TICK FEVER CENTRE, WACOL	SPEC B

COMBAVAC 3 IN 1 LIVE 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 Combavac 3 in 1 live tick fever vaccine produced by the Queensland 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 products

Product code	APVMA registration number	Name of product
CON3I1	53629/0508	Combavac 3 in 1 concentrate
FRZ025	53629/25/0508	25-dose diluent pack for use with Combavac 3 in 1 concentrate
FRZ100	53629/100/0508	100-dose diluent pack for use with Combavac 3 in 1 concentrate

2. Description of pharmaceutical form

Combavac 3 in 1 live 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.

Combavac 3 in 1 is supplied as a two-part system: vaccine concentrate and diluent.

Vaccine concentrate: The vaccine concentrate consists of parasitised bovine blood diluted with an equal volume of 3M glycerol/phosphate buffered saline (PBS) solution containing glucose and antibiotics, which acts as a cryoprotectant. Five (5) mL volumes of vaccine concentrate are dispensed aseptically into sterile polypropylene cryotubes.

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.

Vaccine diluent: Vaccine concentrate is diluted with a sterile isotonic/isosmotic cell-free diluent to provide a minimum number of parasites per dose before use. The diluent consists of a 1.5M glycerol PBS solution containing glucose and antibiotics at the same concentrations used in the vaccine concentrate.

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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 a minimum of: 1 x 10^7 *B bovis*, 1 x 10^7 *A centrale* and 2.5 x 10^6 *B bigemina* organisms.

2.2 The formula

Vaccine concentrate

Bovine blood containing *B bovis*, *B bigemina* and *A centrale*	0.5mL/mL
Glycerol in PBS (Phosphate Buffered Saline)	1.5M
Glucose	5mM
Heparin	2.5 units/mL
Benzylpenicillin	500 units/mL
Streptomycin Sulphate	0.5 mg/mL

Vaccine diluent

Glycerol in PBS	1.5M
Glucose	5mM
Benzylpenicillin	500 units/mL
Streptomycin Sulphate	0.5 mg/mL

2.3 Dose size:

2 mL/animal.

Each 2 mL dose of vaccine contains:

(i) 0.1 mL Bovine blood containing *Babesia bovis, Babesia bigemina* and *Anaplasma centrale*
(ii) Heparin 0.5units
(iii) Antibiotics: Benzylpenicillin 1000 units
Streptomycin sulphate 1000 mg
(iv) Vaccine diluent (1.5M Glycerol in PBS plus glucose and antibiotics) to 2mL.

Infective dose overage (over the label claim for potency):
Each dose exceeds the minimum infective dose (based on infectivity test).

2.4 Dosage form:

Injectable aqueous suspension.

2.5 Route of administration:

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

3 Package details

Packaging specifications

The current packaging materials and methods have been developed to ensure the vaccine concentrate and diluent maintained in a suitable cold chain environment during transport to assure the quality of the vaccine

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Vaccine concentrate:
The Combavac 3 in 1 vaccine concentrate is provided in 5 mL sterile cryotubes and must be stored at minus 196°C in liquid nitrogen.
The Vaccine concentrate can be transported in:

- Liquid nitrogen tanks – containing liquid nitrogen -196°C
- Dry ice eskies- containing sufficient dry ice for transport -70°C
- Dry shippers- no liquid nitrogen in fluid form- 196°C

All recommendations must be followed for relevant transport recommendations and time limitations.

Vaccine diluent: Vaccine diluent is dispensed aseptically into sterile (gamma irradiated) polypropylene packs In two pack sizes

Volume	**Pack size**
50 mL	25 dose
200 mL	100 dose

The Combavac 3 in 1Diluent is refrigerated between 4-8°C during long term storage.
When in transport the diluent is routinely dispatched without cooling agent but it is recommended to refrigerate packs on arrival.
NB: It is important to store the diluent refrigerated between 4-8°C before preparation of the vaccine so its temperature matches the temperature of thawed concentrate of the vaccine.

Exception:

It is recommended to dispatch the diluent with chiller packs when accompanying concentrate vaccine transported in dry ice. (This will allow immediate vaccine preparation on arrival)

Supply of disposable items with each consignment

1. One draw off tube for every 2 packs of diluent (unless otherwise requested).
2. One 19 G x 2" needle with each pack of diluent (unless otherwise requested).
3. One instruction leaflet for each esky (unless otherwise requested) including "NOTE" with 100 dose packs.
4. One 65mL sterile yellow top specimen container for each 100 dose diluent pack.
5. One 20mL syringe for every two 100 doses diluent packs.
6. One 5mL syringe for every two 25 doses diluent packs

Preparation for use
Vaccine is prepared by adding the thawed concentrate to the diluent pack. Concentrate tubes are thawed at 37°C and the contents syringed into a diluent pack to provide vaccine ready for use.

For 1x25 dose diluent pack supply:	**1x 5 mL** Combavac 3 in 1 concentrate tube
For 1x100 dose diluent pack supply:	**4 x 5 mL** Combavac 3 in 1 concentrate tubes

4. Storage and shelf life

Vaccine concentrate: Vaccine concentrate is stored and transported frozen in liquid nitrogen at -196°C and has a shelf life of five (5) years.

Vaccine diluent: Vaccine diluent is stored refrigerated at 4°C and has a shelf life of two (2) years.

Prepared vaccine: Once diluted, prepared vaccine is kept cool (less then 20°C) and has a shelf life of eight (8) hours.

5. Sampling and testing

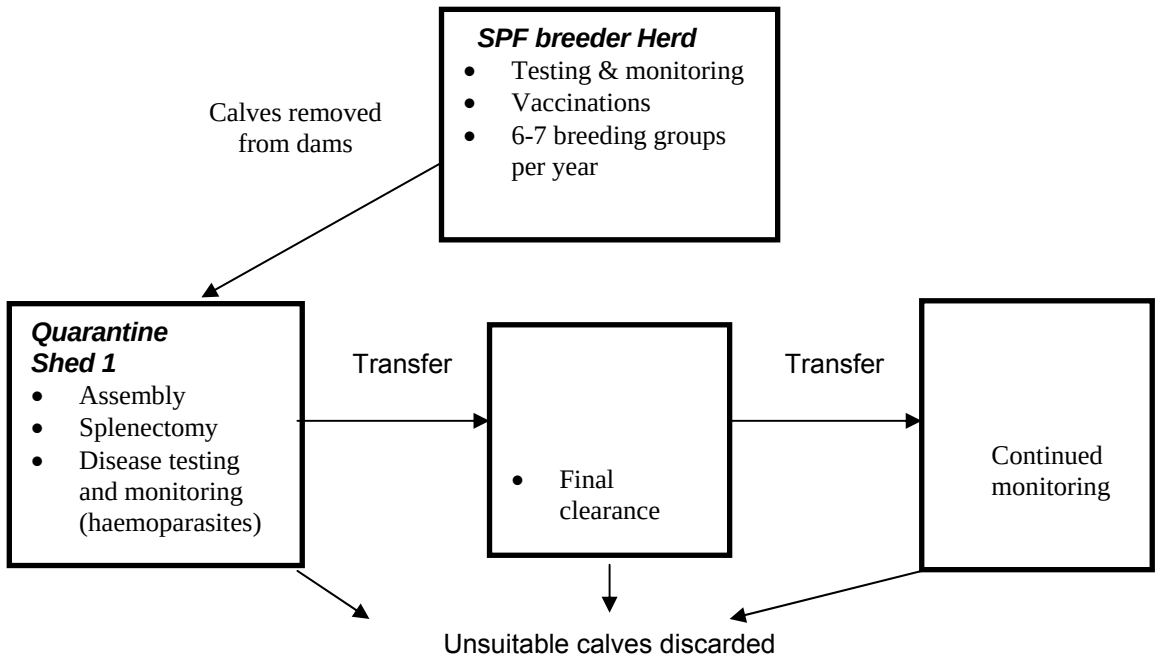
5.1 Calf Quarantine

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 (e.g. 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 BLV 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

5.2 Post production quality control

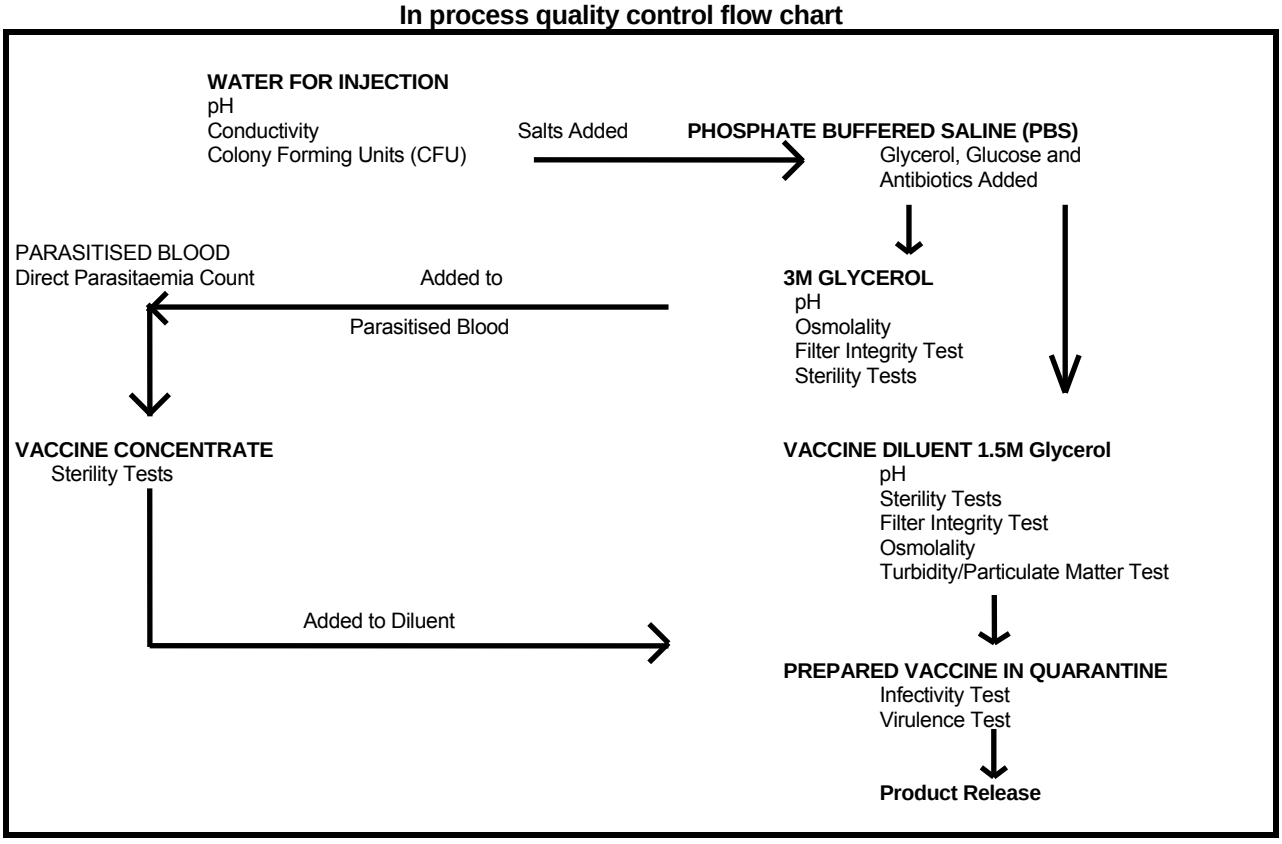
In addition to the rigorous screening of donor calves for disease agents prior to their use for production, calves used in production of Combavac 3 in 1 vaccine are tested for arboviral infections (Bovine ephemeral fever, akabane, aino and bluetongue) by paired serology from the day of collection and 2 weeks post collection. Additional post production monitoring and testing can be carried out on the product for specific disease agents if required by an importing country and if validated tests are available. Retention serum samples are collected from calves at the time of infected blood collection and 2 weeks later and stored for this purpose.

5.3 Post production testing of vaccine for infectivity and virulence

Each batch of vaccine is tested to ensure it is infective and avirulent prior to release from the laboratory. Vaccine concentrate is thawed, diluted 1 in 10 (the recommended dilution rate) and inoculated into susceptible 5-25 group of cattle. The cattle are then monitored for infection by testing of sera for the development of antibodies. Each batch of vaccine must pass the infectivity test before it is released to ensure that it is greater than 95% effective. A batch failing to produce this level of effectiveness is rejected.

5.4 Laboratory quality control procedures

In process quality control flow chart



During Quality Control testing, final product is held in quarantine until all testing is completed and results received by Quality Assurance Officer.

The evaluation of finished product and relevant documentation is concluded with release of "Batch Release Certificate" indicating batch clearance for sale. See Batch Release Specifications below

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Batch Release Specifications

Test	Document name	Record Viewed/ Approved by	Specification
Pre production testing of donor calves			
Haemoparasites general and specific	Vaccine Donor Calves Batch Result Record TFC Serological Results	Veterinary Officer Serologist	No organisms detected No specific antibodies detected
Bovine Leukaemia Virus (BLV)	Laboratory Report BSL	Veterinary Pathologist	No specific antibodies detected
Bovine immunodeficiency Virus (BIV)	Laboratory Report BSL	Vetrinary Pathologist	No viral DNA detected
Bovine sycytial virus (BSV)	Laboratory Report BSL	Veterinary Pathologist	No viral DNA detected
Bovine Pestivirus (MD)	Laboratory Report BSL	Veterinary Pathologist	No rise in specific antibody titres No viral antigen detected
Post production in vivo testing			
Infectivity	TFC vaccines infectivity and virulence results	QA/Veterinary Officer	Overall infectivity for tick fever greater than 95%
Virulence	TFC vaccines infectivity and virulence results	QA/Veterinary Officer	No more than one animal treated for vaccine reactions
Post Production testing of donor calves			
Aino virus (SNT)	Laboratory Report BSL	Veterinary Pathologist	No rise in antibody titre from day 0 to 14 post production
Akabane virus (SNT)	Laboratory Report BSL	Veterinary Pathologist	No rise in antibody titre from day 0 to 14 post production
Bluetongue (AGID)	Laboratory Report BSL	Veterinary Pathologist	No rise in antibody titre from day 0 to 14 post production
Bovine Ephemeral fever	Laboratory Report BSL	Veterinary Pathologist	No rise in antibody titre from day 0 to 14 post production
Documentation check			
3M Glycerol			
pH	Combavac cryoprotectant QC Record	QC /QA Officer	7.1-7.4
Osmolality	Combavac cryoprotectant QC Record	QC/QA Officer	320-380mmol/kg (1:10 dil)
Filter integrity	Combavac cryoprotectant QC Record	QC/QA Officer	Integral to 3.2 bar
Sterility	Combavac cryoprotectant QC Record	QCQA Officer	No growth detected after 48hrs and 35 days @ 35°C and 22˚C
Manufacturing process			
Parasitised blood - Direct Parasitaemia count	Batch manufacturing record	QA Officer	B bovis ≥100 x 10^6/mL A centrale≥100 x 10^6/mL B bigemina≥ 25 x 10^6/mL
Preparation	Batch manufacturing record	QA Officer	Record keeping complete
Dispensing	Batch manufacturing record	QA Officer	Record keeping complete Yield >95%
Volume check	Batch manufacturing record	QA Officer	5.0-5.4mL
Process- Freezing	Batch manufacturing record	QA Officer	Complete
Labelling	Combavac Batch Labelling Record	QA Officer	Correct label, Batch number and Expiry
Sterility – Combavac concentrate	Combavac QC Record	QC/QA Officer	No growth detected after 48hrs and 35 days @ 35°C and 22˚C
Osmolality	Combavac QC Record	QC/QA Officer	150-190mmol/kg (1:10 dil)
Organisms identity- microscopic examination	Combavac QC Record	QC/QA Officer	Relevant organisms confirmed
Vaccine diluent			
pH	Combavac Diluent Batch Release Certificate	QC/QA Officer	7.1-7.4
Osmolality	Combavac Diluent Batch Release Certificate	QC/QA Officer	1980-2060mmol/kg
Filter integrity	Combavac Diluent Batch Release Certificate	QC/QA Officer	Integral to 3.2bar
Sterility	Combavac Diluent Batch Release Certificate	QC/QA Officer	No growth detected after 48hrs and 35 days @ 35°C and 22˚C

5.5 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 these vaccines 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.gow.au (search for 'tick fever')

Authorised by:……Title:……Date:……

Endorsed by: ……Title:……Date:……

COMBAVAC 3 IN 1 LIVE TICK FEVER VACCINE

TECHNICAL SPECIFICATIONS

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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TECHNICAL SPECIFICATIONS

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.

Bock, R.E., de Vos, A.J., Molloy, J.B., 2006, Tick-borne diseases, In: Faragher, J.T. (Ed.) Australian New Zealand Standard Diagnostic Procedures. Subcommittee on Animal Health Laboratory Standards http://www.scahls.org.au/.

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.

Corney, B., 2003, Bovine Pestivirus PCR: Development and Validation - VIR-009., In: Animal Research Institute Laboratory Procedures Manual. Queensland Department of Primary Industries, Brisbane.

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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.

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