TRIVALENT TICK FEVER VACCINE

TECHNICAL SPECIFICATIONS

Tick fever is a collective term used to describe diseases in cattle caused by *Babesia bovis*, *Babesia bigemina* or *Anaplasma marginale.* In Australia, these organisms are transmitted by the cattle tick *Boophilus microplus* and are therefore endemic over the range of this tick. These specifications describe the chilled trivalent tick fever vaccine produced by the Department of Agriculture Fisheries and Forestry (DAFF) at the Tick Fever Centre (TFC), Wacol, Brisbane.

The Tick Fever Centre (TFC) is licensed by the Australian Pesticides and Veterinary Medicines Authority (Licence Number 1018) to produce veterinary immunobiologicals and is committed to providing high quality efficacious vaccines. All vaccines produced at the Laboratory comply with the Australian Code of Good Manufacturing Practice for Veterinary Preparations.

1. Name and code of the product

Product Code	APVMA registration number	Name of product
TRV	49716	**Trivalent tick fever vaccine** (immunises against natural infections with *Babesia bovis, Babesia bigemina* and *Anaplasma marginale)*

2. Description of pharmaceutical form

The trivalent tick fever vaccine is used for control of tick fever (babesiosis and anaplasmosis) in cattle.
Vaccine contains live attenuated strains of tick fever organisms and provides immunity against natural tick fever infection. One vaccination usually provides life long protection. Full immunity to all parasites develops within 8 weeks of vaccination.

The vaccine is manufactured in liquid form and consists of parasitised bovine blood diluted with a cell-free diluent containing 10% bovine serum suspended in an isotonic balanced salt solution.

Parasitised blood is collected from calves artificially infected with attenuated laboratory strains of either *B bovis, B bigemina* or *A centrale* (a related organism which provides cross protection against infection with virulent *A marginale).* Vaccine donor calves are housed in insect-free, air conditioned facilities at TFC. The calves are infected by intravenous inoculation with one of the attenuated laboratory strains and monitored daily for development of parasitaemias by examination of Giemsa stained smears of jugular blood.

Blood from infected calves is collected aseptically when suitable parasitaemias are reached using a closed, sterile collection system. Parasitised blood is diluted with a sterile isotonic cell-free diluent to provide a standard number of parasites per dose.

2.1 Infective dose

Vaccine contains attenuated strains of tick fever organisms and provides immunity against natural tick fever infection. Each dose of vaccine contains: 1×10^7 *B bovis*, 1×10^7 *A centrale* and 2.5×10^6 *B bigemina* organisms.

2.2 Dose size

2 mL/animal.

Each 2 mL dose of trivalent vaccine contains:

(i) Mixed bovine blood containing *B bovis* & *A centrale & B bigemina.*
(10^7 organisms of *B bovis* and *A centrale* and 2. 5 x 10^6 of *B bigemina*).
Maximum blood volume = 1.20mL
Minimum blood volume = 0.03mL
Average blood volume = 0.26mL

(ii) Heparin = 0.5units

(iii) Antibiotics: benzylpenicillin = 1000 units
streptomycin sulphate = 1.0mg

(vi) Vaccine diluent (isotonic balanced salt solution containing 10% bovine serum) to 2ml.

Vaccine diluent formulation

Sodium Chloride (NaCl)	119.8 mM
Magnesium Chloride ($MgCl_2$)	1.5 mM
Glucose ($C_6 H_{12} O_6$)	5.6 mM
Disodium hydrogen orthophosphate (Na_2 HPO4)	17.8 mM
Potassium dihydrogen orthophosphate (KH_2 PO4)	5.1 mM
Sodium hydrogen carbonate (Na HCO_3)	6.1 mM
Benzylpenicillin	500,000 units/L
Streptomycin sulphate	500 mg/L
Sterile bovine serum	100 mL/L

2.3 Dosage form

Injectable aqueous suspension.

2.4 Route of administration

By subcutaneous or intramuscular inoculation (**not intravenous**).

3. Package details

3.1 Packaging specifications

Vaccine is dispensed aseptically into sterile polypropylene packs. A minimum 10% overfill above the stated dosage is included in each pack. This extra volume is allocated for priming of the vaccine injectors.

Pack size		
10 dose pack	20mL	30mL
20 dose pack	40mL	50mL
25 dose pack	50mL	60mL
50 dose pack	100mL	120mL
100 dose pack	200mL	220mL

The current packaging materials and methods have been developed to ensure the vaccine is maintained in a suitable cold chain environment during transit to assure the quality of the vaccine, and that the package is leak proof and strong enough to withstand normal transport handling.

- Vaccine is sealed in a leak-proof primary receptacle (a polypropylene pillow pack)
- The inner packaging is then sealed in a plastic bag (water tight secondary packaging) and polystyrene esky of adequate strength for its capacity, weight and intended use.
- Vaccine must be chilled during transport and 500g or 1000g chiller packs are used

  Small esky 2 x 500g chiller packs
  Large esky 2 x 1kg chiller packs

NB: Chiller packs removed from -5ºC storage must be placed in eskies at least 30 minutes before packing to equilibrate to 0 ºC to ensure vaccine is not frozen. See Cold chain specifications below.

- Each esky consists:
  One draw off tube for every two vaccine pillow packs
  Tick fever vaccine instruction leaflet or other instructional information
- Eskies are placed and sealed in cardboard boxes for dispatch by nominated air or road transport.

3.2 Cold chain specifications

Vaccine is maintained under 20^0C during manufacturing and dispensing process, dispatch and transport (Chilled to touch on arrival)

- Vaccine Starting Materials (Blood and diluent) kept refrigerated between 4-8°C and not to exceed 20°C during the production process
- Minimum temperature of dispensed vaccine = 0°C
- **Freezing point of vaccine is -0.54°C**
- Maximum temperature of vaccine packs after 24 hrs road transport = 20°C

4. Storage and shelf life of vaccine

Chilled vaccines must be refrigerated at 4^oC -8 ^{o}C during storage. (Refrigerate. Do not freeze).
Protect vaccine from the sun light
Chilled tick fever vaccines have a shelf life of four (4) days

Precautions

Freezing vaccine will make it ineffective

- Particular attention should be given to minimising the risk of freezing of the vaccine when repacking.
- Never expose the vaccine packs to chilling agents immediately after removal from -20°C or similar temperatures.

5. Sampling and testing

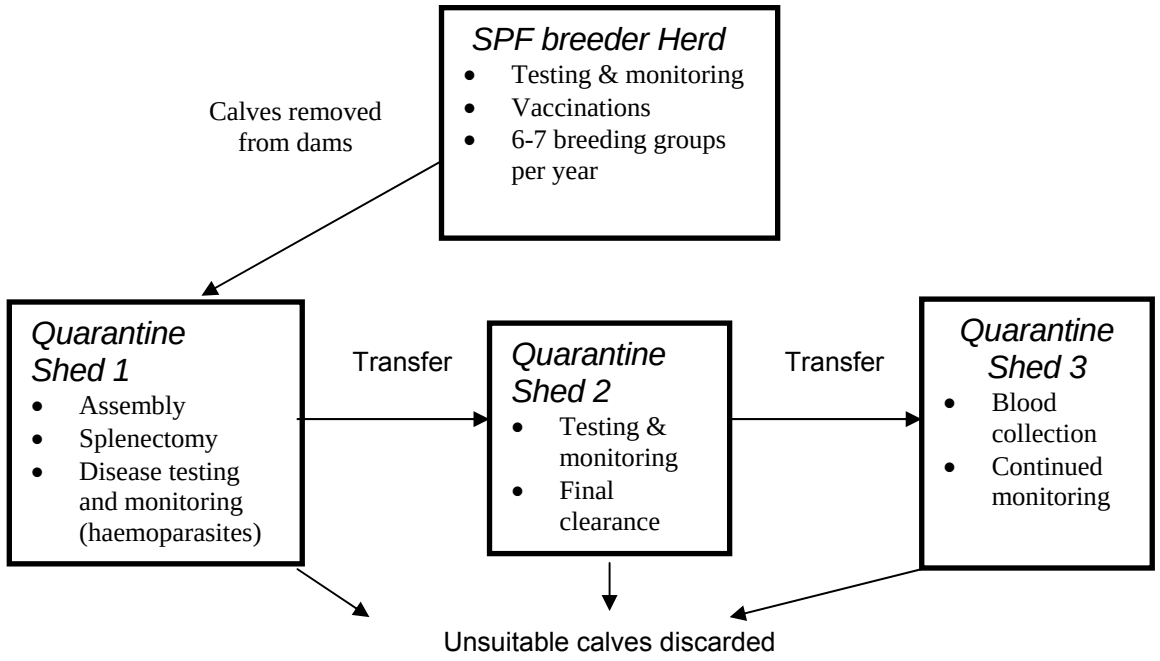
5.1 ***Calf Quarantine***

Calves from the SPF breeder herd are brought into Quarantine Shed 1 (QS1) at an early age. During the Quarantine period in QS 1, each calf is splenectomised to increase the animal's susceptibility to the vaccine organisms, and to induce post splenectomy relapses of any unwanted haemoparasites.

After all calves in a batch have been splenectomised, they are transferred to Quarantine Shed 2 (QS2). During this quarantine period, each calf is tested for specific infectious agents (Bovine Pestivirus, Bovine Lekaemia virus, Bovine immunodeficiency virus and Bovine syncytial virus -See attached Appendix 1). Arboviruses (eg Ephemeral fever, Bluetongue, Aino and Akabane viruses) are excluded by the use of insect-free accommodation. The calves must spend a full 6 weeks in QS2 to ensure freedom from arboviruses.

After this period of stringent quarantine and testing calves are cleared for transfer to Quarantine Shed 3 (QS3)

Calf Quarantine Flow Chart



Any calves showing evidence of infection are unsuitable for vaccine production and discarded. The entire batch of calves is discarded if Bovine Leukaemia Virus is diagnosed.

Animal housing
QS 2 and QS 3 are environmentally controlled, filtered air, insect-free facilities. QS 3 houses the vaccine donor calves. Only disease and parasite-free animals are allowed into the sheds and their nutrition and environment are carefully controlled. Access is limited to authorised personnel.

Testing program for vaccine donor calves used for tick fever vaccine production and References are attached in Appendix 1

Laboratory quality control procedures
In addition to the rigorous screening of donor calves for disease agents prior to their use for production, a comprehensive quality control program is maintained at each stage of vaccine production in the laboratory.

5.2 Vaccine Quality control in process tests and limits

Ingredient	Test type	Release limit/specification
Bovine Serum	Sterility: Membrane filtration for aerobic and anaerobic bacteria	No growth detected after 14 days at 35°C and at 22°C
	Viruses: Calf transmission test for Bovine Leucosis, Bovine Immunodefficiency, Bovine Spumavirus, Aino, Bovine Ephemeral Fever, Mucosal Disease, Bluetongue, Akabane Viruses	No rising antibody titre detected or no proviral RNA detected by PCR..
	Toxicity: *In vivo* infectivity test using trivalent chilled vaccine containing 10% serum	≥95% infectivity (by blood smear examination and/or serology for antibody)
Water for Injection	Conductivity Colony Forming Units (Membrane filtration)	< 1.1μS/cm @20ºC < 1cfu/10mL
Vaccine Diluent	Sterility: Membrane filtration for aerobic and anaerobic bacteria.	No growth detected after 14 days at 35°C and at 22°C.
	Integrity Test: Filtration System Osmolality pH	Integral to 3.2 bar 270 - 300 mOs/kg 7.1 – 7.4
Parasitised Blood	Direct Parasitaemia Count	*B bovis* ≥ 60 x 10^6/mL *A centrale* ≥ 30 x 10^6/mL *B bigemina* ≥ 10 x 10^6mL
	Microscopic confirmation of parasites.	Parasites observed in thin blood smears.
Final Product Vaccine	Sterility Test (Absence of microbes) TSB and FT incubation.	No growth detected after 14 days at 30°C and at 22°C.
	Annual Infectivity and Virulence Tests: Vaccination of susceptible *Bos taurus* steers.	≥95% infectivity (by blood smear examination and/or serology for antibody). Not more than 20% of animals treated for vaccine reactions
	Turbidity / particulate matter test on retention sample.	No turbidity or particulate matter after storage at 4°C for 60 days.

* NB All tests are performed on each batch except vaccine infectivity test which is performed annually.

5.3 Infectious diseases not found in Australia

The Australian Quarantine and Inspection Service (AQIS) has certified that the following infectious diseases are absent from Australia and pose no risk as contaminants of the tick fever vaccine:

Aujeszky's Disease
Bovine Brucellosis
Foot and Mouth Disease
Bluetongue (Clinical)
Bovine Spongiform Encephalopathy
Haemorrhagic Septicaemia
Lumpy Skin Disease
Rabies
Rift Valley Fever
Rinderpest
Contagious Bovine Pleuropneumonia
Heartwater
Jembrana Disease
Pathogenic *Theileria* spp.
Pathogenic *Trypanosoma* spp.
Vesicular Stomatitis

Use of vaccine

Full instructions and recommendations on use of the vaccine are provided with each order (leaflet).
Vaccination against tick fever is the only effective method of controlling losses from the disease.
The Queensland Government has supplied effective vaccines for tick fever since the early 1900's.
Further information can be obtained from the manufacturer.

Manufacturer

Department of Agriculture Fisheries and Forestry
Biosecurity Queensland
Tick Fever Centre
280 Grindle Road
Wacol Queensland 4076 Australia
Telephone: +61 7 3898 9655
Fax: +61 7 3898 9685
E-mail: tfc@daff.qld.gov.au
Visit www.biosecurity.qld.gov.au (search for 'tick fever')

Authorised by:……Title:……Date:……

Endorsed by:……Title:……Date:……

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Appendix 1 Testing programme for vaccine donor calves used for tick fever vaccine production

Agent	Test type	Calf quarantine testing	Test specifications	Test reference
Haemoparasites (general)	Thin blood smear	Weekly examination after splenectomy – QS2 Fortnightly – QS3	No organisms seen	(Bock et al., 2006)
B. bovis	ELISA	1 test - day 21-28 after entry to QS2	No specific antibodies detected	(Bock et al., 2008)
B. bigemina	ELISA	1 test - day 21-28 after entry to QS2	No specific antibodies detected	(Bock et al., 2008)
Anaplasma marginale	cELISA	1 test – day 21-28 after entry to QS2	No specific antibodies detected	(McElwain, 2008; Molloy et al., 1999)
Bovine leukaemia virus	ELISA	2 tests per calf 21-28 days apart	No detectable specific antibodies	(Beier and Vahlenkamp, 2008; Kirkland and Rodwell, 2005)
Bovine Pestivirus	PCR†	1 test - in week of entry to QS2	No viral RNA detected	(Corney, 2003)
Bovine immunodeficiency virus	PCR†	1 test - day 21-28 after entry to QS2	No proviral DNA detected	(Lew et al., 2004)
Bovine spumavirus (Bovine syncytial virus)	PCR†	1 test - day 21-28 after entry to QS2	No proviral DNA detected	(Lew et al., 2004)

† PCR for proviral DNA extracted from calf lymphocytes from each calf

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References for tests

Beier, D., Vahlenkamp, T.W., 2008, Enzootic Bovine Leukosis, In: Manual of standards for diagnostic tests and vaccines for terrestrial animals. Office International des Épizooties, Paris, pp. 729-738.

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Bock, R.E., Jorgensen, W.K., Molloy, J.B., 2008, Bovine babesiosis, In: Manual of standards for diagnostic tests and vaccines for terrestrial animals. Office International des Épizooties. , Paris, pp. 611-623.

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Lew, A.E., Bock, R.E., Miles, J., Cuttell, L.B., Steer, P., Nadin-Davis, S.A., 2004, Sensitive and specific detection of bovine immunodeficiency virus (BIV) and bovine spumavirus (BSV) by 5' Taq nuclease assays with fluorescent 3' minor groove binder-DNA probes (TaqMan-MGB). Journal of Virological Methods 116, 1-9.

McElwain, T.F., 2008, Bovine anaplasmosis, In: Manual of standards for diagnostic tests and vaccines for terrestrial animals. Office International des Épizooties., Paris, pp. 599-610.

Molloy, J.B., Bowles, P.M., Knowles, D.P., McElwain, T.F., Bock, R.E., Kingston, T.G., Blight, G.W., Dalgliesh, R.J., 1999, Comparison of a competitive inhibition ELISA and the card agglutination test for detection of antibodies to *Anaplasma marginale* and *Anaplasma centrale* in cattle. Australian Veterinary Journal 77, 245-249.