COLLEGE OF VETERINARY MEDICINE
& BIOMEDICAL SCIENCES
Department of Veterinary Physiology & Pharmacology

TEXAS A&M UNIVERSITY

Obtaining a Tissue Sample for Cell Culture and Freezing

Equine Embryo Laboratory
College of Veterinary Medicine & 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. To do this, using sterile technique, fill three 5- to 15-ml sterile sealable tubes ¾ full with medium.

2. The tubes should be refrigerated until use. When going to take the tissue sample, the tubes should be placed in an **insulated (e.g. Styrofoam) container (with cover), on ice**.

3. Before going to take the sample, make sure you have something with which to **label the tubes**, and also 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 into the tube.

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, such as the chest or under the belly. 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, as betadine may be less toxic to tissue. 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. To take the biopsy, a sterile **scalpel and forceps** are needed. Make a small skin incision (about 2 cm), and evert the skin edges. Obtain small (~5 mm^3, size of a large grain of rice) samples of subcutaneous connective tissue and place them immediately into the cold cell culture medium. The assistant, using the needle, should help move the tissue from the forceps into the tube 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. Take samples from the subcutaneous connective tissue only -- AVOID fat or muscle. The scalpel / forcep method is the best for obtaining tissue; biopsy punches include the hair remnants, skin and hair follicles, which greatly increases the chance of contamination and exposes the connective tissue to the scrubbing agents used on the skin.

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10. Even if only one cell line is to be cultured, it is best to collect at least 2 samples and place them in separate tubes (label 1, 2, 3 etc). This maximizes the chance that a contamination–free culture will be obtained. If taking samples from multiple areas to culture multiple cell lines, put the samples in different tubes and label with the area sampled. Make sure to include **date, horse's name, and owner's name** on the label.

11. Close incision with a couple of sutures.

12. Keep the medium and samples **cold**, and **cover the container** so the tubes are not exposed to sun or fluorescent light. Transport **on ice**, as quickly as possible to the laboratory. Shipping address: Ms. Kindra Rader, 664 Raymond Stotzer Parkway, VICI 126, Bldg 1814, College Station, TX 77843-4466.

13. The filled-out **Tissue Culture Information Sheet** and **Credit Card Authorization** form should be included with the samples.

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