

USDA APHIS VETERINARY SERVICES STRATEGY & POLICY
PROTOCOL FOR THE IMPORTATION OF BOVINE SEMEN
FROM NEW ZEALAND

August 2008; Updated April 2018

This protocol describes the conditions required to import bovine semen according to regulations found in 9 CFR Part 98.

1. GENERAL REQUIREMENTS

- 1.1. The importer must obtain an import permit from the:

U.S. Department of Agriculture (USDA)
Animal and Plant Health Inspection Service (APHIS) Veterinary Services (VS),
Strategy & Policy (S&P) Unit 39, 4700 River Road
Riverdale, MD 20737-1231

Telephone: (301) 851-3300, option # 2
FAX: (301) 734-6402

The permit application form (VS 17-129, *Application for Import or In-Transit Permit*), may be obtained by writing or telephoning S&P, or by downloading it from the USDA APHIS web site: http://www.aphis.usda.gov/animal_health/permits/

Importers may submit applications via a dedicated email address:
VS.Live.Animals.Import.Permits@aphis.usda.gov.

Please refer to this information for form VS 17-129: [Instructions for Completing and Faxing a VS Form 17-129](#) and [Instructions for Completing and electronically Submitting a VS 17-129](#).

- 1.2. An official health certificate is required and must be issued by a veterinarian designated by the Ministry for Primary Industries (MPI), New Zealand, and must be endorsed by a MPI veterinarian attesting to the certifications and tests as required in this protocol. The health certificate must accompany the semen to the port of entry designated on the USDA import permit.
- 1.3. The semen must originate from a semen collection center (SCC) approved by MPI, and meeting the current criteria of the OIE Terrestrial Animal Code, Chapter 4.5 and 4.6. The Center Veterinarian of the MPI-approved center must supervise the collection and processing of the semen.
- 1.4. For donors collected on more than one occasion for this consignment, testing must be performed according to the criteria specified below for each disease and for each collecting period.
- 1.5. Donors must meet the current criteria listed in Section 3 to be eligible for importation to the United States, either as part of a 'herd' or 'resident herd'. For the purposes of this protocol, APHIS defines a 'herd' as any group of animals held together and isolated from other animals susceptible to ruminant diseases for at least 4 months prior to collection. A 'resident herd' is defined as a herd whose population may change over time, but where only animals of equal or higher health certification status compared to the rest of the herd

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may enter the group.

- 1.6. The tests on the donor sires (and teasers, if used) must be conducted at laboratories recognized by MPI as laboratories approved by the New Zealand National Association of Testing Authorities to conduct the tests.
- 1.7. Unless otherwise specified in Section 3 and 4 below, all assays must be performed according to the current criteria listed in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.

2. HEALTH CERTIFICATIONS to be included in the export health certificate (Note: the MPI veterinarian is responsible for verifying the attestations made by any others, such as a State Government Veterinary Authority, Center Veterinarian, or vendor, who may provide the information below.)

- 2.1. The health certificate must include the following information or statements:
 - 2.1.1. The identification of the donor sire by breed and registry number;
 - 2.1.2. The dates semen was collected for shipment to the United States;
 - 2.1.3. The dates, methods of testing and results of the tests on the donorsires;
 - 2.1.4. The name and address of the consignor and consignee;
 - 2.1.5. The name, address and approval number of the semen collection center where the semen was collected for this shipment;
 - 2.1.6. The number and identification of the straws of semen being shipped.
 - 2.1.7. The number of the official seal applied to the container.
- 2.2. New Zealand is free of Foot-and-Mouth Disease (FMD), Surra, and Contagious Bovine Pleuropneumonia, Bluetongue, Akabane, Aino, and Epizootic Hemorrhagic Disease(EHD), as listed in 9 CFR Part 94 and other official publications.
- 2.3. Cattle from New Zealand are exempt from brucellosis testing.
- 2.4. No cases of disease caused by Schmallenberg virus have been detected or reported in New Zealand.
- 2.5. The donor animals have been part of New Zealand's national herd for a minimum of 60 days prior to collection, with no quarantine or movement restrictions.
- 2.6. All bovine animals are admitted to the SCC herd only after a period of quarantine, isolation, and testing. All bovine animals admitted to the SCC herd must be proven free of tuberculosis, bovine genital campylobacteriosis, and trichomoniasis by testing methods

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recognized by the Office International Des Epizooties (OIE) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals as acceptable for international trade.

Note: specific pre-isolation TB testing is allowed on the farm or similar residence location if the donor bull is isolated for the testing and period before movement to the semen collection center.

- 2.7. The bovine animals admitted to the SCC herd must originate from herds that are officially free of bovine tuberculosis.
- 2.8. All bovine animals admitted to the SCC herd must be proven free of viremia to persistent bovine viral diarrhoea virus infection with negative results before entry into the SCC resident herd. Furthermore, all donor bulls are to be evaluated by a testing program to detect persistent testicular infection prior to semen release.
- 2.9. No clinical or other evidence of tuberculosis, leptospirosis, bovine genital campylobacteriosis, or trichomoniasis has been found in the SCC since the most recent herd test and prior to the export of semen for the United States.
- 2.10. During the 12 months prior to the collection of semen for export to the United States there has been no evidence of tuberculosis found in the donor bulls or on any premises on which the bulls were located during that time.
- 2.11. During the 60 days prior to the collection of semen for export to the United States, the donor bull(s) were not corralled, pastured, or held with animals of lesser health status or under any restrictions which would make them ineligible to provide semen for export to the United States.
- 2.12. The donor bulls were inspected at the time of semen collection for export to the United States and were found to be healthy and clinically free of diseases transmissible in semen.
- 2.13. On the date(s) semen was collected, there was no clinical evidence in the SCC herd of the diseases mentioned in Section 4 below.
- 2.14. During the 60 days prior to the collection of semen for export to the United States, no donor sire has been housed or otherwise had contact with other animals of lesser health status or under restrictions which would make it ineligible for importation to the United States under APHIS' regulations.

3. TESTING

3.1. MOUNT ANIMALS (applicable to all semen collections)

- 3.1.1. Mount animals used during semen collection must be submitted to the same regimen of periodic health tests as bulls in semen production and be maintained continuously in a health testing status equivalent to the bulls.

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3.1.2. Mount animals may not be interchanged between the resident herd and the isolation testing environments.

3.1.3. Areas of contact by the erect penis or of genital secretions upon the hair coat or skin of a mount must be effectively and thoroughly disinfected between successively mounting bulls.

3.2. SEMEN COLLECTION USING DONORS AS PART OF A HERD

3.2.1. Testing prior to entering a collection facility.

3.2.1.1. Bulls and mount animals intended to enter an MPI-approved AI Center shall be healthy and free of infectious or contagious diseases and may not originate from a herd under quarantine. Subsequent to completion of the pre-entry testing (described as above and in section 4), the bulls and mount animals used for collection may not be used for natural service and must be isolated from other cattle. Isolation means no direct contact or fence line contact with other cattle.

3.2.1.2. The pre-entry examination and diagnostic tests must be conducted and results received for each bull and mount animal prior to commencing the isolation interval. These tests should preferably be conducted prior to arrival at the isolation facilities of the AI Center. However, these tests may be conducted in a separate facility at the AI Center, as described below, but the animal isolation interval may not commence until results of the pre-entry tests are known.

3.2.1.3. For purposes of these requirements, pre-entry testing performed at the AI Center means bulls and mount animals were housed in a pre-isolation facility effectively separated from facilities occupied by resident bulls and mount animals, and also separate from bulls and mount animals housed in isolation facilities. Any equipment used to handle bulls and mount animals for semen collection, feeding, watering, and cleaning in isolation or resident herds was used at the pre-isolation facility.

3.2.1.4. Physical Examination: A physical examination must be conducted by an accredited veterinarian within 30 days prior to entry to determine that the bulls or mount animal do not display any clinical symptoms of any infectious, contagious disease.

3.2.1.5. Testing certifications (prior to entering an SCC) for donors that are part of a herd [Note: these statements must be included on the export health certificate].

3.2.2. Testing during isolation in an SCC prior to semen collection (for donors that are part of a herd)

3.2.2.1. For purposes of these requirements, isolation in an SCC means the bulls and

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mount animals are housed in facilities under the control (supervision) of the Center Veterinarian. These facilities must be effectively separated from facilities occupied by resident bulls and mount animals and all equipment used to handle the bulls and mount animals for semen collection, feeding and watering, and cleaning the facilities occupied by the bull or mount animal may not be used for both isolation and resident herds. Semen collection areas for bulls in isolation must be effectively separated from areas used for resident bulls.

3.2.2.2. Testing certifications [Note: these statements must appear on the export health certificate; where testing options are presented, the certifying official should retain the applicable option and strike out non-applicable options].

3.2.2.3. Each bull and mount animal was held in isolation throughout the period of time necessary to conduct the tests listed below. Each bull and mount animal successfully completed the isolation protocol before being permitted to enter the facilities occupied by resident bulls and mount animals and before any semen from the bull was released for use.

3.2.3. Resident herd bulls temporarily taken out of semen production and held at another location must be maintained in a herd of equal health status to the resident herd from which the bull originated, and must be re-tested for bovine trichomoniasis and bovine campylobacteriosis when re-joining the resident herd. The routine testing regimen (as defined for the resident herd) must be resumed prior to the release of semen that was processed after the bull's return to production.

3.2.4. All bulls or mount animals in the resident herd must be maintained in continuous isolation from all animals susceptible to ruminant diseases that have not completed all of the test procedures outlined herein with negative results. At any time that an individual bull or mount animal from the resident tested herd is permitted contact with an untested animal, he must be removed immediately from the resident tested herd and not be permitted re-entry until such time as he has completed another cycle of isolation and the tests prescribed.

4. TESTS

4.1. All bulls and mount animals in the center must be tested to qualify for admittance into the center and once in the center, the animals were tested, with negative results, for the following diseases within a 12 month period prior to the collection of the semen for export to the USA. Test results must be recorded on the health certificate.

4.2. Tuberculosis: Intradermal TB test as prescribed in New Zealand's Pest Management Strategy For Bovine Tuberculosis. Caudal Fold Tuberculin test (CFT) - The intradermal injection of 0.1 ml of bovine purified protein derivative (PPD) tuberculin (1 mg/mL PPD) into either side of the caudal fold with reading by visual observation and palpation 72 hours (plus or minus 6 hours) following injection. A negative test result is the lack of a response that can be seen or palpated. This test must not be conducted within 60 days of any previous

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tuberculin test.

Note: specific pre-isolation TB testing is allowed on the farm or similar residence location if the donor bull is isolated for the testing and period before movement to the semen collection center.

- 4.3. Bovine genital campylobacteriosis: PCR or culture of preputial smegma with negative results.
- 4.4. Trichomoniasis: PCR or a microscopic examination and culture of preputial smegma with negative results.

5. PROCESSING CERTIFICATIONS (to be included on the export health certificate)

- 5.1. The semen was collected and processed under the direction of the Center Veterinarian and placed in individual straws that are permanently marked with the name of the donor, the donor's registration number, and the date of collection. (This information must be recorded on the health certificate, and may be coded provided that a key to the code accompanies the import permit and health certificates.)
- 5.2. Semen collection equipment which comes into contact with bulls, or their secretions and excretions, shall be thoroughly disinfected after each use. Good laboratory practices shall be followed during collection and processing of semen in order to minimize the possible introduction of microbial contamination.
- 5.3. Ruminant products used in commercial semen extenders must be sourced from countries considered by USDA as free from FMD and rinderpest, as listed in 9 CFR Part 94 and other official publications: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-and-animal-product-import-information/import-live-animals/ct_foot_and_mouth_and_rinderpest
- 5.4. Antibiotics shall be added to neat semen and extender in amounts and combinations approved by APHIS [Note: an example of an approved antibiotic combination is one in which the concentration of antibiotics at the time of initial extension of the semen is: 100 ug/ml tylosin, 500 ug/ml gentamycin, 300 ug/ml lincomycin, and 600 ug/ml spectinomycin and whose final concentration in the processed and frozen semen is : 50 ug/ml tylosin, 250 ug/ml gentamycin, 150 ug/ml lincomycin, and 300 ug/ml spectinomycin for a two-step extender and processing or whose final concentration in the processed and frozen semen is : 100 ug/ml tylosin, 500 ug/ml gentamycin, 300 ug/ml lincomycin, and 600 ug/ml spectinomycin for a one step extender and processing. For further details, see **Appendix I**.
- 5.5. Only virgin liquid nitrogen will be used to export semen to the United States. No biological products other than frozen semen qualified for shipment to the USA may be shipped in the containers.
- 5.6. Prior to being used for exporting semen to the United States, the semen shipping container was examined by the Center Veterinarian and found empty of semen and any other biological material; and is either new or has been cleaned and disinfected.

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- 5.7. The semen was maintained securely in the custody of the Center Veterinarian until it was placed in the shipping container and sealed with Government of New Zealand seals. (The seal numbers must be recorded on the health certificate.)
 - 5.8. The semen must be stored under lock and key or in the custody of the center veterinarian, and segregated from other semen of lesser health status, until it is placed in the shipping container and sealed with official seals of New Zealand. The seal numbers must be recorded on the health certificate.
 - 5.9. It is acceptable for semen from different approved SCC units in New Zealand to be included in a single shipment, as long as the official veterinarian can certify the integrity of the total shipment, and continuity of storage conditions. MPI must certify that none of the semen for export to the USA has been stored or transported in containers with semen produced under less than equivalent animal health conditions
 - 5.10. Semen must be routed directly to the United States from New Zealand with no stops en route other than those provided on the USDA APHIS import permit.
- 6. ADDITIONAL REQUIREMENTS:** Importers are advised that individual states may have stricter requirements than USDA APHIS. It is the importer's responsibility to verify these conditions and to meet them. The importer should contact the U.S. State veterinarian ([State Regulations and Import Requirements](#)) of the destination state to determine the requirements.
- 7. ARRIVAL AND INSPECTION AT THE PORT OF ENTRY**
- 7.1. The shipment must be routed directly to the United States from with no stops en route other than those provided on the USDA import permit. This shipment may not transit a region considered by USDA APHIS to have foot and mouth disease (FMD) as noted on the USDA APHIS webpage: (<https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-and-animal-product-import-information/animal-health-status-of-regions>).
 - 7.2. On arrival at the port of entry the importer or the importer's agent must present the USDA port veterinarian with the original health certificate and the original import permit for the semen.
 - 7.3. All shipping containers and all the straws or ampules containing semen must be made available to the USDA port veterinarian for inspection at the port of entry. The shipment may not be removed from the port of entry until the inspector determines that the semen is eligible for importation in accordance with this protocol and releases the shipment.
 - 7.4. The shipping containers must be sealed with an approved seal from MPI, and that seal must be intact upon the shipping container arriving in the United States. If the seal is broken the shipping container will automatically be refused entry into the United States and returned to the exporting country or destroyed. The seal number must be recorded on the health certificate.

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8. SEMEN SHIPMENTS REFUSED ENTRY

If any semen shipments are determined to be ineligible for importation into the United States upon arrival at the port of entry, the importer must remove such shipments from the United States within 30 days, or the shipment will be destroyed.

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APPENDIX I ANTIBIOTICS AND SEMEN PROCESSING

1. Antibiotics should be added to the neat semen and extender according to the specifications in this section in order to provide effective microbiological control of: Mycoplasmas, Ureaplasmas, *Haemophilus somnus*, *Campylobacter fetus* subsp. *venerealis*
2. Effective microbiological control is the condition in which the number of organisms potentially present is reduced to below the threshold of infectivity.
3. An acceptable protocol is the treatment of semen and extender with the antibiotics gentamicin, tylosin, lincomycin and spectinomycin (GTLS) as. Synopsized in these appendices.
4. Acceptable alternative protocols must provide effective microbiological control of the organisms in point
1 above based on scientific evidence that should be submitted to and approved by APHIS prior to use in semen exported to the United States. An example of an approved alternative protocol is the 1-step procedure described in **Section II** of **Appendix I**.

ANTIBIOTIC PROCEDURES/CONDITIONS SECTION I

A. Antibiotics/Stock Solutions

- (1) Antibiotics:
 - a) Gentamicin sulfate: powder, micronized, non-sterile, U.S.P. (veterinary grade), 100 grams per bottle.
 - b) Tylosin: labeled as Tylan Soluble, product of Elanco Products Company, 100 grams per bottle.
 - c) Linco-Spectin: product of the Upjohn Company, 20 ml per vial, each ml contains 50 mg lincomycin and 100 mg spectinomycin.

NOTE: Antibiotics obtained from some sources have not been tested and may contain deleterious agents that may harm or kill sperm cells. For recommended sources, contact APHIS.

- (2) Stock solutions of individual antibiotics (gentamicin and tylosin) may be prepared and stored separately at 5°C for eight days or stored frozen in LN vapor for up to six months. Linco-Spectin as supplied by distributor should be maintained at 5°C after it is opened.
- (3) Stock solutions of individual antibiotics will be combined on day of use, and not held over.
- (4) Extenders must be used on the day the combined antibiotics are added.

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B. Neat Semen Treatment

- (1) 100 µg of tylosin, 500 µg gentamicin and 300/600 µg of Linco-Spectin dissolved in .02 ml of double distilled sterile water will be added and carefully mixed with each ml of neat semen.

NOTE: All of the antibiotic concentrations expressed herein are for active units of antibiotic. Potency values may vary between batches of antibiotic. Therefore, amounts of raw material have to be adjusted for each batch in order to obtain the required concentrations of active antibiotic.

- (2) The addition of these antibiotics should be scheduled so as to allow a three to five minute time period for the antibiotics to be in contact with the neat semen before the addition of any extender.

C. Non-Glycerol Fraction of Extender

- (1) All non-glycerol fractions of any of the five extenders listed below will be prepared to contain the following concentrations of antibiotics before being added to semen:

Tylosin 100 µg per ml Gentamicin 500 µg per ml Linco-Spectin 300/600 µg per ml

- (2) A volume of this extender (up to 50 percent of the planned final extended volume) is added to the neat semen prior to cooling. All semen must be held in contact with the non-glycerol extender for a minimum of two hours prior to the addition of any glycerol containing extender.

D. Glycerol Containing Fraction of Extender

- (1) This fraction of the extender may contain 5-10 percent of the antibiotic concentration listed under C.
 - Non-Glycerol Fraction of Extender.
 - The glycerol fraction of the extender should be added to the non-glycerol fraction of extender plus semen at a 1 to 1 ratio.

E. Final Concentration of Antibiotics

Following the above procedures will yield a final concentration of 50 µg tylosin, 250 µg gentamicin and 150/300 µg of Linco-Spectin in each ml of frozen semen.

F. Required Processing Procedures

It has been shown that processing procedures, extender composition, and antibiotic combinations may affect efficacy of microbial control or fertility. Therefore, deviation from the following may require additional efficacy testing:

- (1) Use of extender other than that specified in this appendix.

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- (2) Antibiotic/neat semen contact of less than three minutes.
- (3) Cooling of semen and non-glycerol fraction less than two hours to 5°C.
- (4) Glycerol is not used as an extender component until after cooling to 5°C.

G. Tested and Approved Extenders

The following five extenders have been tested for efficacy of control of microbial organisms. Use of the antibiotic combination in extenders 1 and 3 did not adversely affect post-thaw motility or fertility (extenders 2, 4, and 5 were not evaluated). Other extenders may be approved by APHIS. Antibiotics dissolved in double distilled sterile water should be included in the preparation of extenders to yield the final volumes shown under **Section I, E** of **Appendix I**. The final composition of each extender is as follows:

- (1) Egg Yolk Citrate 20% Egg yolk
2.12 gm % sodium citrate dihydrate
0.183 gm % citric acid monohydrate 7.0% glycerol
- (2) 20% Egg Yolk-Tris 20% egg yolk
2.42 gm % tris (hydroxymethyl aminomethane)
1.38 gm % citric acid monohydrate
1.0 gm % fructose 7.0% glycerol
- (3) Heated Whole Milk 7.0% glycerol
- (4) Plus-X
Plus-X, as supplied by distributor. 7.0% glycerol
- (5) 28% Egg Yolk-Tris 28% egg yolk
1.92 gm % tris (hydroxymethyl aminomethane)
1.10 gm % citric acid monohydrate
1.00 gm % glucose 7.0% glycerol

SECTION II: Alternative One-Step Method

I. General Description

This processing protocol is approved only for 20% Egg Yolk Tris extender (see Section I, H, 2 of **Appendix I**). It requires the same preparation of antibiotics/stock solutions (see **Section I, A** of **Appendix I**); and neat semen treatment (see **Section I, B** of **Appendix I**) as the standard 2-step protocol. However the main differences from a 2-step protocol are as follows:

- (1) The extender is not fractionated into a non-glycerol and glycerol component. The complete

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extender contains 7.0% glycerol.

- (2) The concentration of GTLS antibiotics in each ml of extender is the same as that prescribed for neat semen treatment (i.e., 100 µg tylosin, 500 µg gentamicin, 300/600 µg Linco-Spectin. Thus the final concentration of antibiotics is essentially doubled compared to the standard 2-step protocol.

II. Neat Semen Treatment

Identical to that for the standard 2-step protocol. See Section I, B, 1 and 2 of **Appendix I**.

III. Final Concentration of Antibiotics

The 1-step protocol will yield a final concentration of 100 µg tylosin, 500 µg gentamicin, and 300/600 µg of Linco-Spectin in each ml of frozen semen.

IV. Required Processing Procedures

It has been shown that processing procedures, extender composition, and antibiotic combinations may affect efficacy of microbial control or fertility. Therefore, deviation from the following may require the organization to conduct additional efficacy testing:

- (1) Use of extender other than one listed in this appendix.
- (2) Antibiotic/neat semen contact of less than three minutes.