Supplies that will be sent to the cooperator:

- Impact Shipper with instructions for use (see Impact Shipper Protocol);
- Cooling packs;
- Sample tubes;
- Zip lock bags;
- Cooling media.

Prior to collection

1. Thaw the frozen media in a 37°C water bath
2. Ensure that the cooling packs are at 5 °C (They should not be frozen at time of use but at 5 °C.)

Collection Process

1. Collect semen from sexually mature bulls via an artificial vagina or electroejaculation.
2. Check sample to make sure it is free of urine and other contaminants.
3. Determine the sample volume and sperm concentration.
4. Multiplying the two (volume x count) will give the sperm count.
5. Divide the sperm count by 120 x 10⁶ sperm/mL which will determine the final volume that the sample should be diluted to.
6. The final volume minus the sample volume will determine the amount of the 37°C cryopreservation media to add to the sample.

Here is an example of the dilution math:

<table>
<thead>
<tr>
<th>Sample volume:</th>
<th>4.5 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm concentration:</td>
<td>300 x 10⁶ sperm/mL</td>
</tr>
<tr>
<td>Sperm count (volume x concentration):</td>
<td>1350 x 10⁶ sperm/mL</td>
</tr>
<tr>
<td>Final diluted volume (count ÷ 120 x 10⁶ sperm/mL):</td>
<td>11.25 mL</td>
</tr>
<tr>
<td>Amount of cryopreservation media to add (Final volume less sample volume):</td>
<td>6.75 mL</td>
</tr>
</tbody>
</table>

After dilution of the sample with the 37 °C cryopreservation media, label the tube with the bull’s name and/or identification number and place the sealed tube in a refrigerator or 5 °C environment. After collection, dilution and cooling of all semen samples, pack the Impact Shipper with the samples according to the instructions in the Impact Shipper Protocol and seal the box.