



Foot-and-mouth disease vaccines from the Animal Sciences Group



Introduction

The Animal Sciences Group (Products Division) develops, produces, stores and delivers foot-and-mouth disease antigens and vaccines against different strains for emergency use. In the event of an emergency, millions of doses of mono or multivalent FMD vaccine can be produced from the stored antigen stocks and transported, delivered and distributed to the field within a few weeks. This enables a national authority to protect its livestock population at risk from spread of the disease, and protects trade, when according to the international regulations, the region at risk can be effectively controlled. Currently, ASG provides the FMD antigen banks and vaccines for the Netherlands, and delivers FMD vaccines worldwide.

The main activities are:

- developing new strains that may pose a threat to the cattle in the country,
- production and storage of millions of doses of antigen for each of 7 different FMD subtypes,
- capability to produce within a short period of time emergency FMD vaccines in a GMP formulation and filling unit.

Requirements for protection against FMD outbreaks

There are several pre requisites for FMD vaccine banks that must be fulfilled in order to provide sufficient protection.

1. new FMD strains that pose a threat for a country or region must be adapted for production purposes
2. sufficient amount of antigen must be manufactured and stored, necessary to produce sufficient FMD emergency vaccines to protect a region at risk
3. the quality, safety and efficacy of the vaccine must be proven according to (inter-) national registration requirements
4. the quality of the manufacturing process must be in compliance with internationally recognized GMP standards
5. formulation and filling facilities should be available to guarantee the production and supply of a large number of doses of the finished product within a few days after request

New FMD strains

The selection of new strains to be included in FMD vaccines, is usually based on the advice of (inter) national research institutes and by consultation of the World Reference Laboratory for FMD in Pirbright. ASG has close links with national FMD institutes and international organizations such as OIE, FAO and the world reference laboratory for FMD in Pirbright. The final choice for a specific strain is always made by the national authority. ASG has experience in the adaptation of new FMD field strains to BHK cells, in the production of seed lot systems (MSV and WSV) and in the testing of these new seed lots for the absence of extraneous agents.

Manufacturing

FMD virus is cultured on BHK suspension cells using a Seed Lot System in cGMP compliant facilities. Virus is cultured on BHK cells, inactivated using binary ethylene imine (BEI), purified, concentrated and stored at ultra low temperatures until formulation into FMD DOE vaccine. The purification steps incorporated in the downstream processing are designed to



separate complete FMD virus capsid (146S antigen) from proteins that have a significantly different molecular mass. Non-structural proteins (NSP) are significantly smaller than 146S antigen and are therefore removed. The vaccine is formulated by the primary emulsification of an aqueous solution of 146S antigen in special, light mineral oil, followed by a second emulsification of this water/oil emulsion in the outer aqueous phase. The resulting water/oil/water emulsion enhances the immunogenicity of the antigen and results also, in an ease to administer vaccine due to a low viscosity. Cedivac-FMD vaccines have a shelf life of 12 months when stored at 2-8°C.

Quality, Efficacy and Safety

Cedivac-FMD vaccines meet the requirements for quality, efficacy and safety as stated in European Pharmacopoeia monograph 04/2005:0063 Foot-And-Mouth Disease (Ruminants) Vaccine (Inactivated). Storing of emergency antigen stocks and performing potency tests on representative trial blends allows rapid release of 6 PD50 vaccines in the event of an emergency. This is in accordance with the Position Paper on Requirements for Vaccines against Foot-and-Mouth Disease EMEA/CVMP/775/02-final.

Products and Services of ASG



Cedivac-FMD DOE vaccine is registered in the Netherlands (REG NL 8265). The FMD vaccine is administered subcutaneously in the dew lap of cattle, subcutaneously behind the elbow of sheep and goats and intramuscularly in the neck of pigs. One dose consists of 2 ml but 1 ml is recommended for lambs, young goats and piglets. Cedivac-FMD is available in ultra-light vials of 50, 100 and 250 ml.

Cedivac-FMD vaccine properties

- ≥ 6 PD50 per dose of 2 ml, water-in-oil-in-water (DOE) emulsion
- Suitable for cattle, sheep, pigs and goats
- Monovalent or multivalent vaccines using inactivated, purified FMD antigens:
 - O1 Manisa
 - O1 BFS
 - O Taiwan 3/97
 - A Turkey 14/98 (A Iran 96 analogue)
 - A22 Iraq
 - A24 Cruzeiro
 - Asia1 Shamir
- A single dose confers at least 6 month duration of immunity
- Reduces virus transmission in vaccinated herds
- "Marker Vaccine" principle is subject of ongoing studies: based on the first results Cedivac-FMD allows the detection of FMD infection in vaccinated herds within 7 days.

ASG can deliver the following products and services. Each product or service can also be provided separately. All activities are performed in compliance with the GMP guidelines

Development

- Field Strain Selection
- Field strain adaptation to BHK cell
- Adventitious virus removal step by a solvent detergent treatment
- Production of a seed lot system (Master Seed Virus and Working Seed Virus)
- Scale up to fermentor level in homogenous BHK suspension cell culture
- Potency testing conform European Pharmacopoeia
- both potency by 50 per cent cattle protective dose (PD50) challenge studies but also routine batch potency tests by validated sero-neutralisation tests can be performed
- Complete testing of Master Seed Virus/ Working Seed Virus for absence of extraneous agents conform the registration requirements and European Pharmacopoeia

Antigen production

FMD antigens are manufactured in a dedicated cGMP High Containment Unit

- Cultivation of suspension cells, based on a Cell Bank System
- Inoculation of suspension cell culture with FMD virus, based on a Seed Lot System
- Cultivation of virus
- Removal of cellular material
- Inactivation; during inactivation, the virus titre is monitored by a sensitive and reproducible technique. The inactivation procedure is not satisfactory unless the decrease in virus titre, plotted logarithmically, is linear and extrapolation indicates that there is less than 1 virus particle per 10^4 litres at the end of the inactivation process.
- The inactivation process is validated for each individual antigen batch to safeguard complete inactivation and to guarantee that no live virus is present.
- Down Stream Processing
 - Concentration and purification of antigen
 - Sterile filtration and filling of the antigen in containers
- Quality Control
 - Antigen content (146S), sterility, safety and identity

Storage of antigen stock and replacement of stock

- Storage of FMD antigen containers at ultra low temperature
- Potency check
- Replacement of stock
- Full batch documentation
- Release certificate

Vaccine production/ supply of emergency vaccines

- Antigen selection (mono- or multivalent)
- Production of the internal and external aqueous phase and the oil phase (adjuvant preparation)
- Formulation of the Double Oil Emulsion (DOE) vaccine
- Automated filling in clean rooms
- Capping, labeling and packaging
- Transport

- ASG offers contract storage of dedicated emergency antigen stocks and guarantees rapid delivery of emergency vaccines.
- ASG is capable to formulate several hundreds of thousands of doses of FMD vaccine within a few days. Millions of doses can be made available within 1-2 weeks.
- On request of the customer technical support and training can form a part of the services supplied.

Biosafety and Quality

Biosafety as well as quality are critical factors in FMD vaccine production.

Biosafety

Both the up-stream and down-stream process of FMD virus (antigen production) requires a state of the art High Containment Unit to protect escape of live virus to the environment. The state of the art FMD production plant in Lelystad, is constructed according to the most modern technologies with a separate media production cell culture unit with positive air pressure, a separate virus culture unit with negative air pressure and a purification and concentration unit (down stream process unit) including a separate clean room for the sterile filtering and filling of concentrated antigen.

The virus culture unit is provided with double absolute HEPA filters, and access to the units via sealed air locks, is restricted to qualified personnel, exit by showering, and a strict quarantine system for all personnel that has worked in the units. Automated systems reduce costs and the number of personnel required in the production facilities, thus limiting the risk of in adventitious contamination of the environment.

FMD history

- 1929 Start of FMD research in the Netherlands, raising of the State Veterinary Institute (SVOI).
- 1930 Start with research on the in-vitro proliferation of FMD virus by dr. Frenkel.
- 1943 First vaccinations with inactivated vaccine in the Netherlands using the Waldmann method (vaccine from virus produced in live cattle).
- 1947 Successful use of tongue epithelium as growth substrate for the proliferation of FMD virus (Frenkel process).
- 1953 Start of large scale FMD vaccination in the Netherlands.
- 1959 Raising of the CDI (Central Veterinary Institute) in Amsterdam.
- 1967 Start with research on BHK cells as growth substrate for FMD proliferation
- 1972 Move to Lelystad, High Containment Unit.
- 1991 End of FMD vaccination in the Netherlands
- 1993 Commercial FMD vaccine production for Intervet BV
- 2003 FMD Emergency vaccine bank establishment for the Dutch Ministry of Agriculture

Arrangements for FMD vaccine banks

After a thorough risk assessment and prioritization process, countries should decide how they want to protect their livestock . ASG can offer development, antigen production, storage and supply of FMD emergency vaccines at high quality. A country can apply alone or in a consortium. Experts of ASG are available to customize the needs of a particular country, to safeguard the livestock against the devastating effects of an outbreak, using state-of-the-art technology.

Quality

The quality of the FMD antigen and the FMD manufacturing process is a key success factor of FMD vaccine from Lelystad. The FMD production unit and process is GMP certified; Standard Operation Procedures (SOPs), validated processes, maintained and calibrated equipment and extensive documentation and recording systems are in use during manufacturing.

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