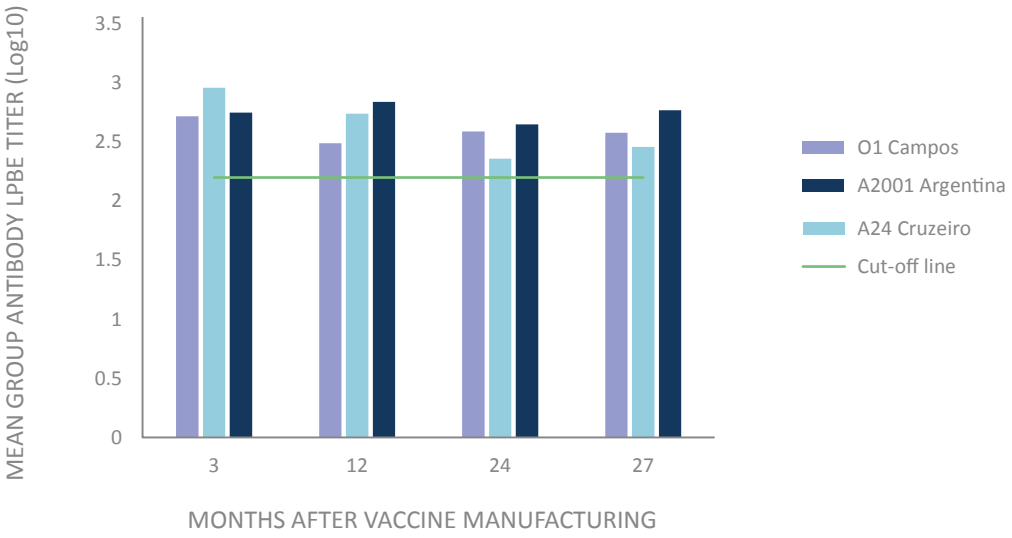
5.3.Safety

All vaccine batches produced are officially controlled in target species by sanitary health authorities, by observing and recording any local or general adverse reactions. Due to the oil adjuvant, small local inflammatory reactions are normal and assure an effective immune response. These reactions, when they occur, dissapear without treatment in 4-8 weeks after vaccination. Biogénesis Bagó FMD Vaccine displays a satisfactory safety profile in vaccinated animals when the product is used following the product label recommendations.

5.4.Stability

Biogénesis Bagó FMD Vaccine showed antigenic stability for at least 24 months from its manufacturing date. For stability studies, potency tests in cattle were carried out at different times after vaccine manufacturing. Similar studies were performed in pigs. FMD vaccine stored at 2-8ºC for 24 months induce equivalent antibody levels to those developed in the potency test carried out when the vaccine was recently manufactured and approved.

ANTIGENIC STABILITY OF FMD VACCINE IN CATTLE

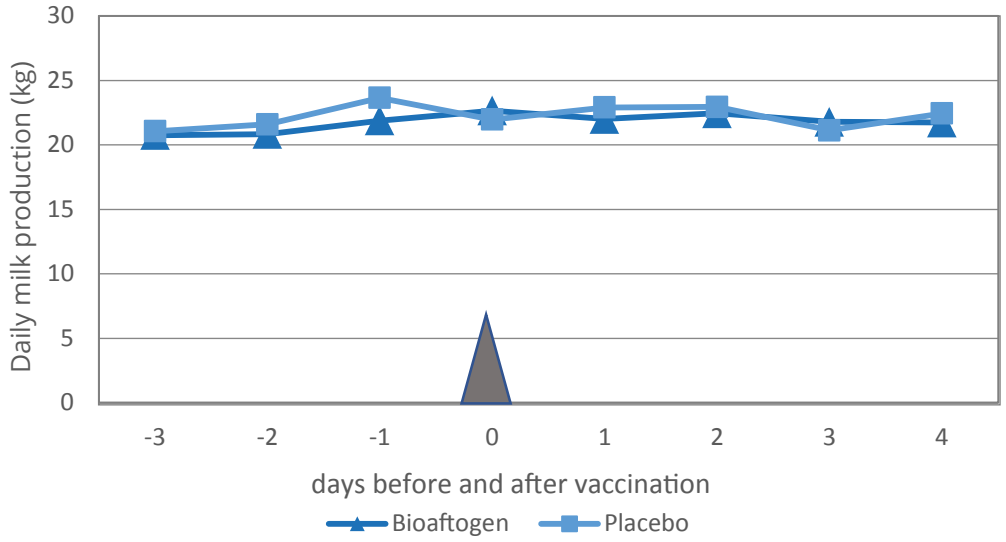


Potency test in cattle were carried out at 0, 12, 24 and 27 months after vaccine manufacturing. Four groups of 17 cows free from FMDV antibodies were vaccinated, one for each storage interval, with vaccine stored at 2-8°C by intramuscular route (2 mL/dose). Antibody titer in serum samples of each individual cattle were assessed by LPBE against each vaccine strain. Cut-off indicates protection value

5.5. Effect on milk production

The effect of Bioaftogen vaccine on milk production in dairy cattle was examined. Holstein cows 130 to 290 days in milk were used. Animals were blocked according to days in milk, milk production and number of lactancies and randomly allocated to the two treatments: Bioaftogen vaccine and placebo (saline sterile solution). There were no significant differences on milk production between vaccinates and the control cows after vaccination.

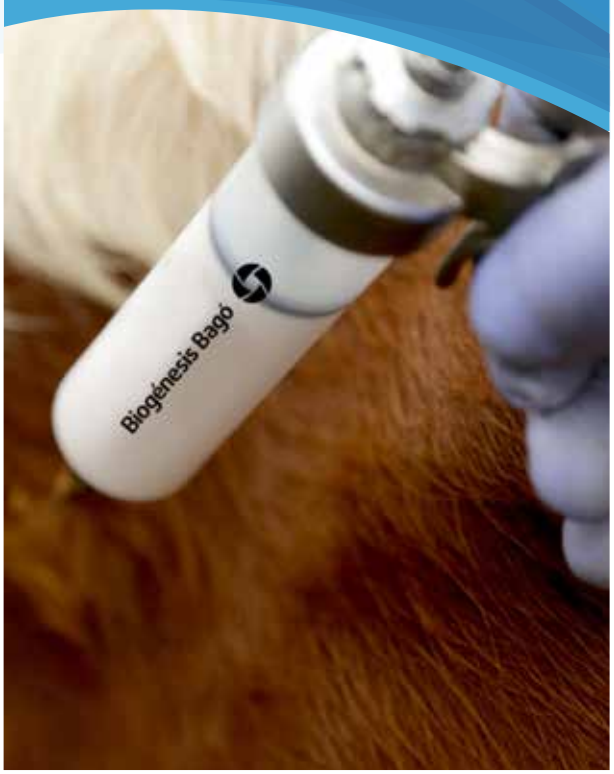
MEAN DAILY MILK PRODUCTION BY TREATMENT GROUP



Animals: Holstein cows 130 to 290 days in milk, 19 cows in each group
Vaccination: 2 mL/dose by SC route following label directions.
Daily milk production (kg) was recorded for each animal (two records/day), 3 days prior to vaccination, day of vaccination and 4 days after vaccination in the vaccinated (Bioaftogen) and placebo-treated (Control) groups. Each point indicates the mean daily milk production of each group. There were no significant differences between Bioaftogen vaccinates and the placebo inoculated cows.

CHAPTER VI

Vaccine strain selection and vaccine matching



The selection of the most appropriate strains of FMD vaccines to use in control programmes and to store in vaccine antigen reserves is based on the matching of representative field isolates from outbreaks around the world to available vaccine strains.

Successful control programs in countries that eradicated the disease have been based on strong vaccination campaigns with potent vaccines with few changes in vaccine strains.

The most direct and reliable method to measure cross-protection is to vaccinate relevant target species and then to challenge them by exposure to the virus isolate against which protection is required (PGP or PD50). However, such an approach requires the use of live FMDV and appropriate biosecurity procedures. Additionally, the use of animals for such studies should be avoided where possible by the use of *in-vitro* alternatives.

Vaccine matching tests, mainly based on *in-vitro* methods are performed in a relatively small number of laboratories around the world. These tests are used to quantify antigenic differences between FMDV strains and thereby estimate the likely cross-protection between a vaccine strain and a field isolate.

The table summarizes the methodologies being used for vaccine matching purposes.

Vaccine matching tests

STUDY	METHODOLOGY	DETAILS	VACCINE STRAIN ACCEPTANCE CRITERIA
r1 values	2D-VNT	serological relationship between a field isolate and a vaccine virus	≥ 0.3
	LPBE		≥ 0.4
Expectancy of Protection (EPP)	VNT	The titers of sera against a field isolate are used to estimate the immunological coverage of the vaccine strain in relation to the field virus.	≥ 75% EPP in booster-vaccinated animals
	LPBE		
Determination of heterologous neutralization titers	VNT	Assessment of VNT titers against field virus in serum samples of vaccinated animals (post vaccination or revaccination).	Select the vaccine strain that induced the highest heterologous titer

EPP: Expectance percentage of protection
2D: double dimension
VNT: virus neutralization test
LPBE: Liquid phase blocking sandwich ELISA

Issues related to vaccine matching

- The use of r1 as a selected method to infer protection has many limitations.
- A better choice would be to look for the vaccine that induces the highest VNT titer against the field virus.
- Broad spectrum vaccine strains formulated in high quality vaccines protect against a wide spectrum of viruses
- Booster doses of the vaccine and the effect of additional serotypes and strains also play an important role in the protection outcome.

High potency Biogenesis Bago vaccine showed wide antigenic spectrum against several field FMDV of different regions

Biogénesis Bagó vaccines include reference strains that performed successfully in South America and in many countries of Asia against a wider variety of strains. This relies on the use of immunogenic and cross reactive vaccine strains incorporated in high potency vaccines.

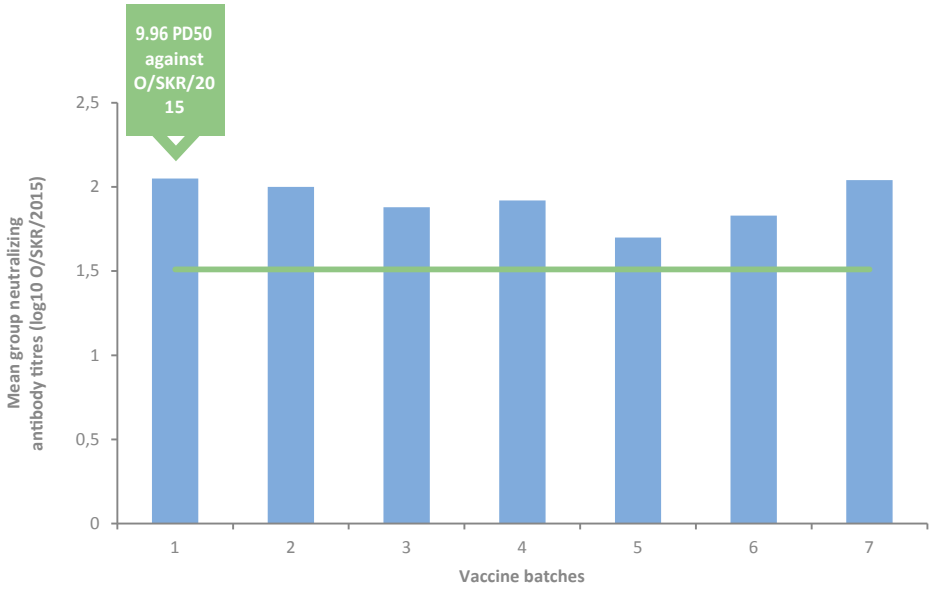
Biogenesis Bagó FMD vaccines containing O1 Campos, A24 Cruzeiro and A2001 Argentina vaccine strains conferred high heterologous antibody response to the major circulating serotype O and A FMD viruses in Middle East, and East Asia.

Protection against heterologous challenge

Challenge studies conducted in pigs evidenced high heterologous protection against O/SEA/Mya 98 (O/SKR/2015) and A/ASIA/Sea 97 (A/SKR/2010, A/SKR/2017, A/SKR/2018) field strains.

A clear example of cross reactivity is the performance of the BB vaccine in South Korea. Vaccine batches containing O1 Campos vaccine strain performed successfully in official potency tests against heterologous O/SKR/2015 Jincheon strain, a prototype strain that caused massive outbreaks in 2014/2016 in South Korea. Additionally it showed 9.96 PD50 in challenge test.

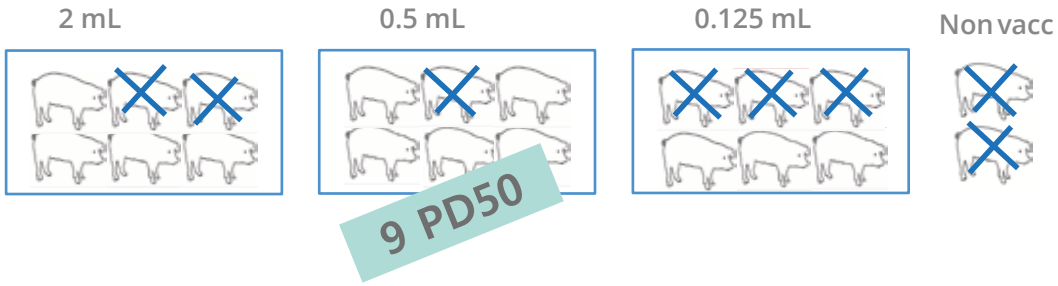
OFFICIAL POTENCY TESTS OF INDUSTRIAL VACCINE BATCHES CONTAINING O1 CAMPOS VACCINE STRAIN AGAINST O/SKR/2015



Results of the official potency control tests of industrial vaccine batches containing O1 Campos vaccine strain expressed as mean group neutralizing antibody titers against O/SKR/2015, Jincheon FMDV strain. Potency tests were carried out in groups of 10-14 two-month-old pigs free from specific antibodies to FMDV. Pigs were vaccinated by the intramuscular route with 2 mL/dose. Horizontal line indicates protection cut-off value established by South Koreans Health authorities (log 10=1,51)

Also vaccines containing O1 Campos, A24 Cruzeiro and A 2001 Argentina showed a wide cross reactivity against serotype A viruses circulating in South Korea, that was confirmed by challenge tests.

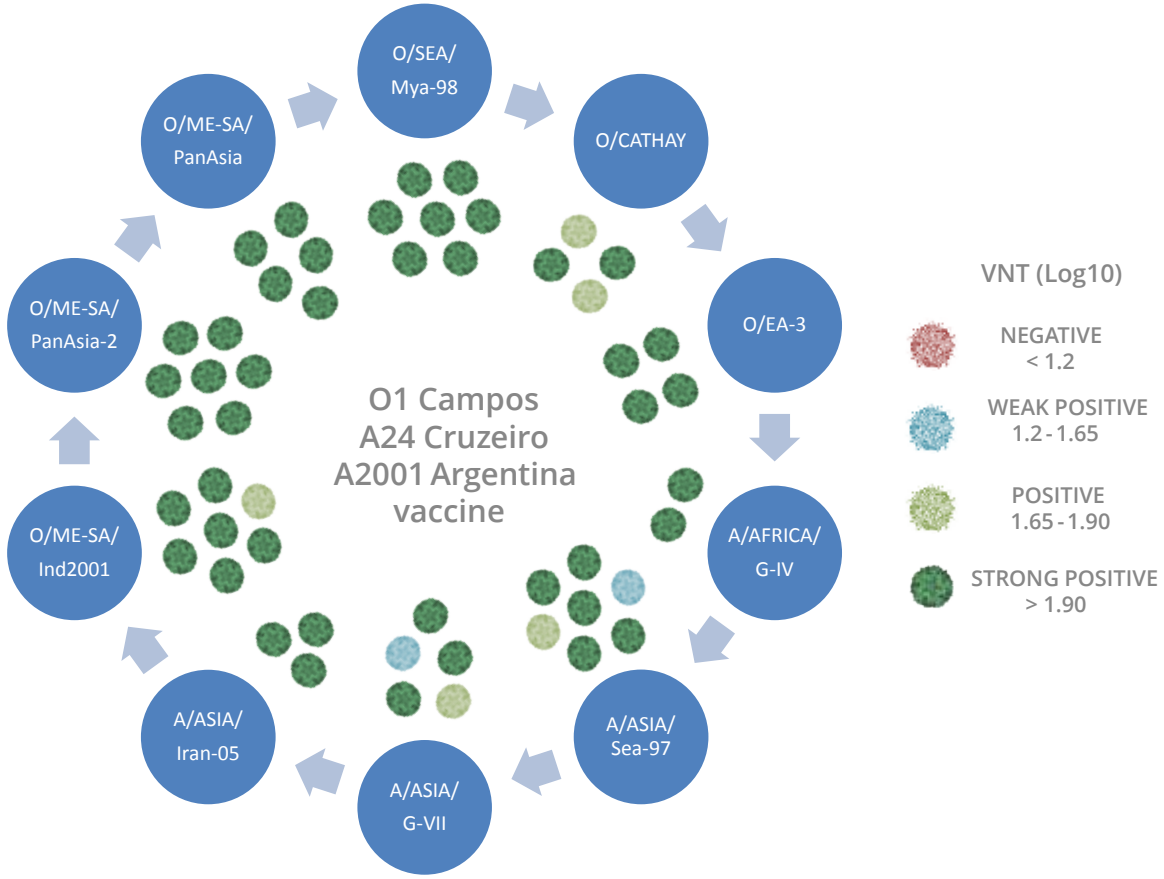
BIOAFTOGEN (O1 CAMPOS / A24 CRUZ / A 2001 ARG) HIGH POTENCY VACCINE CONFERS HETEROLOGOUS PROTECTION AGAINST A/SKR/4/2018 (GIMPO STRAIN- A/ASIA/SEA 97) IN PIGS



SENASA FMD OIE Ref Lab

Results of the challenge test in pigs at 28 dpv using an industrial Bioaftogen vaccine batch (O1 Campos, A24 Cruzeiro and A 2001 Argentina) against A/SKR/2018, Gimpo FMDV strain. Pigs were vaccinated by the intramuscular route with 2 mL/dose.

Vaccine matching against representative FMDV virus from topotypes and lineages that circulated in Asia and North Africa during last years. Each viral particle corresponds to a field isolate tested. This data is supported by determination of virus neutralization titers (VNT) 28 days after vaccination against the field viruses. Values greater than 1.65 were considered acceptable.

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Glossary

ADVENTITIOUS: Foreign agents that contaminate cells or virus seeds.

ADJUVANT: Substance that, when administered with an antigen, increases the immune response to it.

ANAPHYLACTIC SHOCK: Acute, immediate, systemic and serious reaction due to the action of certain organic substances when an organism is repeatedly exposed to the same substance.

ANTIGEN: Any foreign substance that can develop an immune response.

ANTIBODY: Substance produced in response to and counteracting a specific antigen.

BINARY ETHYLENEIMINE (BEI): First order inactivant that guarantees a quick and complete inactivation of the virus without altering its antigenic properties. It is an aziridine derivative.

BSE: Bovine Spongiform Encephalopathy. It is a degenerative disease of the central nervous system of cattle, and is characterised by neurological symptoms in adult animals that progressively worsen until death.

BIOSAFETY: Measures adopted to prevent spreading of infectious agents to the operator, the product or the environment.

BHK 21 CELLS: Cell line derived from baby hamster kidney cells, which are highly susceptible and allow replication of several viruses including FMD virus, rabies virus, etc. The cell lines are characterized for maintaining their original properties through successive passages.

INACTIVATION KINETICS: Study of the rate of virus inactivation.

EFFICACY: Specific capacity of the biological product to generate its desired effect when used under the conditions recommended by the manufacturer.

CONICET: National Council of Scientific and Technical Investigations (Consejo Nacional de Investigaciones Científicas y Técnicas). Ministry of Science, Technology and Innovation. National Government of Argentina.

ELISA: Enzyme-linked immunosorbent assay is a technique in which an immobilised antigen or antibody is detected with an antibody conjugated to an enzyme that generates a detectable product and is quantified with a spectrophotometer.

EMULSION: Liquid with a milky appearance that keeps an insoluble substance in suspension (oil) finely divided by an emulsifier.

EPP: Expected percentage of protection, predicted value of protection obtained by correlation tests between antibody levels of vaccinated cattle and protection data collected after the viral challenge.

SCALING UP: Combination of techniques that allows a small scale process up to achieve the required levels of production.

GENOMIC STUDY: Study of gene characteristics of a species/agent.

GMP: Good Manufacturing Practices.

HPLC: High Performance Liquid Chromatography. Equipment used to separate viral components according to their size.

INACTIVATION: Elimination of the infection capacity of a virus or other agents.

INFECTIOUS Titer: Method used to quantify a virus stock by using cells susceptible to infection.

INTA: National Institute of Agricultural Technology (Instituto Nacional de Tecnología Agropecuaria). Ministry of Agriculture, Livestock and Fisheries. National Government of Argentina.

ISO: International Organization for Standardization.

LAMINAR FLOW: It provides an aseptic area that protects the working environment from dust and other contaminants by maintaining a constant, unidirectional air flow over the area.

LPBE: Liquid phase blocking sandwich ELISA.

MUTATION: Change or alteration of inheritable and measurable genetic material.

NAFMDVB: North American Foot and Mouth Disease Vaccine Bank.

NSP: Non Structural Proteins of the virus, the proteins not associated to the viral capsid.

OIE: Office International des Epizooties. World Organization for Animal Health.

PD50: The 50% protective doses (PD50) analysis is the quantitative method for calculating the vaccine potency by challenge

PGP: Protection against generalization, a method used to directly determine the vaccine's efficacy, where vaccinated cattle or pigs are challenged with the virus.

POTENCY: Quantitative measure of the vaccine's capacity to induce protection.

SENASA: National Health and Agrifood Quality Service (Servicio Nacional de Sanidad y Calidad Agroalimentaria). Ministry of Agriculture, Livestock and Fisheries. Argentina.

RNA: Ribonucleic acid.

SEROEPIDEMIOLOGICAL SURVEYS: Assessment of specific antibodies by using statistical samplings in an animal population.

VNT: virus neutralization test. Serological study to assess neutralizing strain specific antibodies

Links

http://www.oie.int

http://www.fao.org/eufmd/en/

http://www.wrlfmd.org/

http://www.aphis.usda.gov/

http://www.ars.usda.gov/GFRA/reports.htm

https://www.ema.europa.eu/en

http://ec.europa.eu/food/index_en.htm

https://bioportal.ucdavis.edu/

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