

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

January 2014, Updated May 2020

This protocol describes the conditions required to import embryos from domestic bovines (*Bos taurus*, *Bos indicus*, *Bison bison*) according to regulations found in 9 CFR Part 98. **The following requirements apply *in vivo* derived bovine embryos.**

## **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 NIES, 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 by a veterinarian designated by the Australian Department of Agriculture, and must be endorsed by an Australian Department of Agriculture veterinarian. The health certificate must accompany the embryos to the port of entry designated on the USDA import permit. The health certificate must show:
- 1.2.1. The identification of each donor dam and sire by breed and registry number.
  - 1.2.2. The dates of recovery of all embryos in the shipment.
  - 1.2.3. The name and address of the laboratory conducting the tests.
  - 1.2.4. The dates and methods of testing and results of the tests on the donor dam and sire.
  - 1.2.5. The name and address of the consignor and consignee.
  - 1.2.6. The name and address of the embryo collection center where the donor dam was inseminated or bred, and where the embryos were collected and processed for this shipment.
- 1.3. The embryos must originate from an embryo collection center (ECC) approved by the

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Australian Department of Agriculture, and meeting the current criteria of the OIE Terrestrial Animal Code, Chapter 4.7 and 4.8.

- 1.4. Any animal leaving the ECC must fully comply again with these requirements before re-entry into the embryo collection facilities.
  
2. **CERTIFICATIONS:** The following certifications must be included in the export health certificate [Note: the Australian Department of Agriculture veterinarian is responsible for verifying the attestations made by any others, such as a State Government Veterinary Authority, or Center Veterinarian who may provide the information below.]
  - 2.1. Australia is free of foot-and-mouth disease (FMD), surra, and contagious bovine pleuropneumonia.
  - 2.2. Bovine tuberculosis is a notifiable disease in Australia.
  - 2.3. No cases of disease caused by Schmallenberg virus have been detected or reported in Australia.
  - 2.4. Within the previous 12 months, no cases of bovine tuberculosis have been reported in the donor animal herd, or from any group of animals susceptible to bovine TB with which the donor(s) was (were) commingled.
  - 2.5. During the 12 months prior to the collection of embryos for export to the United States, there has been no evidence of tuberculosis found in donor dams (or sires where natural breeding or fresh semen is used) or on any premises the donors have been on during that time.
  - 2.6. The donors showed no signs of bovine tuberculosis on the day of collection of the embryos (or semen, where natural breeding or fresh semen is used) and either:
    - 2.6.1. Were kept in an ECC free from bovine tuberculosis and only accepts animals from free herds; or
    - 2.6.2. Showed negative results to tuberculin tests carried out annually and were kept in a herd free from bovine tuberculosis.
    - 2.6.3. 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 due to animal health reasons placed during that time.
  - 2.7. On the date(s) the embryos were collected, there was no clinical evidence in the ECC herd of the diseases mentioned below.
  - 2.8. During the 60 days prior to the creation of embryos for export to the United States, no donor dam has been housed or otherwise had contact with other animals of lesser health status or under restrictions which would make the embryos ineligible for importation to the United States under APHIS' regulations.

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- 2.9. During the 60 days prior to the collection of embryos all donor dams were inspected by the Team Veterinarian and found clinically free of contagious diseases. Where natural breeding or fresh semen is used to inseminate the donor dam, all donor sires were inspected by the Team Veterinarian during the 60 days prior to collection and found to be clinically free of contagious diseases.
- 2.10. During the 60 days prior to the collection of embryos for export to the United States, all donor dams (and sires where natural breeding or fresh semen is used) have not been corralled, pastured, or held with other animals of lesser health status or under restrictions which would make them ineligible for export to the United States.
- 2.11. All donor dams (and donor sires, if the semen was collected in Australia or natural service was performed) were: born, raised, and continuously resident in Australia or were part of Australia's national herd for a minimum period of time. Paragraphs 2.6.1 and 2.6.2 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.
  - 2.11.1. 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 ECC.
  - 2.11.2. If the animals are offspring of animals imported from a country not recognized by 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 ECC.
- 2.12. The ova were fertilized as follows:
  - 2.12.1. Either donor sires used for natural service were of an equivalent isolation and health status to the donor dams OR
  - 2.12.2. Semen used for insemination was eligible for export to the United States of America OR
  - 2.12.3. Semen used for insemination was imported into Australia in accordance with Australia's import conditions on an Australian Department of Agriculture import permit number (list permit number).

### **3. TESTING REQUIREMENTS**

- 3.1. Arthropod-vectored viruses:
  - 3.1.1. **EITHER** the donor animals were kept in an Australian Department of Agriculture - approved embryo collection center (ECC) located in a region certified free from bluetongue, bovine ephemeral fever, Akabane, and Aino for a minimum of 60

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consecutive days before commencement of, and during, collection of the embryos for export. [Note: the dates of this period of residency must be included on the export health certificate.]

- 3.1.2. **OR** were tested on the day of collection using the following tests:
  - 3.1.2.1. Akabane - Negative to a serum neutralization test at 1:4 serum dilution.
  - 3.1.2.2. Bluetongue - Negative to an agar gel immune-diffusion test or the competitive ELISA (cELISA) test.
  - 3.1.2.3. Aino - Negative to a serum neutralization test at a 1:10 serum dilution.
  - 3.1.2.4. Bovine ephemeral fever - Negative to a serum neutralization test at a 1:10 serum dilution OR Virus isolation test or PCR on blood samples collected at the start and conclusion of, and at least every 7 days during the semen collection for export to the US.
- 3.1.3. If collected on more than one occasion for this export, the donor dams were tested as described above for the first test, on blood samples collected on the first and last day of collection, and at least every 7 days (for virus isolation) or at least every 28 days (for PCR assays) during embryo collection for this consignment.
- 3.1.4. The pre-collection and post-collection tests on the donors were conducted in laboratories designated by the Australian Department of Agriculture as laboratories approved to conduct the tests.

#### **4. PROCESSING CERTIFICATION**

- 4.1. The artificial insemination or natural breeding of the donor dams was performed under the direction of an Australian Department of Agriculture- approved veterinarian. The Australian Department of Agriculture- approved veterinarian personally observed the flushing of the embryos from the donor dams and the processing of the recovered embryos for shipment to the United States.
- 4.2. Embryos were processed in accordance with the latest published edition of the Manual of the International Embryo Transfer Society (IETS).
- 4.3. Each embryo must be washed at least 10 times. Each wash must be accomplished by transferring the embryo into an aliquot of fresh medium that is 100 times the volume of the embryo, plus any fluid transferred from the previous wash.
- 4.4. No more than 10 embryos from the same flush may be washed together.

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- 4.5. A sterile micropipette must be used for each transfer, and the embryos must be well agitated throughout the entire volume of the wash before the next transfer. Embryos from different donors may not be washed together.
- 4.6. After the last wash, each embryo were microscopically examined over its entire surface at not less than 50x magnification and all zona pellucida were found to be intact and free from any adherent material.
- 4.7. After washing and examination of the zona pellucida, embryos were individually packaged in sterile ampules or straws and frozen in virgin liquid nitrogen.
  - 4.7.1. The donor dam's and sire's identification and breed, the date of embryo collection, the name and address of the place where the embryos were collected, and an identification number for the straw or ampule must be recorded with indelible markings on each ampule or straw.
  - 4.7.2. If any of this information is provided in code, deciphering information must be attached to the health certificate for the embryos.
- 4.8. All equipment used to handle the embryos was replaced by new or sterilized equipment between each washing, and all standard sterile procedures were observed.
- 4.9. The shipping container were examined by the veterinarian issuing the health certificate and found empty of embryos and other biological materials prior to use for export of embryos to the United States. Only fresh liquid nitrogen has been used to export embryos to the United States.
- 4.10. The embryos were maintained under lock and key or in the custody of the veterinarian issuing the health certificate until they were placed in the shipping container and sealed with Government of Australia seals. The seal numbers must be recorded on the health certificate.
- 4.11. The shipment is routed directly to the United States with no stops en route other than those provided for on the USDA import permit.

**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 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

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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 embryos.
- 6.3. All shipping containers and the complete embryo shipment 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 shipment 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 of the exporting country, and that seal must be intact when the shipping container arrives in the United States. If the seal is broken or has been opened, the shipping container will automatically be refused entry into the United States, and will be returned to the exporting country or destroyed.

## **7. EMBRYOS REFUSED ENTRY**

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