

Protocol for Antibiotic Sensitivities & Analysis Using the Equine Express II Cooled Semen & Embryo Transport System

1. Pre-set your laboratory refrigerator to 8° C or 40° F that will assimilate a passive cooling system, so that you can test multiple doses at the same time inexpensively.

a. This will take about three (3) days to accomplish, in order to accurately set the temperature.

2. Have an Equine Express II for the control group so that you have a legitimate transport system in place.

Prepare each dose of semen to the Exodus Protocol

a. 750 million progressively motile sperm to extend to a 5:1 ratio

b. 25 million progressively motile sperm/ml in a total dose volume of 30 milliliters.

3. You should always test using at least two different extenders and as many antibiotics as possible.

4. Simply draw a syringe for each antibiotic and one un-extended dose of semen as your control group.

5. Each dose should be drawn up as outlined above in figure 2, marking the end of the piston of each syringe with the extender type and antibiotic. Abbreviate where necessary so that identification can easily be made during the testing phase, mark as your control group with a "C".

6. It would be suggested that the following antibiotics be considered for this trial, maximum dosage is exemplified as follows;

a. Amikacin: (1 mg/ml) & Potassium (K) Penicillin: (100,000 units/ml)

b. Timentin: (1 mg/ml)

c. Naxcel: (1 mg/ml)

d. Ampicillin: (1 mg/ml)

7. Mark each syringe according to the individual antibiotic in each dosage.

8. Using a new Equine Express II cooled semen & embryo transport system, place the control syringe of each type extender in a syringe and place in the bottom of each container. Lay the *Thermoregulator* over the syringes and place a fresh and frozen *special coolant pac* on top, push the Styrofoam lid in place and finish by placing the Styrofoam body into the corrugated box and slide the lid into the sides of the box securing the package.

9. At 12, 24 & 36 hours, pull out each syringe from the refrigerator and/or the Equine Express II and place one drop of semen on a clean & warmed microscope slide from the incubator. Compare and analyze each syringe for total progressive motility.

a. From our website, download the motility monitoring form and post the total progressive motility of each syringe, for each seminal review and place out of site in a separate folder until the entire test is finished. This will prevent a bias opinion if the results are known from the previous motility analysis.

10. After all of your testing has been completed, begin to assemble the results of the hourly seminal review so that you can make a qualified analysis of the stallions motility. Some stallions are better at 24 hours than they are at 12 and so on and so fourth, for this reason, it is important not to allow previous test results interfere with the overall values of the total progressive motility.

