

Obtaining a Tissue Sample for Cell Culture and Freezing

Equine Embryo Laboratory College of Veterinary Medicine and Biomedical Sciences Texas A&M University

1. Cells should be collected and placed directly into cold culture medium (DMEM/F-12 with 10% fetal bovine serum and antibiotics). We can send the medium; it is sent chilled in three 15-ml conical tubes. Alternatively, chilled embryo holding medium may be used; using sterile technique, fill three 5- to 15-ml sterile sealable tubes $\frac{3}{4}$ full with medium.
2. The tubes should be refrigerated until use. They should be placed in an insulated (e.g. Styrofoam) container (with cover), on ice, when going to take the tissue sample.
3. Before going to take the sample, make sure you have something to label the tubes with, and that there is someone ready to open the tubes, who has a 1.5 inch 18- or 20-ga needle ready to help get the tissue off the forceps.
4. We typically take tissue from the neck under the mane for cosmetic reasons and because this may have had less sun exposure. For an animal that may be terminal, it is recommended that two different areas be sampled, and the tissue grown as two separate cell lines. In this case take another sample from the gum if possible, or if not, from any other place easy to get to, which has been out of the sun. Try to avoid really fatty areas such as the tail head.
5. Shave an area approximately 7 x 7 cm (unless gum), and scrub and rinse as for surgery. We typically use betadine scrub rather than nolvasan. Do a last wipe with only sterile saline to remove remnants of scrub.
6. Glove and maintain sterility; contamination is the most common problem with tissue biopsies.
7. Perform an inverted U block with lidocaine, so that the lidocaine is not in the tissue being sampled.
8. Make a small skin incision (about 2 cm), and evert the skin edges. Obtain small ($\sim 5 \text{ mm}^3$) samples of subcutaneous connective tissue and place them immediately into the cooled cell culture medium. Place 1 or 2 pieces of tissue in each tube. The assistant, using the needle, should help move the tissue quickly, and should make sure the tissue goes down into the medium. If you think any contamination may have taken place, start with a new tube, and new instruments if necessary.
9. If taking samples from two areas, put the samples in different tubes and label accordingly.
10. Keep the medium and samples cold, and cover the container so the tubes are not exposed to sun or fluorescent light. Transport as quickly as possible to the laboratory. Shipping address: Dr. Katrin Hinrichs, Large Animal Front Desk, Texas A&M University College of Veterinary Medicine, College Station, TX 77843-4457.
11. Close incision with a couple of sutures.

