FINAL SCRIPT: APPROVED FOR FILMING

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Title: Harvest of Vestibular End-Organs under Physiologic Conditions During Labyrinthectomy

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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: 9
Number of Shots: 17

Introduction

NOTE: Only the interview was filmed by the videographer

1.1. **Nicholas Andresen or Bryan Ward:** This protocol describes a new technique for harvesting human inner ear tissue during labyrinthectomy using an underwater technique. We are using this technique to better understand inner ear disorders such as Meniere's disease.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.2.2*

What research gap are you addressing with your protocol?

1.2. **Nicholas Andresen or Bryan Ward:** Human inner ear diseases are difficult to study because the human inner ear is encased in dense bone. Previous studies have primarily relied upon post-mortem tissue analysis or high-resolution imaging. This protocol offers a new means of directly studying human inner ear tissue.

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

What advantage does your protocol offer compared to other techniques?

1.3. **Nicholas Andresen or Bryan Ward:** The inner ear tissue is kept under physiologic conditions using balanced salt solution. Additionally, the underwater technique and increased magnification afforded by the endoscope allow the easy visualization of a membranous labyrinth, aiding in trauma-free dissection.

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*

Videographer: Obtain headshots for all authors.

Ethics Title Card

This research has been approved by the Institutional Review Board at Johns Hopkins University School of Medicine

NOTE: The protocol shots were filmed by the authors themselves and they have given timestamps. The writer has not reviewed the footage.

2. Fresh Human Vestibular End Organ Isolation

Demonstrators: Nicholas Andresen and Bryan Ward

~~2.1. To begin, perform mastoidectomy and identify the semicircular canals inside it under the operating microscope **[1]**.~~

~~2.1.1. WIDE: Talent examining the sample under the operating microscope~~. **NOTE**: Not filmed

2.2. To begin, perform mastoidectomy and submerge the mastoid cavity in a balanced salt solution **[1]** and visualize the labyrinth using a zero-degree endoscope with a lens-cleaning sheath irrigation system **[2]**.

2.2.1. Talent placing the mastoid cavity in a dish filled with balanced salt solution.

2.2.2. Talent using the zero-degree endoscope for visualization, with the lens-cleaning sheath system visible.

2.3. Then, irrigate the mastoid cavity with balanced salt solution to wash away blood and improve visualization of the labyrinth **[1]**.

2.3.1. Talent irrigating the mastoid cavity. Author provided timecode: CH2_004_CH001 1:10-1:20

2.4. Using the endoscope for visualization, carefully drill away the otic capsule bone **[1]**.~~ with a 3-millimeter diamond burr until the semicircular canals appear as a bluish line **[1]**. Irrigate intermittently with balanced salt solution to wash away blood and ensure adequate visualization of the semicircular canals **[2]**~~.

2.4.1. TEXT ON PLAIN BACKGROUND:
Use a 3-millimeter diamond burr for drilling

Irrigate intermittently with balanced salt solution

~~2.4.2. Talent using the endoscope and holding the 3-millimeter diamond burr while drilling the otic capsule bone.~~. **NOTE**: Not filmed

2.4.3. ~~Talent using the lens-cleaning sheath irrigation system to flush the area~~.

2.5. Under balanced salt solution, enter the dome of the lateral semicircular canal and follow it anteriorly until its ampullae are identified **[1]**. Enter the superior semicircular

canal and follow it medially to its ampullae **[2]**. Then, cut the lateral semicircular canal duct sharply to facilitate removal **[3]**.

2.5.1. Shot of entering the dome of the lateral semicircular canal with the endoscope. Author provided timecode: CH2_004_CH001 1:20-1:25

2.5.2. Shot of following the superior semicircular canal with precise drilling. Author provided timecode: CH2_004_CH001 4:00-14:00

2.5.3. Shot of cutting the lateral semicircular canal duct. Author provided timecode: CH2_004_CH001 02:30

2.6. Next, employing a Rosen needle, elevate the horizontal and superior semicircular canal ampullae off the crista **[1]**. Harvest the required structures and place all tissues in balanced salt solution **[2]**.

2.6.1. Shot of elevating the ampullae using a Rosen needle. Author provided timecode: CH2_004_CH002 7:50-15:00 and CH2_004_CH003 0:00-10:10

2.6.2. TEXT ON PLAIN BACKGROUND:

- Separate the afferent fibres from the epithelia and the membranous labyrinth
- Transect the dome of the posterior semicircular canal
- Harvest the ampullae

2.6.3. ~~Talent removing the ampullae and separating the afferent fibers~~. . **NOTE**: Not filmed

~~2.7. Transect the dome of the posterior semicircular canal and follow it inferiorly and anteriorly to its ampullae **[1]**. Harvest the ampullae and place all tissues in balanced salt solution on ice **[2]**.~~

~~2.7.1. Shot of transecting the posterior semicircular canal.~~

2.7.2. ~~Talent placing harvested tissues into a dish containing balanced salt solution on ice~~. . **NOTE**: Not filmed

2.8. Remove the bone between the horizontal and posterior semicircular canal ampullae to expose the vestibule **[1]**. Elevate and remove the macula while maintaining the fluid level **[2]**.

2.8.1. Shot of removing bone to expose the vestibule. Author provided timecode: CH2_004_CH003 12:50-15:00 and CH2_004_CH004 0:00-2:30

2.8.2. Shot of macula being elevated and removed. Author provided timecode: CH2_004_CH004 2:30-4:15

2.9. Finally, sharply elevate and remove the saccule from the spherical recess and place the tissue samples in balanced salt solution on ice **[1-TXT]**.

2.9.1. Shot of elevating and removing the saccule from the recess. Author provided timecode: CH2_004_CH005 2:00-3:00 **TXT: Perform immunohistochemistry and imaging**

2.9.2. ~~Talent placing harvested saccule tissue into a dish with balanced salt solution on ice~~. . **NOTE**: Not filmed

Results

3. Representative Results

3.1. The human utricle **[1]** and lateral and superior canal ampullae were harvested intact with minimal trauma **[2]**.

3.1.1. LAB MEDIA: Figure 2. *Video editor: Focus on the image labeled A*.

3.1.2. LAB MEDIA: Figure 2. *Video editor: Focus on the image labeled B*.

3.2. Immunofluorescent labeling showed intact type 1 vestibular hair cells in the utricle **[1]**. Hair cell density was recorded at 82 cells per 10,000 square micrometers **[2]**.

3.2.1. LAB MEDIA: Figure 3. *Video editor: Highlight the red-stained hair cells (type 1 vestibular cells) pointed by the white arrows*.

3.2.2. LAB MEDIA: Figure 3. *Video editor: Focus on the image B*.

Pronunciation Guide

1. Mastoidectomy

Pronunciation link:
https://www.merriam-webster.com/medical/mastoidectomy
IPA: /ˌmæstɔɪˈdɛktəmi/
Phonetic Spelling: ma-stoy-dek-tuh-mee

2. Semicircular

Pronunciation link:
https://www.merriam-webster.com/dictionary/semicircular
IPA: /ˌsɛm.iˈsɝː.kjə.lɚ/
Phonetic Spelling: seh-mee-sur-kyuh-lur

3. Labyrinth

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

IPA: /ˈlæbəˌrɪnθ/
Phonetic Spelling: la-buh-rinth

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4. Endoscope

Pronunciation link:
https://www.merriam-webster.com/dictionary/endoscope
IPA: /ˈɛn.dəˌskoʊp/
Phonetic Spelling: en-duh-skohp

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5. Irrigation

Pronunciation link:
https://www.merriam-webster.com/dictionary/irrigation
IPA: /ˌɪrəˈgeɪʃən/
Phonetic Spelling: ih-ruh-gay-shun

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6. Ampullae

Pronunciation link:
https://www.howtopronounce.com/ampullae
IPA: /ˈæmpjʊˌliː/
Phonetic Spelling: am-pyuh-lee

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7. Crista

Pronunciation link:
https://www.howtopronounce.com/crista
IPA: /ˈkrɪstə/
Phonetic Spelling: kris-tuh

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8. Afferent

Pronunciation link:
https://www.merriam-webster.com/dictionary/afferent
IPA: /ˈæfərənt/
Phonetic Spelling: af-uh-ruhnt

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9. Epithelia

Pronunciation link:
https://www.howtopronounce.com/epithelia
IPA: /ˌɛpəˈθiːliə/
Phonetic Spelling: eh-puh-thee-lee-uh

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10. Membranous

Pronunciation link:
https://www.merriam-webster.com/dictionary/membranous
IPA: /ˈmɛmbrənəs/
Phonetic Spelling: mem-bruh-nuhs

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11. Vestibule

Pronunciation link:
https://www.merriam-webster.com/dictionary/vestibule
IPA: /ˈvɛstɪˌbjuːl/
Phonetic Spelling: veh-stuh-byool

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12. Macula

Pronunciation link:
https://www.merriam-webster.com/dictionary/macula
IPA: /ˈmækjʊlə/
Phonetic Spelling: mak-yuh-luh

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13. Saccule

Pronunciation link:
https://www.merriam-webster.com/medical/saccule
IPA: /ˈsæk.juːl/
Phonetic Spelling: sak-yool

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14. Utricle

Pronunciation link:
https://www.merriam-webster.com/medical/utricle
IPA: /ˈjuːtrɪkəl/
Phonetic Spelling: yoo-tri-kuhl

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15. Immunofluorescent

Pronunciation link:
https://www.howtopronounce.com/immunofluorescent
IPA: /ɪˌmjunəʊflʊˈrɛsənt/
Phonetic Spelling: ih-myoo-noh-floo-reh-suhnt