

**USDA APHIS VETERINARY SERVICES,
STRATEGY & POLICY PROTOCOL FOR THE IMPORTATION
OF BOVINE SEMEN FROM AUSTRALIA**

July 2012, Update Oct 2019

This protocol describes the conditions required to import semen from domestic bovines (*Bos taurus*, *Bos indicus*, *Bison bison*) 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)
4700 River Road, Unit 39
Riverdale, MD 20737-1231

Telephone: **(301) 851-3300, Option 2**
Fax: **(301) 734-4704**

Web Site: [APHIS Imports](#)

The application, **VS Form 17-129, “Application for Import or in Transit Permit,”** may be obtained by writing or telephoning S&P, or by downloading it from the APHIS web site: [Animal Health Permits](#)

1.2. An official health certificate is required. The official health certificate must be issued and endorsed by a full-time, salaried veterinarian employed by the Australian Department of Agriculture. The health certificate must accompany the semen to the port of entry designated on the USDA import permit.

1.3. The health certificate must contain:

1.3.1. The identification of the donor sire by breed and registry number;

1.3.2. The dates on which semen was collected for shipment to the United States;

1.3.3. The dates and methods of testing and results of the tests on the donor sires;

1.3.4. The name and address of the consignor and consignee;

1.3.5. The name and address of the semen collection center (SCC) where the semen was collected for this shipment;

1.3.6. The certification statements as designated below.

1.4. The semen must originate from a semen collection center (SCC) approved by the Australian Dept. of Agriculture, and meeting the current criteria of the OIE Terrestrial

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Animal Code, Chapter 4.5. The Center Veterinarian of the approved SCC must supervise the collection and processing of the semen.

- 1.5. - 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.
 - 1.6. 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 may enter the group.
 - 1.7. The tests on the donor sires (and teasers, if used) must be conducted at laboratories recognized by the Dept. of Agriculture as laboratories approved by the Australia National Association of Testing Authorities to conduct the tests.
 - 1.8. Unless otherwise specified in Section 3 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 Dept. of Agriculture 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. Australia is free of foot-and-mouth disease (FMD), surra, and contagious bovine pleuropneumonia.
 - 2.2. No cases of disease caused by Schmallenberg virus have been detected or reported in Australia.
 - 2.3. The donor animals have been part of Australia's national herd for a minimum of 60 days prior to collection, with no quarantine or movement restrictions.
 - 2.4. Paragraphs 2.4.1 and 2.2.2 describe how the United States defines "part of the national herd" and the length of time the animals must be part of the national herd.
 - 2.4.1. If the animals were imported from countries recognized by USDA as free of FMD, then these animals must have been free of any import quarantine restrictions and able to move freely within Australia's national herd for a minimum of 60 days prior to entering the SCC.
 - 2.4.2. If the animals are offspring of animals imported from a country not recognized by

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USDA as free of FMD, then the animals for export must have been free of any import quarantine restrictions and able to move freely within Australia's national herd for a minimum of 90 days prior to entering the SCC.

- 2.5. On the date(s) semen was collected, there was no clinical evidence in the SCC herd of the diseases mentioned in Section 3 below.
- 2.6. 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 (teasers) 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.
- 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 thoroughly and effectively disinfected between successively mounting bulls.

3.2. SEMEN COLLECTION USING DONORS THAT ARE PART OF A HERD

3.2.1. Testing prior to entering a collection facility

- 3.2.1.1. Bulls and mount animals that are intended to enter a government - 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 below, 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 following 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 SCC. However, these tests may be conducted in a separate facility at the SCC, as described below, but the animal isolation interval may not commence until results of the pre-entry tests are known.

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3.2.1.3. For purposes of these requirements, pre-entry testing performed at the SCC means the 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]:

3.2.1.5.1. Tuberculosis: The donor sires originated from a herd that tested negative to a whole herd test for tuberculosis within 1 year prior to the date of collection for export to the United States and the donor has a negative individual test performed a minimum of 60 days after the whole herd test, and prior to collection. (A negative test means no detectable response using both visual examination and palpation when read 72 hours following injection with intradermal tuberculin.) This test was performed by a veterinarian designated by the Dept. of Agriculture.

3.2.1.5.2. Bovine Leptospirosis: A blood test for serotypes *L. pomona*, *L. hardjo*, *L. canicola*, *L. icterohaemorrhagiae*, and *L. grippotyphosa* was conducted within 30 days prior to entry. Any animal with a significant titer was subjected to a second blood test within two to four weeks after the first. An end or limiting titer (1:100 or greater) was run on both samples. [Note: only cattle with a stabilized low titer (negative at 1:400) on both tests may be considered satisfactory to enter the isolation facility.]

3.2.1.5.3. Bovine Viral Diarrhea Virus (BVDV): A blood test for BVDV with negative results was conducted within 30 days prior to entry. The test for BVDV was either a viral isolation test of whole blood or serum performed in bovine cell culture followed by staining of the cell culture by immunofluorescence (IFA) or immunoperoxidase (IP) methods, **OR** an antigen capture ELISA, **OR** a PCR test.

3.2.2. **Testing during isolation in an SCC prior to semen collection (for donors that**

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are part of a herd)

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

NOTE: 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. Arthropod-vectored viruses

- 3.2.3.1. The donor sires **EITHER** were kept in a Dept. of Agriculture.-approved semen collection center located in a region certified free from bluetongue, bovine ephemeral fever, Akabane, and Aino for a minimum of 60 consecutive days before commencement of, and during, collection of the semen for export. [Note: the dates of this period of residency must be included on the export health certificate.]
- 3.2.3.2. **OR** were tested on two occasions, the first test occurring within 180 days prior to collection, and the second test between 30 and 180 days after collection of semen, using the following tests:
- 3.2.3.2.1. Akabane - Negative to a serum neutralization test at 1:4 serum dilution.
- 3.2.3.2.2. Bluetongue - Negative to an agar gel immunodiffusion (AGID) test or the competitive ELISA (cELISA) test.
- 3.2.3.2.3. Aino - Negative to a serum neutralization test at a 1:10 serum dilution.
- 3.2.3.2.4. Bovine ephemeral fever - Negative to a serum neutralization test at a 1:10 serum dilution.

3.2.4. Other disease testing

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- 3.2.4.1. Tuberculosis: The donor sires originated from a herd that tested negative to a whole herd test for bovine tuberculosis within 1 year prior to the date of collection for export to the United States, and the donor has a negative individual bovine TB test performed a minimum of 60 days after the whole herd test, and prior to collection. (A negative test means no detectable response using both visual examination and palpation when read 72 hours following injection with intradermal tuberculin). This test was performed by a veterinarian designated by the Department of Agriculture.
- 3.2.4.2. Bovine Leptospirosis: within 180 days prior to collection, and again between 30 and 180 days after collection, all donors tested negative (at 1:400 dilution) by serology for the 5 serotypes of importance in the USA (*L. pomona*, *L. hardjo*, *L. canicola*, *L. icterohaemorrhagiae*, *L. grippotyphosa*).
- 3.2.4.3. *Campylobacter fetus* ssp. *venerealis*: within 30 days prior to collection, and again between 30 and 180 days after collection, all donors tested negative by culture of preputial smegma for bovine genital campylobacteriosis.
- 3.2.4.4. Bovine Venereal Trichomoniasis: within 30 days prior to collection, and again between 30 and 180 days after collection, all donors tested negative by a microscopic examination of a culture of preputial smegma for trichomoniasis.
- 3.2.4.5. Bovine Viral Diarrhea/Mucosal Disease (BVD-MD): All donors tested negative by a viral isolation test of whole blood or serum performed in bovine cell culture followed by staining of the cell culture by immunofluorescence (FA) or immunoperoxidase (IP) methods.
- 3.2.4.6. The following test methods and schedules are to be used to test for persistent BVD viremic infection.
 - 3.2.4.6.1. Diagnostic test: The animal must be subjected to a PCR test on whole blood **OR** a virus isolation test with one pass performed in bovine cell culture with a negative result as demonstrated by staining of the cell culture by immunofluorescence (FA) or immunoperoxidase (IP) methods.
 - 3.2.4.6.2. Diagnostic specimens: PCR test on whole blood. Virus isolation on **EITHER** whole blood **OR** serum, but whole blood must be used for animals less than 6 months of age.
 - 3.2.4.6.3. Confirmation of persistent BVDV infection: If BVDV is demonstrated by FA **OR** IP in cell culture **OR** by PCR, the

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animal is to be isolated from other cattle and retested in not less than 21 days by PCR (serum in this case, regardless of the age of the animal) **OR** inoculation of bovine cell cultures with an appropriate specimen (whole blood or serum depending on the age of the animal). Demonstration of BVDV a second time is considered confirmation of persistent infection and the animal is not eligible to enter the resident herd of the Department of Agriculture- approved AI center.

- 3.2.4.6.4. Confirmation that an animal is not persistently infected: Animals from which BVDV has been isolated or demonstrated must remain in isolation apart from other cattle until proven free of BVDV by 2 consecutive negative virus isolation tests conducted at least 10 days apart and performed on the appropriate specimen (whole blood or serum).
- 3.2.4.7. Samples of any semen collected and processed from bulls from which BVDV has been isolated, but which are later proven to be free of persistent infection (as stated above in 3.2.8.1.iv.), must be subjected to BVDV isolation tests or PCR, with negative results from any collection within 30 days preceding or following the date of virus isolation to be eligible for importation to the U.S.
- 3.2.4.8. The following test shall be conducted for all bulls before their semen is released. If the bulls are not of semen producing age during the isolation period, this test may be conducted after the isolation period is completed:
 - 3.2.4.8.1. Bovine Viral Diarrhea Virus (BVDV): One of the following test methods and schedules is used to test for persistent testicular BVDV infection.
 - 3.2.4.8.2. Test all bulls anytime during the isolation interval for BVDV by the serum neutralization (SN) test for both types I and II. All bulls that test positive must have one negative PCR test on processed semen, or a virus isolation test on processed semen with one pass performed in bovine cell culture with a negative result as demonstrated by staining of the cell culture by immunofluorescence (FA) or immunoperoxidase (IP) methods. Processed semen is semen that is completely extended and frozen.
 - 3.2.4.8.3. **OR** All bulls must have one negative PCR test on processed semen or a virus isolation test with one pass of processed semen performed in bovine cell culture with a negative result

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as demonstrated by staining of the cell culture by immunofluorescent (FA) or immunoperoxidase (IP) methods. Processed semen is semen that is completely extended and frozen.

Any bulls with a positive virus isolation test of semen should have additional processed semen tested to confirm persistent testicular infection. [Note: Any bull that has a persistent testicular infection for BVDV is not eligible for semen collection and is not permitted to remain in the herd.]

3.3. RESIDENT HERD COLLECTIONS

3.3.2. Once a bull or mount animal has completed the isolation testing outlined above, he may enter the resident herd with a status where he shall continue to be tested and certified in accordance with the following procedures performed at 6 month intervals:

- 3.3.2.1. Tuberculosis: The donor is negative to a whole-herd test for bovine tuberculosis (A negative test means no detectable response using both visual examination and palpation when read 72 hours following injection with intradermal tuberculin.) This test must be performed by a veterinarian designated by the Australian Department of Agriculture.
- 3.3.2.2. Bovine Leptospirosis: the donor is negative to a serological test for serotypes *L. pomona*, *L. hardjo*, *L. canicola*, *L. icterohaemorrhagiae*, and *L. grippotyphosa*. If result is not negative, the bull must have a stabilized low titer (1:400).
- 3.3.2.3. Bovine Venereal Trichomoniasis: the donor is negative to a single microscopic examination of cultured preputial material.
- 3.3.2.4. Bovine Campylobacteriosis: the donor is negative to a single culture test of preputial material as an alternative procedure, the preputial material may be examined using the fluorescent antibody (FA) technique. Any positive FA test shall be followed by culture of preputial material, with a negative result.

3.3.3. 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.

3.3.4. 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

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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 therefore..

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

- 4.1. 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 if a key to the code accompanies the import permit and health certificates.)
- 4.2. 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.
- 4.3. 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 Australia seals. (The seal numbers must be recorded on the health certificate.)
- 4.4. The semen shipment is routed directly on the waybill or other export documents to the United States, with no stops en route other than those provided for on the USDA import permit.
- 4.5. 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.
- 4.6. 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. 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

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contact the U.S. State veterinarian ([State Regulations and Import Requirements](#)) of the destination state to determine the requirements.

6. ARRIVAL AND INSPECTION AT THE PORT OF ENTRY

- 6.1. The shipment must be routed directly to the United States from Australia 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>.
- 6.2. 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.
- 6.3. 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.
- 6.4. The shipping containers must be sealed with an approved seal from the Australian Department of Agriculture, 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.

7. SEMEN SHIPMENTS REFUSED ENTRY:

If any semen shipments are determined to be ineligible for importation into the United States on 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

I. Antibiotics/Stock Solutions

A. Antibiotics:

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

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- Stock solutions of individual antibiotics will be combined on day of use, and not held over.
- Extenders must be used on the day the combined antibiotics are added.

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 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/mL Gentamicin 500 µg/mL Linco-Spectin 300/600 µg/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% of the antibiotic concentration listed under C.
2. 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

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the following may require additional efficacy testing:

1. Use of extender other than that specified in this appendix.
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 m % 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
1.10 m % tris (hydroxymethyl aminomethane)
1.10 gm % citric acid monohydrate
1.0 gm % glucose 7.0% glycerol

**ANTIBIOTIC PROCEDURES/ CONDITIONS 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

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2-step protocol. However, the main differences from a 2-step protocol are as follows:

- A. The extender is not fractionated into a non-glycerol and glycerol component. The complete extender contains 7.0% glycerol.
- B. 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:

- A. Use of extender other than one listed in this appendix.
- B. Antibiotic/neat semen contact of less than three minutes.