



**Hamilton Research, Inc.**

## **Instructions for Use**

# ***EQUITAINER***

## **Equitainer II for Transported Semen**

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# 1. Introduction

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Successful Transported Semen requires attention to detail. Some procedures may be different than your customary artificial insemination practices.

The instructions in this booklet provide only the minimal direction needed to use the Equitainer. For more detailed information about collecting and shipping cooled stallion semen, we recommend our *Educational CD-ROM* or *Easy Breeder CD-ROM* that contain an updated digital version of the *Transported Semen Handbook for the Stallion Owner and Veterinarian*. The digital handbook provides greater detail and should answer most of the questions arising in using Transported Semen. It is available from Hamilton Research, Inc.

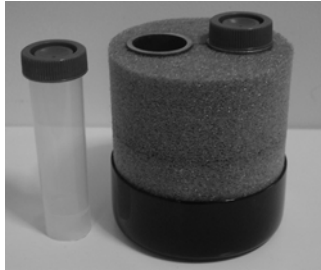
A note of caution: Not all stallions are good candidates for Transported Semen. Before shipping a sample to the mare owner, be sure to perform a trial storage in the Equitainer and check the motility after 24 hours.

As a service to our customers, Hamilton Research, Inc. offers an evaluation service. For more information, please call (800) 367-0266 or visit the Hamilton Research, Inc. website at [www.equitainer.com](http://www.equitainer.com).

## 2. New Isothermalizer

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The Isothermalizer has been redesigned to use two 50 ml centrifuge tubes for shipping the extended semen sample. This new format eliminates the need for Whirlpak bags, baby bottle liners, ballast bags and the sterile cup. The new design incorporates two central wells, into which the centrifuge tubes are placed, a cupped rubber base and built-in x-ray protection.



*Figure 1: New Isothermalizer*

### **Benefits:**

- No need to replace ballast bags
- Tight-fitting, screw top tubes prevent sample leakage
- Easier to package semen dose
- Cupped rubber base for increased durability
- Built-in x-ray protection for increased confidence in airline shipping

### **Important Facts to Remember:**

- Both centrifuge tubes **must** be filled with either semen or water to achieve the proper cooling rate
- Both centrifuge tubes must be filled to the top (50 ml) for proper cooling

### **3. Description of the Equitainer II Components**

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In addition to the Equitainer itself, the following components are included with each system.

**Isothermalizer: (One)** The Isothermalizer provides crucial temperature control and protection from potentially harmful x-rays. The Isothermalizer consists of:

- A blue, foam core for insulation
- Two lined, internal wells for sample cooling and protection against x-rays
- An internal x-ray protectant base, which also assists in achieving the proper cooling rate
- An external cupped rubber base for thermal control

Each well accepts one 50 ml centrifuge tube (see below). **Both wells must hold a filled centrifuge tube to achieve the proper cooling rate.**

**Freezer Cans: (One)** The Freezer Cans provide the exact amount of frozen liquid needed to cool the sample and maintain it at the proper temperature, up to the time limits of the system. The Freezer Cans must be kept in a deep freezer for at least 24 hours prior to use.

**Centrifuge Tubes: (Two)** The 50 ml centrifuge tubes are used to hold the insemination dose. **Important:** Both tubes must be filled to the top (50 ml) for proper cooling. **If only one tube of semen is shipped, the second tube must be filled with water (water ballast) for proper cooling.**

*Note: The centrifuge tubes are single-use only. They should either be discarded after each shipment or used only for water ballast in future shipments.*

#### **4. Preparation for Collection**

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1. Store the Freezer Can in a DEEP FREEZE for at least 24 hours. Some small freezers are not always cold enough. However, if you do not have access to a deep freeze, store the Freezer Can in a standard freezer for 48 hours.
2. The centrifuge tubes and any equipment that will come in contact with the semen must be stored in an incubator set at 37°C (99°F) for at least 4 hours prior to use. Equine sperm are extremely sensitive to temperature shock so be sure that the temperature is accurate.
3. The Equitainer and Isothermalizer may be left at room temperature.
4. Mix or thaw the semen extender. Maintain the semen extender at 37°C (99°F) until ready to extend the semen. If the semen extender does not already contain antibiotics, add the appropriate antibiotic to the semen extender and mix gently.
5. The stallion's penis should be washed gently with warm water. It should be allowed to air dry or be patted dry with a paper towel. Research has shown that washing with disinfectants or soaps should be avoided, since this upsets the natural balance of bacteria and lets pathogens multiply.
6. Prepare the AV as usual for collection. Pay particular attention to the temperature of the AV, especially the tip and collection pouch during cold weather. Be sure to use a sterile filter, non-spermicidal lubricating gel and take care to avoid any types of contamination.

In cold weather, a standard AV cover or quilted mitt should be placed over the collection bag or bottle.

## 5. After the Collection

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1. If using a collection pouch or bag, transfer the ejaculate into a new sterile cup. If a collection bottle was used, this is not required.
2. Maintain the ejaculate at 37°C (99°F) until ready to extend. Dilution should occur within 5 minutes after collection for best results. If left unextended for greater than 15 minutes, sperm survivability will be greatly decreased.
3. Record the volume of the raw ejaculate. Determine the concentration of the raw ejaculate and calculate the proper dilution ratio to achieve a final sperm concentration of 40 million sperm per ml of extended semen (see chart below for help). **Important: The new Isothermalizer design requires a total sample volume of 50 ml in the centrifuge tube. To achieve the recommended insemination dose of 2 billion total sperm, samples may now be diluted to 40 Million/milliliter (M/ml).\***

Initial Concentration	Dilution Ratio*
80 M/ml	1 part semen to 1 part extender**
120 M/ml	1 part semen to 2 parts extender
160 M/ml	1 part semen to 3 parts extender
200 M/ml	1 part semen to 4 parts extender
240 M/ml	1 part semen to 5 parts extender
280 M/ml	1 part semen to 6 parts extender
320 M/ml	1 part semen to 7 parts extender

\* Using the older Equitainer system, a dose volume of 40 ml was used, at a concentration of 50 M/ml, giving the recommended 2 billion total sperm. You may still dilute the ejaculate to 50 M/ml if desired, but you must ship 50 ml in the centrifuge tube. In this circumstance, a total sperm dose of 2.5 billion will result. See page 8 for an alternate dilution chart.

\*\* A dilution of 1 part semen to 1 part ejaculate may not provide enough protection for the sperm and result in adverse effects on sperm survival. Thus, it may be best to dilute the sample 1:2 or 1:3 and send a larger insemination dose.

## 6. Extending the Semen

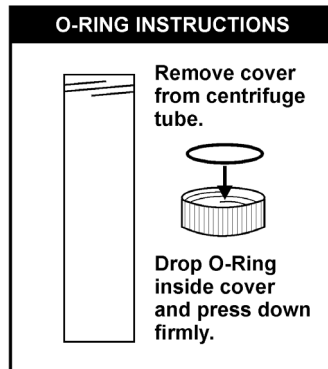
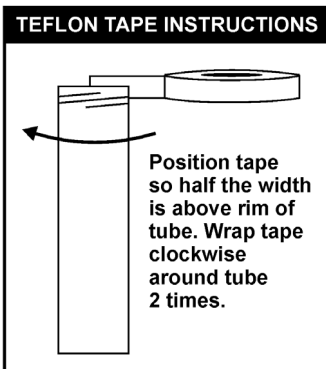
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1. Based on the determined dilution ratio, add the appropriate amount of semen extender to the semen slowly by pouring down the side of the cup, 1/4 at a time, mixing gently.
2. The extended semen should be left at room temperature during the packaging process.

## 7. Packaging the Semen

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1. For added protection against sample leakage, one of the following options should be used:
  - a) **Teflon Tape:** with half the width of the tape above the rim of the tube, wrap the Teflon tape **twice** around the centrifuge tube in a **clockwise** direction (with the threads). Pull the tape with enough force while wrapping so that it **stretches** slightly.
  - b) **O-Ring:** remove the cap from the centrifuge tube, drop the O-Ring into the cap and press down firmly.



2. Pour 50 ml of the extended semen into the centrifuge tube. It is vital that the full 50 ml is used – no excess air should be in the centrifuge tube.

3. Cap the tube tightly.
4. Using indelible ink, label the tube with the stallion's name and other pertinent information.

## 8. Loading the Isothermalizer

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**EXTREMELY IMPORTANT:** Both wells must be occupied with filled centrifuge tubes for proper cooling rates. Even if you are only sending one dose of semen, ~~vital~~ that the second well is also filled. This may be accomplished using a tube filled with water. (See Figure 2 on following page.)

1. Place the labeled centrifuge tube filled with extended semen into one of the wells.
2. If shipping two doses, place the second labeled centrifuge tube filled with extended semen into the remaining well.
3. If you are only shipping one dose of semen, fill an empty centrifuge tube to the top with 50 ml of water and cap tightly. Label the tube to indicate that the tube contains only water and should not be used for insemination. Place the tube into the empty well.

Figure 2. Properly Loaded Isothermalizer.  
Both wells MUST be filled, either with sample or water.

## 9. Loading the Equitainer II

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1. Place one (1) frozen Freezer Can, with arrows pointing UP, into the ziplock bag.
2. Put the loaded Isothermalizer on top of the Freezer Can.
3. Slide the entire ziplock bag into the central well of the Equitainer II. Close the Equitainer lid and secure the latch.

## 10. Alternate Dilution Chart

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Use this chart if you prefer to dilute sample to a final dose concentration of 50 M/ml. If you use this dilution, you still must fill the centrifuge tube with 50 ml of extended semen. This will give a 2.5 billion total sperm concentration per insemination dose.

Initial Concentration	Dilution Ratio*
100 M/ml	1 part semen to 1 part extender**
150 M/ml	1 part semen to 2 parts extender
200 M/ml	1 part semen to 3 parts extender
250 M/ml	1 part semen to 4 parts extender
300 M/ml	1 part semen to 5 parts extender
350 M/ml	1 part semen to 6 parts extender
400 M/ml	1 part semen to 7 parts extender

## 11. Time / Temperature in Equitainer

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Time (hours)	Temperature	
	°C	°F
0.0	37	99
0.5	31	88
1.0	26	79
1.5	22	71
2.0	19	66
2.5	16	61
3.0	14	57
3.5	12	54
4.0	11	51
4.5	10	49
5.0	9	48
5.5	8	47
6.0	7	45
6.5	7	45
7.0	7	44
7.5	6	43
8.0	6	43
8.5	6	43
9.0	6	42
9.5	6	42
10.0	5	42

