FINAL SCRIPT: APPROVED FOR FILMING

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Title: Large Volume Blood Collection from Swine for In Vitro Applications: Use of Intracardiac Cannulation and A Vacuum Pump as a Terminal Procedure

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 12
Number of Shots: 24

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Ediane Silva:** This procedure allows us the opportunity to collect a large volume of blood that maintains cell integrity for use in master seed virus development.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.8.1*

What are the current experimental challenges? NOTE: Changed to John A. Hall

1.2. **John A. Hall:** There is variation in the pressure applied by personnel that may compromise, damage, disrupt, or impair the cells. Our efficient technique provides excellent cell viability and constant vacuum suction to maximize collection volume.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.7.2*

What advantage does your protocol offer compared to other techniques? NOTE: Changed to John A. Hall

1.3. **John A. Hall:** Large volume blood draws can be performed manually, but there may be variation in the pressure applied. Our efficient technique provides excellent cell viability and constant vacuum suction to maximize collection volume and quality.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.10.1*

What research questions will your laboratory focus on in the future?

1.4. **Ediane Silva:** This technique maximizes cell viability and collection volume, decreases variability in technique between users, and is simple, yet highly efficient.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at Midwest Veterinary Services

Protocol

2. Large Volume Blood Collection from a Porcine Left Ventricle Using a Roller Bottle Assembly

Demonstrator: Kelli Millsap, Leeanna Burton

2.1. To begin, assemble the tubing to the stainless-steel straws **[1]** and connect the hose end to the bubble tubing **[2]**. Attach the tubing assembly to the roller bottle using a rubber stopper fitted with two uneven metal straws and a glass bottle **[3]**.

2.1.1. WIDE: Talent assembling plastic tubing to the stainless-steel straws.

2.1.2. Shot of the other end being attached to the bubble tubing.

2.1.3. Talent inserting the tubing into the rubber stopper and securing it into the roller bottle along with a glass bottle.

2.2. Aseptically instil 10 milliliters of sodium heparin solution through the suction tubing and catheter stylet to prevent clotting **[1-TXT]**. Then add 50 milliliters of sodium heparin solution at the same concentration into each roller bottle **[2]**. Confirm the vacuum pump is set to no more than 7 pounds of draw **[3]**.

2.2.1. Talent instilling 10 milliliters of heparin through suction tubing using a syringe. **TXT: Sodium heparin: 1000 USP/mL**

2.2.2. Talent pouring 50 milliliters of heparin into roller bottles aseptically. NOTE: This was done before 2.2.1.

2.2.3. Shot of the vacuum pump control panel showing 7 psi.

2.3. Next, place an anesthetized pig on a lift table **[1-TXT].** Clip the thoracic hair over the heart near the fourth and fifth intercostal space, just caudal to the point of the elbow **[2]**.

2.3.1. Shot of an anesthetized pig on a lift table. **TXT: Anesthesia: Telazol (30 - 5.0 mg/kg), ketamine (8 - 12 mg/kg), and xylazine (4.0 - 6.0 mg/kg) injection (i.m)**

2.3.2. Talent clipping hair on the thorax with electric clippers.

2.4. If available, use ultrasound to identify the left ventricle **[1]**. If ultrasound is not available, locate the left ventricle by auscultating and palpating near the fourth or fifth intercostal space medial to the point of maximal impulse **[2]**.

2.4.1. Shot of ultrasound showing the left ventricle.
AND
Shot of the heart fluttering

Videographer : Please capture instrument screen for this shot

2.4.2. Talent palpating the chest to locate the point of maximal impulse manually.

2.5. Next, use a 4 by 4-inch gauze soaked in 2 percent chlorhexidine scrub and 70 percent isopropyl alcohol **[1]**, to aseptically scrub the injection site in a circular motion, starting at the center and rotating outwards **[2]**. Repeat this process at least three times, alternating between the two disinfectants **[3]**.

2.5.1. Talent soaking gauze in either disinfectant.

2.5.2. Talent scrubbing the injection site with chlorhexidine-soaked gauze.

2.5.3. Talent repeating the scrub with alcohol-soaked gauze in alternating cycles.

2.6. Verify that the animal remains under surgical anaesthesia by checking for absent palpebral reflex, loose jaw tone, and absence of pedal reflex **[1]**.

2.6.1. Talent assessing palpebral reflex, jaw tone, and pedal reflex on the anesthetized pig.

2.7. Now, remove the stylet from the intravenous catheter **[1]**. Position the stylet over the left ventricle **[2]** and insert it perpendicularly through the skin into the ventricle **[3]**.

2.7.1. Talent removing the stylet from the catheter.

2.7.2. Shot of the stylet being positioned over the left ventricle.

2.7.3. Talent inserting the stylet perpendicular to the skin over the heart.

2.8. When blood begins to pulse out of the stylet **[1]**, connect it to the bubble tubing hose end attached to the roller bottle and activate the vacuum pump **[2]**.

2.8.1. Shot of the blood pulsing out of the stylet.

2.8.2. Talent connecting the tubing and turning on the vacuum pump.

2.9. Confirm a steady stream of blood flowing into the roller bottle **[1]**. As blood is pulled into the roller bottle, gently swirl the roller bottle to mix the blood with the anticoagulant **[2]**.

2.9.1. Shot of the blood flowing through the tubing in a steady stream.

2.9.2. Talent gently swirling the roller bottle as blood is collected.

2.10. When a litre of blood has been collected, use haemostats to clamp the bubble tubing near the roller bottle **[1]**. Then disconnect the rubber stopper **[2]** and attach it to the next roller bottle **[3]**. Release the haemostat to resume flow and continue swirling gently **[4]**.

2.10.1. Talent clamping the tubing with haemostats.

2.10.2. Talent detaching the stopper.

2.10.3. Talent securing the rubber stopper onto the next roller bottle.

2.10.4. Talent unclamping the tubing and swirling the bottle.

2.11. Continue collecting blood in this manner until the desired volume is reached **[1]**. Clamp the tubing and turn off the pump to end the collection **[2-TXT]**.

2.11.1. Shot of multiple blood filled roller bottles.

2.11.2. Talent clamping the tubing and switching off the vacuum pump. **TXT: Fix a syringe with sodium pentobarbital to induce euthansia**

~~2.12. Using a tube brush, clean visible blood from the stainless-steel components like straws and connectors **[1-TXT]**.~~

~~2.12.1. Talent scrubbing stainless-steel tools with a tube brush. **TXT: Autoclave for future use**~~

2.13. Place the sterilized rubber stoppers, straws, hose end connectors, and hemostats into a new autoclave pouch and sterilize before the next use **[1]**.

2.13.1. Talent placing sterilized items into a fresh autoclave pouch.