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Title: A Simple Double Centrifugation Tube Method to Obtain Platelet-rich Plasma from Equine Blood

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

√ Correct

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

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

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **05/20/2025**

When you are ready to submit your video files, please contact our Content Manager, Utkarsh Khare.

Current Protocol Length

Number of Steps: 14
Number of Shots: 30

Introduction

REQUIRED:

1.1. **Jorge U. Carmona:** Our research focuses on developing a simple, cost-effective method to produce platelet-rich plasma (PRP) from horse blood for treating musculoskeletal disorders like osteoarthritis and tendon injuries.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What are the current experimental challenges?

1.2. **Jorge U. Carmona:** Key challenges include maintaining sterility during manual processing and achieving consistent leukocyte reduction, which varies more than platelet concentration. NOTE: Jorge U. Carmona delivered this statement.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1*

What research gap are you addressing with your protocol?

1.3. **Claudia Jurado-Grisales:** Our protocol fills the gap of lacking standardized, video-demonstrated techniques for equine PRP preparation, especially for clinics without access to advanced kits.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.3*

What advantage does your protocol offer compared to other techniques?

1.4. **Claudia Jurado-Grisales:** Our method is affordable, uses basic lab equipment, and avoids costly kits, making PRP accessible for routine equine practice while maintaining therapeutic efficacy. NOTE: Claudia Jurado-Grisales delivered this statement.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.8.2*

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

1.5. **Jorge U. Carmona:** We'll explore PRP's long-term clinical outcomes and refine protocols to improve platelet yield and consistency for diverse equine musculoskeletal conditions.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.6.1*

Video editor: This is not an ethics statement, but the authors wish to display this statement after interviews (before protocol begins)

This study was conducted in accordance with the internal protocols for the preparation and use of PRP in horses at the Veterinary Teaching Hospital of the Universidad de Caldas, Manizales, Colombia. This study did not require Internal Animal Care Committee approval as the data were obtained from lame horses treated clinically at our institution.

Protocol

2. Restraining and Positioning the Horse for Blood Collection

Demonstrator: Jorge U. Carmona

2.1. To begin, restrain the horse securely using either a clinical examination chute or a bridle depending on its behavior **[1]**. Clip a 5 by 5-centimeter area of hair using an electric shaver in the mid-third of one side of the neck over the jugular vein **[2].**

2.1.1. WIDE: Talent restraining the horse with a bridle. NOTE: This shot was slightly modified during the shoot.

2.1.2. Talent using an electric shaver to clip a 5 by 5 centimeter area on the neck over the jugular vein.

2.2. Using non-sterile gloves and gauze, perform a preliminary non-sterile skin preparation over a 10-centimeter length of the jugular area for approximately 3 minutes **[1]**. Apply several pieces of gauze soaked in antiseptic foam in a circular motion **[2]**.

2.2.1. Talent rubbing the skin with antiseptic-soaked gauze over the jugular area.

2.2.2. Talent applying circular strokes using foam-soaked gauze on the clipped region.

2.3. Now, using sterile gloves, scrub the skin with gauze soaked in disinfectant in a circular motion for approximately 5 minutes **[1]**. Then, clean the area with sterile gauze soaked in 95 percent ethyl alcohol **[2]**.

2.3.1. Talent scrubbing the neck area using disinfectant-soaked gauze. NOTE: 2.3.1 and 2.3.2 are combined.

2.3.2. Talent wiping the same area with alcohol-soaked gauze.

3. Aseptic Blood Collection from the Horse

Demonstrator: Catalina López

3.1. Place sixteen 4.5-milliliter sodium citrate tubes, previously wiped with gauze soaked in 95 percent ethyl alcohol, in a clean plastic rack **[1-TXT]**. Disinfect the rubber caps of the vacuum tubes, especially at the needle coupling recess, with 95 percent ethyl alcohol **[2]**.

3.1.1. Talent lining up 16 alcohol-cleaned tubes in a rack. **TXT: Wipe the tubes with gauze soaked in 95% ethyl alcohol beforehand**

3.1.2. Talent wiping the tube caps carefully.

3.2. Now, sterilely insert a butterfly needle into the prepared jugular vein site **[1]** and attach a 4.5-milliliter sodium citrate tube to the needle holder to collect blood **[2]**. Then, gently shake the filled tube and place it back in the rack **[3]**.
   3.2.1. Talent inserting butterfly needle into the disinfected jugular vein site.
   3.2.2. Talent attaching vacuum tube to the holder and collecting blood. NOTE: 3.2.2 and 3.2.3 are combined.
   3.2.3. Talent shaking and placing the tube into the rack.

4. Obtaining Platelet-Rich Plasma from the Whole Blood

Demonstrator: Catalina López NOTE: Catalina López demonstrated the procedure.

4.1. Place the tubes containing whole blood in a four-place swing-out rotor at a 90-degree angle in a bench-top centrifuge **[1]**. Centrifuge the tubes at 120 *g* for 5 minutes at room temperature **[2]**. After centrifugation, place a sterile impervious drape in a class 2 laminar flow hood **[3]**. NOTE: The VO has been edited.
   4.1.1. Talent placing tubes in centrifuge.
   4.1.2. Close-up of centrifuge setting being adjusted and turned on.
   4.1.3. Talent placing the drape inside the hood.
   ~~4.1.4. Talent arranging tubes on a rack inside the laminar hood over the drape.~~ NOTE: This shot was not filmed.

4.2. Wearing a face mask and sterile gloves, place a sterile 10-milliliter luer syringe with a 21 gauge, 5 by 8 inch sterile needle on the dominant hand side **[1]**.
   4.2.1. Talent placing syringe and needle setup on dominant side of laminar hood.

4.3. Insert the sterile needle 3 millimeters above the buffy coat in each centrifuged citrated blood tube **[1]** and gently aspirate 50 percent of the plasma without disturbing the buffy coat **[2]**. Continue aspirating the plasma from the tubes and distribute it into 5 to 10-milliliter sterile plain tubes **[3]**. NOTE: The VO has been edited.
   4.3.1. Talent inserting needle above buffy coat.
   4.3.2. Talent gently aspirating plasma to avoid turbulence. NOTE: 4.3.1 and 4.3.2 are combined.
   4.3.3. Talent continues gently aspirating plasma and distributing collected plasma into sterile plain tubes. NOTE: Additional actions were performed in this shot during the shoot. 4.3.3 and 4.4.1 are combined.

4.4. Close the tubes with rubber stoppers **[1]** and place them in a four-place swing-out rotor in the centrifuge **[2]**. Centrifuge at 240 *g* for 5 minutes at room temperature **[3]** to obtain small cellular buttons **[4]**. NOTE: The VO has been edited.

4.4.1. Talent sealing tubes with rubber stoppers. NOTE: 4.3.3 and 4.4.1 are combined.
4.4.2. Talent placing tubes in centrifuge. NOTE: 4.4.2 and 4.4.3 are combined.
4.4.3. Close-up of centrifuge setting being adjusted and turned on.

Added shot: 4.4.4: Talent opening the centrifuge and showing the centrifugate tubes with small cellular buttons.

4.5. After placing the tubes back on a rack inside the laminar flow hood, place two sterile 10-milliliter Luer syringes, a sterile spinal needle, and a sterile plastic cap inside the hood **[1].** NOTE: 4.5, 4.6, 4.7, and 4.8 are combined.
~~4.5.1. Talent placing tubes on the rack inside the laminar hood.~~ NOTE: This shot was not filmed.

Added shot: Talent placing two sterile 10-milliliter Luer syringes, a sterile spinal needle, and a sterile plastic cap inside the hood.

4.6. Open them **[1]** and use the non-dominant hand to remove the rubber caps **[3]**. With the sterile dominant hand, insert an 18 gauge, 90-millimeter spinal needle attached to a 10-milliliter sterile luer syringe into the tube **[4]** and aspirate the top 75 percent of the plasma **[5]**. NOTE: The VO has been edited. NOTE: 4.5, 4.6, 4.7, and 4.8 are combined.

Added shot: Talent sterilely opening two 10 mL siringes, one spinal catheter, and one plastic cap.
4.6.1. Talent removing rubber caps using non-dominant hand.
4.6.2. Talent inserting the syringe into the tube.
4.6.3. Shot of aspirating and discarding the top plasma layer as platelet-poor plasma.

4.7. Then, aspirate the remaining 25 percent of the plasma using the same needle attached to a fresh 10-milliliter sterile syringe **[1-TXT]**. NOTE: 4.5, 4.6, 4.7, and 4.8 are combined.
4.7.1. Talent aspirating the remaining 25 percent plasma and displaying it. **TXT: This is the platelet-rich plasma (PRP) fraction**

4.8. Finally, wrap the sterile luer syringe containing PRP in a small sterile, impermeable drape **[1]** and cap with a sterile needle or closure to prevent contamination **[2]**. NOTE: 4.5, 4.6, 4.7, and 4.8 are combined.
4.8.1. Talent wrapping PRP-filled syringe in sterile drape.
4.8.2. Talent sealing the syringe with sterile cap.

Results

5. Results

5.1. The mean platelet count was 2.41-X higher in PRP **[1]** than in whole blood **[2]**. In contrast, the mean leukocyte concentration in PRP was significantly lower **[3]** than that in whole blood **[4]**.

5.1.1. LAB MEDIA: Table 1 *Video editor: Highlight the value 360.95 in the row "mean"*

5.1.2. LAB MEDIA: Table 1 *Video editor: Highlight the value 149.68 in the row "mean"*

5.1.3. LAB MEDIA: Table 1 *Video editor: Highlight the value 3.05 in the row "mean"*

5.1.4. LAB MEDIA: Table 1 *Video editor: Highlight the value 7.02 in the row "mean"*

5.2. TGF-β_1 *(T-G-F-Beta-1)* concentrations were significantly different between plasma, PRP supernatants, and PRP lysate **[1]**, with the highest concentrations for PRP lysate **[2].** On the other hand, PDGF-BB concentrations were significantly higher in PRP supernatants and PRP lysate **[3]** compared to plasma **[4]**.

5.2.1. LAB MEDIA: Figure 5A

5.2.2. LAB MEDIA: Figure 5A *Video editor: Highlight the bar for PRP-L*

5.2.3. LAB MEDIA: Figure 5B *Video editor: Highlight the bar for PRP-S and PRP-L*

5.2.4. LAB MEDIA: Figure 5B *Video editor: Highlight the bar for plasma*

Pronunciation Guides:

1. Jugular

Pronunciation link:
https://www.merriam-webster.com/dictionary/jugular
IPA: /ˈdʒʌgjələr/
Phonetic Spelling: juhg-yuh-lur

2. Centimeter

Pronunciation link:
https://www.merriam-webster.com/dictionary/centimeter
IPA: /ˈsɛntəˌmiːtər/
Phonetic Spelling: sen-tuh-mee-ter

3. Antiseptic

Pronunciation link:
https://www.merriam-webster.com/dictionary/antiseptic

IPA: /ˌæn.ʈɪˈsep.tɪk/
Phonetic Spelling: an-tih-sep-tik

4. Disinfectant

Pronunciation link:
https://www.merriam-webster.com/dictionary/disinfectant
IPA: /ˌdɪs.ɪnˈfek.tənt/
Phonetic Spelling: dis-in-fek-tuhnt

5. Citrate

Pronunciation link:
https://www.merriam-webster.com/dictionary/citrate
IPA: /ˈsɪˌtreɪt/
Phonetic Spelling: sih-trayt

6. Aseptic

Pronunciation link:
https://www.merriam-webster.com/dictionary/aseptic
IPA: /ˌeɪˈsep.tɪk/
Phonetic Spelling: ay-sep-tik

7. Buffy coat

Pronunciation link:
https://www.howtopronounce.com/buffy-coat
IPA: /ˈbʌfi koʊt/
Phonetic Spelling: buh-fee koht

8. Centrifuge

Pronunciation link:
https://www.merriam-webster.com/dictionary/centrifuge
IPA: /ˈsɛn.trəˌfjuːdʒ/
Phonetic Spelling: sen-truh-fyooj

9. Laminar

Pronunciation link:
https://www.merriam-webster.com/dictionary/laminar
IPA: /ˈlæm.əˌnɑr/
Phonetic Spelling: lam-uh-nar

10. Plasma

Pronunciation link:
https://www.merriam-webster.com/dictionary/plasma
IPA: /ˈplæz.mə/
Phonetic Spelling: plaz-muh

11. Leukocyte

Pronunciation link:
https://www.merriam-webster.com/dictionary/leukocyte
IPA: /ˈluː.kəˌsaɪt/
Phonetic Spelling: loo-kuh-syte

12. Platelet

Pronunciation link:
https://www.merriam-webster.com/dictionary/platelet
IPA: /ˈpleɪt.lɪt/
Phonetic Spelling: plate-lit