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Title: Through-the-Wall Blood Sampling Method to Minimize Sleep Disruption in Clinical Settings

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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: 11
Number of Shots: 37

Introduction

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

1.1. **Grissy Simé Mora:** We study how sleep and circadian rhythms impair cardiometabolic health. Ultimately, we would like to find strategies to reduce the risk of diseases associated with sleep and circadian disruption.

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

What are the current experimental challenges?

1.2. **Catherine Lowry:** To study things that change in the body over 24-hours we need to have a window into the system during the entire period. This can be tough because we don't want to disturb sleep and circadian rhythms by going into a participant's room while they're sleeping to get a blood sampling. To get around that we use a through-the-wall blood sampling protocol.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.6.1., 2.6.2.*

What research gap are you addressing with your protocol?

1.3. **Grissy Simé Mora:** We address the gap by using a long IV line that we place through the wall from which we can sample blood from outside of the participant room. This allows us to sample blood with minimal disruption to sleep or circadian rhythms.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.3.1, 3.3.2.*

How will your findings advance research in your field?

1.4. **Catherine Lowry:** Our findings will allow researchers to assess metabolic health markers, such as glucose and insulin, across 24 hours without disrupting sleep, which can help clarify the influence of sleep and circadian rhythms on disease risk.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 2.*

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

Ethics Title Card

This research has been approved by the Institutional Review Board of Colorado State University and the Colorado Multiple Institutional Review Board

Protocol

2. Setting Up the Extension Line

Demonstrators: Grissy Simé Mora and Cat Lowry

Videographer's note: My apologies for the shots 2.1.2. - 2.2.4. on the white table, they are blown out and I didnt realize that at the time. I hope they are recoverable.

2.1. To begin, attach the extension line for through-the-wall sampling by connecting the three-way stopcock to the female Luer lock end of the extension line **[1]**. After breaking the seals, place the 10-milliliter saline flush syringe **[2]** and the 3-milliliter sample syringe onto the stopcock **[3]**.

2.1.1. WIDE: Talent connecting the three-way stopcock to the female Luer lock end of the extension line.

2.1.2. Talent placing the saline flush syringe onto the stopcock.

2.1.3. Talent placing the sample syringe onto the stopcock.

2.2. Twist the **OFF** *(Off)* tab of the stopcock to close the extension line to the 3-milliliter syringe and open it to the 10-milliliter saline flush **[1]**. Push saline through the extension line until it reaches the end **[2]**. Carefully remove the cap and allow the saline to drip into a cup or onto the absorbent pad **[3]**. Twist the **OFF** tab to close the extension line to both syringes, keeping them attached to the stopcock **[4]**.

2.2.1. Talent twisting the **OFF** tab of the stopcock to redirect the saline flow.

2.2.2. Talent pushing saline through the line using the 10-milliliter syringe and the saline reached the end of the line.

2.2.3. Talent removing the cap and the saline dripping into a cup or onto the pad.

2.2.4. Talent twisting the **OFF** tab to close the line to both syringes, ensuring they remain attached.

2.3. Feed the capped end of the extension line through the wall port into the participant room, ensuring the cap remains in place to maintain sterility **[1]**.

2.3.1. Talent feeding the capped end of the extension line through the wall port.

2.4. In the participant's room, clean the distal end of the extension line and the attachment site of the IV *(I-V)* extension set using a sterile alcohol prep pad **[1]**. Remove the cap

from the extension line **[2]** and attach it to the IV extension set connected to the participant **[3]**.

2.4.1. Talent wiping both the extension line and the IV set with an alcohol prep pad.

2.4.2. Talent removing the cap from the extension line.

2.4.3. Talent connecting the extension line to the participant's IV extension set connected to the participant.

2.5. Now, return to the antechamber **[1]**. Remove and discard the used saline flush from the stopcock **[2]** and attach a warmed 10 milliliter saline flush **[3]**. Twist the **OFF** tab to close the line to the sample syringe and open it to the new saline flush **[4]**. Push saline through the extension line to verify the absence of leaks and confirm that the IV *(I-V)* line remains patent **[5]**. Then, twist the **OFF** tab to close the line to both syringes **[6]**. Slowly continue pushing the saline flush while simultaneously turning the **OFF** tab to fully seal the system **[7]**.
Videographer's Note: Shots 2.5.6. and 2.5.7. were confusing to the authors, we did our best to interpret the instructions

2.5.1. Talent returning to the antechamber.

2.5.2. Talent removing the used saline flush from the stopcock.

2.5.3. Talent attaching a new warmed saline flush to the stopcock.

2.5.4. The **OFF** tab being turned to close the line to the sample syringe and open it to the new saline flush.

2.5.5. Talent pushing saline through the extension line, observing for any leakage, and checking the intravenous line.

2.5.6. Talent twisting the **OFF** tab to close both syringe lines.

2.5.7. Talent slowly pushing saline while simultaneously turning the **OFF** tab to fully seal the system.

2.6. Once the extension line is confirmed to be functioning, return to the participant's room to wrap the IV *(I-V)* extension set with a self-adherent, elastic bandage to prevent dislodgment during sleep **[1]**. Tape the extension line securely to the bed frame **[2-TXT]**.

2.6.1. Talent wrapping the IV extension set with an elastic bandage.

2.6.2. Talent taping the extension line to the bed frame. **TXT: Discuss sleeping preferences with the participant and provide extra tubing accordingly**

3. Sampling Blood Through the Extension Line

3.1. Attach a 5-milliliter waste syringe, ensuring that the extension line is saline primed **[1-TXT]** and a 3-milliliter sample syringe to the stopcock **[2]**. Twist the **OFF** tab to open the line to the 5-milliliter waste syringe **[3]**.

3.1.1. Talent connecting a 5-milliliter waste syringe to the stopcock. **TXT: Ensure that the OFF tab blocks both syringes; Flush 4.4 mL warm saline if blood appears in the line**

3.1.2. Talent connecting a 3-milliliter sample syringe to the stopcock.

3.1.3. The OFF tab being turned to open the line to the 5 milliliter syringe.

3.2. Using the 5-milliliter syringe, collect at least 4.4 milliliters of waste **[1]**, then continue drawing until whole blood appears **[2]**. Now, twist the **OFF** tab of the stopcock to close the line to the 5-milliliter waste syringe **[3]**.

3.2.1. Talent collecting the waste fluid with the 5-milliliter syringe.

3.2.2. A shot of the visible whole blood in the waste syringe while drawing.

3.2.3. The **OFF** tab being twisted to close access to the 5-milliliter waste syringe.

3.3. Use the 3-milliliter syringe to draw the desired amount of blood **[1]** and twist the **OFF** tab to close the extension line to both syringes **[2]**.

3.3.1. Talent drawing blood into the 3 milliliter syringe.

3.3.2. Talent twisting the **OFF** tab to close both lines.

3.4. Remove the 5-milliliter waste syringe **[1]** and replace it with a warm 10 milliliter saline flush **[2]**. Twist the **OFF** tab to close the line to the empty 3-milliliter syringe, opening it to the saline flush **[3]**. Push up to 10 milliliters of warmed saline into the extension line, ensuring no blood remains in the line **[4]**. Twist the **OFF** tab to close the line to both syringes **[5]**.

3.4.1. Talent removing the 5-milliliter waste syringe.

3.4.2. Talent attaching the warm saline flush where the waste syringe was attached.

3.4.3. The **OFF** tab being turned to redirect flow to the saline flush.

3.4.4. Talent pushing saline into the extension line.

3.4.5. Talent twisting the **OFF** tab to close both syringe ports.

3.5. Slowly continue to push the saline flush while turning the **OFF** tab to maintain pressure in the extension line and minimize blood backflow **[1]**. Use any remaining saline to flush through the stopcock into the empty 3 milliliter syringe **[2]**. Then, remove the saline syringe and pull back on the 3-milliliter syringe to extract any remaining liquid from the stopcock **[3]**. Replace all syringes to prepare for the next blood draw **[4]**.
Videographer's Note: 3.5.1 was accidentally with shot 3.4.5

3.5.1. Talent slowly pushing the saline flush while adjusting the **OFF** tab.

3.5.2. Talent directing the remaining saline into the empty syringe through the stopcock.

3.5.3. Talent removing the saline syringe and pulling back on the 3 milliliter syringe to extract remaining liquid from the stopcock.

3.5.4. Talent replacing all syringes.

Results

4. Results

4.1. The 24-hour plasma melatonin concentration profile of a participant was measured under dim light conditions **[1]**.

4.1.1. LAB MEDIA: Figure 2.

4.2. The findings suggest that the melatonin levels follow a distinct rise-and-fall pattern **[1]** consistent with a normal circadian rhythm, confirming that the through-the-wall blood sampling method accurately captures hormonal changes during the biological night. **[2]**.

4.2.1. LAB MEDIA: Figure 2. *Video Editor: Highlight the plot in the graph*.

4.2.2. LAB MEDIA: Figure 2.

4.3. In a recent study, the success rate for through-the-wall blood sampling was 67%, corresponding to 304 out of 454 total blood draw attempts **[1]**.

4.3.1. LAB MEDIA: Figure 3. *Video editor: Highlight the black section of the vertical bar at the left labeled "67% / 304/454 draws".*

4.4. Unsuccessful blood draws accounted for 33% of attempts **[1]**, with 94% of these failures attributed to positional issues such as the participant lying on or occluding the extension line **[2]**.

4.4.1. LAB MEDIA: Figure 3. *Video editor: Highlight the red section of the vertical bar at the left labeled "33% / 150/454 draws".*

4.4.2. LAB MEDIA: Figure 3. *Video editor: Highlight the large red portion of the **pie chart** labeled "94% Positional issues".*

4.5. During an easy blood draw, polysomnography recordings showed stable electroencephalogram, electrooculogram, electromyogram, and electrocardiogram signals with no evidence of arousal or sleep stage change **[1].**

4.5.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the black square boxed region*.

4.6. A difficult blood draw was associated with disrupted electroencephalogram, electrooculogram, and electromyogram activity, along with an elevated heart rate indicative of arousal **[1]**.

4.6.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the black square boxed region.*

Pronunciation Guide:
1. Circadian
Pronunciation link:
https://www.merriam-webster.com/dictionary/circadian
IPA: /sərˈkeɪ.di.ən/
Phonetic Spelling: sir-KAY-dee-uhn

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2. Cardiometabolic
Pronunciation link:
https://www.merriam-webster.com/dictionary/cardiometabolic
IPA: /ˌkɑr.di.oʊˌmɛt̬.əˈbɑː.lɪk/
Phonetic Spelling: kar-dee-oh-met-uh-BAH-lik

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3. Insulin
Pronunciation link:
https://www.merriam-webster.com/dictionary/insulin
IPA: /ˈɪn.sə.lɪn/
Phonetic Spelling: IN-suh-lin

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4. Glucose
Pronunciation link:
https://www.merriam-webster.com/dictionary/glucose
IPA: /ˈgluː.koʊs/
Phonetic Spelling: GLOO-kohs

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5. Institutional
Pronunciation link:
https://www.merriam-webster.com/dictionary/institutional
IPA: /ˌɪn.stəˈtuː.ʃən.əl/
Phonetic Spelling: in-stuh-TOO-shun-uhl

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6. Antechamber
Pronunciation link:
https://www.merriam-webster.com/dictionary/antechamber
IPA: /ˈæn.t̬iˌtʃeɪm.bɚ/
Phonetic Spelling: AN-tee-chaym-ber

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7. Stopcock
Pronunciation link:
https://www.merriam-webster.com/dictionary/stopcock
IPA: /ˈstɑpˌkɑk/
Phonetic Spelling: STOP-kok

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8. Luer lock
Pronunciation link:
https://www.howtopronounce.com/luer-lock
IPA: /ˈluː.ər lɑːk/
Phonetic Spelling: LOO-er lok

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9. Saline
Pronunciation link:
https://www.merriam-webster.com/dictionary/saline
IPA: /ˈseɪ.liːn/
Phonetic Spelling: SAY-leen

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10. Patency (as in "line remains patent")
Pronunciation link:
https://www.merriam-webster.com/dictionary/patency
IPA: /ˈpeɪ.tən.si/
Phonetic Spelling: PAY-tuhn-see

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11. Melatonin
Pronunciation link:
https://www.merriam-webster.com/dictionary/melatonin
IPA: /ˌmɛl.əˈtoʊ.nɪn/
Phonetic Spelling: mel-uh-TOH-nin

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12. Polysomnography
Pronunciation link:
https://www.merriam-webster.com/dictionary/polysomnography
IPA: /ˌpɑː.li.sɑmˈnɑː.grə.fi/
Phonetic Spelling: pah-lee-sahm-NAH-gruh-fee

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13. Electroencephalogram
Pronunciation link:
https://www.merriam-webster.com/dictionary/electroencephalogram
IPA: /ɪˌlɛk.troʊ.ɪnˈsɛf.ə.ləˌgræm/
Phonetic Spelling: ih-LEK-troh-in-SEF-uh-loh-gram

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14. Electrooculogram
Pronunciation link:
https://www.merriam-webster.com/dictionary/electrooculogram
IPA: /ɪˌlɛk.troʊˈɑː.kjə.loʊˌgræm/
Phonetic Spelling: ih-LEK-troh-AH-kyuh-loh-gram

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15. Electromyogram

Pronunciation link:
https://www.merriam-webster.com/dictionary/electromyogram
IPA: /ɪˌlɛk.troʊˈmaɪ.oʊˌgræm/
Phonetic Spelling: ih-LEK-troh-MY-oh-gram

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16. Electrocardiogram
Pronunciation link:
https://www.merriam-webster.com/dictionary/electrocardiogram
IPA: /ɪˌlɛk.troʊˈkɑr.di.oʊˌgræm/
Phonetic Spelling: ih-LEK-troh-KAR-dee-oh-gram

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17. Colorado (place name)
Pronunciation link:
https://www.merriam-webster.com/dictionary/Colorado
IPA: /ˌkɑː.ləˈræd.oʊ/ or /ˌkɑː.ləˈrɑːd.oʊ/
Phonetic Spelling: kah-luh-RAD-oh or kah-luh-RAHD-oh

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18. Anschutz (as in Anschutz Medical Campus)
Pronunciation link:
https://www.howtopronounce.com/anschutz
IPA: /ˈæn.ʃuːts/
Phonetic Spelling: AN-shoots