FINAL SCRIPT: APPROVED FOR FILMING

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Title: High-Density Lipoprotein-Specific Phospholipid Efflux Assay

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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? **YES**

   If **Yes**, how far apart are the locations? Different areas of common lab space – 20 ft.

4. **Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length
Number of Steps: 16
Number of Shots: 42

Introduction

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

INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

1.1. **Edward Neufeld:** We are developing a surrogate assay to measure HDL-mediated removal of coronary artery plaque lipids to assess coronary artery disease risk.

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. **Edward Neufeld:** The current challenge is to develop a simple, rapid and robust HDL-lipid efflux assay for both research and clinical applications.

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

CONCLUSION:

~~What advantage does your protocol offer compared to other techniques?~~

1.3. **Edward Neufeld:** Our simple, rapid protocol, which predicts incident cardiovascular disease risk, can be used for both research and clinical diagnostic studies.

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

~~What new scientific questions have your results paved the way for?~~

1.4. **Edward Neufeld:** Our findings demonstrate that our assay can elucidate the role of both cellular and extracellular plaque lipids in cardiovascular disease.

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.

Protocol

2. Preparation of Fluorescent Phospholipid-Labeled Donor Lipid Particles

Demonstrator: Edward Neufeld

2.1. To begin, add all the required contents into a glass under a chemical fume hood **[1]** and vortex the lipid mixture briefly to mix the solutions **[2]**.

2.1.1. WIDE: Talent pipetting stock solutions into a borosilicate glass tube inside a fume hood.

2.1.2. Talent briefly vortexing the glass tube to mix the solutions.

2.2. Dry the mixture in a glass tube under a gentle stream of nitrogen for 1 hour to form a dry lipid film at the bottom of the tube **[1]**.

2.2.1. Talent placing the glass tube in a holder inside the fume hood, under a gentle nitrogen stream.

2.3. For coating calcium silicate hydrate, insert a weighing paper cone into the glass tube containing the dried lipids **[1]** and add 80 milligrams of calcium silicate hydrate powder into the tube using the cone to direct the powder to the base **[2]**. Immediately add 2 milliliters of normal saline to the tube **[3]** and cover the top with parafilm **[4]**.

2.3.1. Talent inserting a weighing paper cone into the glass tube.

2.3.2. Talent pouring calcium silicate hydrate powder through the cone into the glass tube.

2.3.3. Talent pipetting 2 milliliters of normal saline into the tube.

2.3.4. Talent sealing the top of the glass tube with parafilm.

2.4. Vortex the glass tube by hand to dislodge the bulk of the lipid from the bottom **[1]**. Place the glass tube into a hole made in a Styrofoam platform attached to a vortex **[2]** and secure the tube to the vortex platform using reinforced tape **[3]**.

2.4.1. Talent vortexing the glass tube by hand to dislodge lipid.

2.4.2. Talent inserting the glass tube into the Styrofoam platform mounted on a vortex.

2.4.3. Talent fastening the tube to the vortex with reinforced tape.

2.5. Vortex the glass tube for 10 minutes **[1]** and confirm that no lipid remains on the wall of the tube **[2]**. Transfer the contents of the glass tube to a 15-milliliter conical plastic tube using a 1 milliliter pipette **[3]**. Wash the glass tube with 3 milliliters of saline and transfer the wash into the same 15 milliliter tube **[4]**.

2.5.1. Talent operating the vortex with the glass tube secured.

2.5.2. Close-up of the inside of the glass tube showing no lipid residue on the wall.

2.5.3. Talent transferring the suspension from the glass tube into a 15 milliliter conical plastic tube.

2.5.4. Talent adding the glass tube with 3 milliliters of saline and transferring the wash into the same 15 milliliter tube.

2.6. Next, place the 15-milliliter plastic tube containing lipid-coated calcium silicate hydrate into a centrifuge and spin at 935 *g* for 2 minutes at 4 degrees Celsius **[1]**. Use a long and fine pipette tip to aspirate the supernatant **[2]**.

2.6.1. Talent placing the 15 milliliter conical tube into the centrifuge and starting the spin.

2.6.2. Talent picking up a pipette with long fine tip.

2.7. Starting from the top of the supernatant, glide the pipette tip slowly along the side of the tube while aspirating **[1]**. Tip the tube slightly during aspiration and leave approximately 200 microliters of saline above the pellet to avoid disturbing it **[2]**. Then, add saline to bring the total volume to 5 milliliters **[3]**.

2.7.1. Talent carefully aspirating the supernatant from the top down along the side of the tube.

2.7.2. Close-up of the pipette leaving a small volume of saline above the pellet.

2.7.3. Talent pipetting saline into the tube until the total volume reaches 5 milliliters.

2.8. Repeat the centrifugation and supernatant removal four times **[1]**. After the final wash, add saline to bring the total volume to 2.5 milliliters **[2]**.

2.8.1. Talent placing the tube in centrifuge.

2.8.2. Talent pipetting saline into the tube after the last wash to adjust the volume to 2.5 milliliters.

3. Incubation of Plasma/Serum Samples with Fluorescent Phospholipid-Labeled Donor Lipid Particles (LC-CSH)

3.1. Pipette 75 microliters of saline into each well of a 0.3 milliliter 96-well plate according to the number of wells needed for the sample set **[1-TXT]**.

3.1.1. Talent pipetting saline into the wells of a 96-well plate using a multi-well pipette. **TXT: Triplicate negative control (NC) wells: 100 µL saline; Duplicate positive control (PC) wells: 75 µL of saline**

3.2. Vortex the 15-milliliter plastic stock tube containing LC-CSH three times for 10 seconds each **[1]**. Using a single-well pipette, dispense 50 microliters of LC-CSH along the right side of the saline-containing wells **[2]**. After 3 wells, repeat the vortexing protocol before dispensing into the next three wells **[4]**.

3.2.1. Talent vortexing the 15 milliliter stock tube.

3.2.2. Talent pipetting 50 microliters of LC-CSH along the right side of a saline-containing well.

3.2.3. Talent vortexing the LC-CSH tube.

3.3. Rotate the 96-well plate by 180 degrees so that the left side of the wells is now to the right **[1]**. Pipette 25 microliters of plasma or serum samples along the right side of each well **[2]**. For the positive control wells, add 25 microliters of reference standard human normolipidemic plasma or serum **[3-TXT]**.

3.3.1. Talent rotating the 96-well plate by 180 degrees on the bench.

3.3.2. Talent pipetting 25 microliters of plasma or serum samples along the right side of the wells.

3.3.3. Talent pipetting 25 microliters of reference standard plasma or serum into the positive control wells. **TXT: Ensure that the total volume per well is 150 µL**

3.4. Next, seal the 96-well plate tightly with adhesive film **[1]** and incubate the sealed plate in the dark for 1 hour at 37 degrees Celsius and 1200 revolutions per minute in a thermomixer **[2]**. After incubation, remove the plate from the thermomixer and place it on ice **[3]**. Centrifuge the plate for 2 minutes at 935 *g* at 4 degrees Celsius to pellet the donor particles and stop the transfer reaction **[4]**.

3.4.1. Talent sealing the 96-well plate with adhesive film.

3.4.2. Talent placing the sealed plate into a thermomixer set to 37 degrees Celsius and 1200 revolutions per minute.

3.4.3. Talent removing the plate after incubation and placing it on ice.

3.4.4. Talent placing the 96-well plate into a centrifuge and starting the spin.

4. Measurement of Fluorescent Phospholipid Efflux to Plasma/Serum HDL

4.1. Now, carefully remove the adhesive film from the 96-well plate without disturbing the LC-CSH pellet **[1]**. Transfer 50 microliters of supernatant from the reaction plate into the wells of a black 96-well flat-bottom polystyrene plate using a multi-well pipette **[2]**. Fill a reservoir with normal saline and add 50 microliters of saline to each well using a multi-well pipette **[3]**.

4.1.1. Talent peeling off the adhesive film from the 96-well plate with steady hands.

4.1.2. Talent transferring 50 microliters of supernatant into a black polystyrene plate using a multi-well pipette.

4.1.3. Talent dispensing 50 microliters of saline into each well with a multi-well pipette.

4.2. Prepare 1 percent Triton X-100 in a 125-milliliter plastic Erlenmeyer flask **[1]**. Fill a reservoir with the prepared 1 percent Triton X-100 **[2]** and add 100 microliters of the same solution at room temperature into each well **[3]**. Mix by gently pipetting up and down 2 to 3 times with a multi-well pipette **[4]** and pop any bubbles using air from a 3-milliliter transfer pipette **[5]**.

4.2.1. Talent pouring 90 milliliters of distilled water into a 125 milliliter Erlenmeyer flask, followed by 10 milliliters of 10 percent Triton X-100.

4.2.2. Talent filling a liquid reservoir with the prepared 1 percent Triton X-100 solution.

4.2.3. Talent pipetting 100 microliters of Triton X-100 into the wells of the black plate.

4.2.4. Close-up of talent gently pipetting up and down in the wells to mix without creating bubbles.

4.2.5. Talent holding a 3 milliliter transfer pipette and using air to pop bubbles in the wells.

4.3. Finally, measure Lissaminerhodamine fluorescence using a fluorimeter with excitation at 540 nanometers and emission at 600 nanometers **[1]**.

4.3.1. Talent placing the sample in a fluorimeter.

Results

5. Results

5.1. A standard curve was generated using serially diluted LRh-PE-labeled LC-CSH and showed a highly linear fluorescence response **[1]**.

5.1.1. LAB MEDIA: Figure 4.

5.2. In the plasma concentration curve study, increasing volumes of pooled human plasma were dispensed in triplicate wells alongside saline controls **[1]**.

5.2.1. LAB MEDIA: Figure 6A.

5.3. The plasma dose response curve showed excellent linearity between percent PE efflux and plasma volume, with a strong correlation in the 15 to 35 microliter range **[1]**.

5.3.1. LAB MEDIA: Figure 6B.

Pronunciation Guide:

- **High-Density Lipoprotein**
  Pronunciation link: https://www.merriam-webster.com/dictionary/high-density%20lipoprotein
  IPA: /ˌhaɪ ˈdɛn.sɪ.ti ˈlɪp.oʊˌproʊ.tiːn/
  Phonetic Spelling: hy·den·si·tee lip·oh·proh·teen
- **Phospholipid**
  Pronunciation link: https://www.merriam-webster.com/dictionary/phospholipid
  IPA: /ˌfɑːs.foʊˈlɪp.ɪd/
  Phonetic Spelling: fahs·foh·lip·id
- **Efflux**
  Pronunciation link: https://www.merriam-webster.com/dictionary/efflux
  IPA: /ˈiːˌflʌks/
  Phonetic Spelling: ee·fluhks
- **Lipoprotein**
  Pronunciation link: https://www.merriam-webster.com/dictionary/lipoprotein
  IPA: /ˌlɪp.oʊˈproʊ.tiːn/
  Phonetic Spelling: lip·oh·proh·teen
- **Surrogate**
  Pronunciation link: https://www.merriam-webster.com/dictionary/surrogate
  IPA: /ˈsɝː.ə.gət/
  Phonetic Spelling: sur·uh·git
- **Cardiovascular**
  Pronunciation link: https://www.merriam-webster.com/dictionary/cardiovascular
  IPA: /ˌkɑːr.di.oʊˈvæs.kjə.lɚ/
  Phonetic Spelling: kar·dee·oh·vas·kyuh·ler
- **Coronary**
  Pronunciation link: https://www.merriam-webster.com/dictionary/coronary
  IPA: /ˈkɔːr.əˌner.i/
  Phonetic Spelling: kor·uh·nerr·ee
- **Fluorescent**
  Pronunciation link: https://www.merriam-webster.com/dictionary/fluorescent
  IPA: /flʊˈrɛs.ənt/
  Phonetic Spelling: floor·eh·suhnt

- **Borosilicate**
  Pronunciation link: https://www.merriam-webster.com/dictionary/borosilicate
  IPA: /ˌbɔːr.oʊˈsɪl.ɪ.keɪt/
  Phonetic Spelling: bor·oh·sil·ih·kayt
- **Calcium Silicate Hydrate**
  Pronunciation link: https://www.merriam-webster.com/dictionary/calcium%20silicate
  IPA: /ˈkæl.si.əm ˈsɪl.ɪ.keɪt ˈhaɪ.dreɪt/
  Phonetic Spelling: kal·see·um sil·ih·kayt hy·drayt
- **Centrifuge**
  Pronunciation link: https://www.merriam-webster.com/dictionary/centrifuge
  IPA: /ˈsɛn.trəˌfjuːdʒ/
  Phonetic Spelling: sen·truh·fyooj
- **Supernatant**
  Pronunciation link: https://www.merriam-webster.com/dictionary/supernatant
  IPA: /ˌsuː.pɚˈneɪ.tənt/
  Phonetic Spelling: soo·per·nay·tuhnt
- **Pellet**
  Pronunciation link: https://www.merriam-webster.com/dictionary/pellet
  IPA: /ˈpɛl.ɪt/
  Phonetic Spelling: pel·it
- **Normolipidemic**
  Pronunciation link: https://www.merriam-webster.com/dictionary/normolipidemic
  IPA: /ˌnɔːr.moʊˌlɪp.ɪˈdiː.mɪk/
  Phonetic Spelling: nor·moh·lip·ih·dee·mik
- **Thermomixer**
  Pronunciation link: https://www.howtopronounce.com/thermomixer
  IPA: /ˈθɝː.moʊˌmɪk.sɚ/
  Phonetic Spelling: ther·moh·mik·ser
- **Polystyrene**
  Pronunciation link: https://www.merriam-webster.com/dictionary/polystyrene
  IPA: /ˌpɑː.liˈstaɪ.riːn/
  Phonetic Spelling: pah·lee·sty·reen
- **Triton X-100**

Pronunciation link: https://www.howtopronounce.com/triton
IPA: /ˈtraɪ.tɑːn/
Phonetic Spelling: try·ton

- **Erlenmeyer**
  Pronunciation link: https://www.merriam-webster.com/dictionary/Erlenmeyer
  IPA: /ˈɝː.lənˌmaɪ.ɚ/
  Phonetic Spelling: er·luhn·my·er

- **Fluorimeter**
  Pronunciation link: https://www.merriam-webster.com/dictionary/fluorimeter
  IPA: /flʊˈrɑː.mə.tɚ/
  Phonetic Spelling: floor·ah·mih·ter

- **Lissaminerhodamine**
  Pronunciation link: https://www.howtopronounce.com/lissamine
  IPA: /ˈlɪs.ə.miːn ˈroʊ.də.miːn/
  Phonetic Spelling: lis·uh·meen roh·duh·meen