

- 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**
- 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. **Yes**

Current Protocol Length

Number of Steps: 19

Number of Shots: 41

Interviews

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

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

Authors: The answers are slightly edited in accordance with the journal guidelines, and trimmed to the five statements the video can carry.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Susanne Brueckner:** Our research focuses on quantifying fluoride concentrations in the serum, bone, and teeth of fluoride-treated mice.

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

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Susanne Brueckner:** Existing methods need large samples or expensive instruments, whereas this protocol measures fluoride in milligram or microliter samples.

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

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Susanne Brueckner:** This protocol accurately measures fluoride concentrations in serum and mineralized tissues from very small sample volumes.

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

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Susanne Brueckner:** It is critical that the precisely weighed ash transfers fully into the diffusion dish, as any loss compromises the result.

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

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Maiko Suzuki:** We hope future studies use this approach to uncover how fluoride accumulation influences tissue health, disease risk, and aging.

- 1.5.1. 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.

Promotional Interview Questions:

Videographer: Please capture these shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Statements will be presented live by the authors, sharing their spontaneous perspectives.

Were there any surprising findings or observations during the development or validation of this protocol, particularly when comparing adolescent and mature mice?

1.6. : (authors will present their testimonial statements live)

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

If another laboratory were implementing this protocol for the first time, what advice would you give them to ensure reliable and reproducible results?

1.7. **Enter author name:** (authors will present their testimonial statements live)

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

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee at
Nova Southeastern University

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Specimen Preparation for Fluoride Measurement

Demonstrator: Susanne Brueckner

2.1. To begin, excise the femora from adolescent and mature C57BL/6J (*see-fifty-seven-B-L-six-J*) mice given either sodium fluoride-supplemented or fluoride-free drinking water [1-TXT]. After drying, transfer the femora into an alumina crucible [2]. Then, ash them in a muffle furnace at 600 degrees Celsius for 6 hours, using a ramp rate of 150 degrees Celsius per hour [3].

2.1.1. WIDE: Talent showing the extracted femur specimens. **TXT: See the text protocol for detailed specimen preparation**

2.1.2. Talent placing the bone in alumina crucible.

2.1.3. Talent placing the sample in a furnace.

Authors: The blood-centrifuge shot is removed and the bone alone now goes into the furnace. The bone dwell time is corrected to 6 hours as per manuscript step 2.2.5

3. Diffusion Dish Preparation and Bone Ash Loading

3.1. Place a 60 by 15 millimeter untreated Petri dish on a clean surface [1].

3.1.1. WIDE: Talent placing a 60 × 15 millimeter untreated Petri dish on the work surface.

3.2. Using a heated 18-gauge syringe needle, melt a small hole into the lid of the Petri dish [1].

3.2.1. Talent melting a small hole into the lid of the Petri dish with a heated 18-gauge syringe needle.

3.3. Next, with a 10-milliliter syringe fitted with a blunt 14-gauge needle [1], coat the inner rim of the Petri dish lid with a continuous petrolatum seal [2]. ~~Dispose of all the used needles in an approved institutional sharps waste container [3].~~

3.3.1. Close up of the talent pointing to a 10 milliliter syringe fitted with a blunt 14-

gauge needle.

3.3.2. Talent applying a continuous petrolatum seal around the inner rim of the Petri dish lid with the syringe.

~~3.3.3. Talent disposing of all the used needles in an approved institutional sharps waste container.~~ **NOTE: Not filmed**

3.4. Using a micro spatula, grind the ashed bones inside the 1.5 milliliter microcentrifuge tubes into a fine powder [1], and proceed to process the pulverized bone ash immediately. Dispose of the consumables contaminated with the bone ash in the institutional chemical waste stream [2].

3.4.1. Talent grinding the ashed bones inside a 1.5 milliliter microcentrifuge tube with a micro spatula.

3.4.2. Talent disposing of the consumables contaminated with the bone ash in the institutional chemical waste stream.

3.5. Transfer the milled bone ash into a small porcelain dish to minimize the static charge and prevent powder dispersion [1].

3.5.1. Talent transferring the milled bone ash from the microcentrifuge tube into a small porcelain dish. **Authors: This step is moved here**

3.6. Next, place the empty bottom of the Petri dish onto an analytical balance with a readability of at least 0.1 milligram [1]. Using a micro spatula, add approximately 5 milligrams of the bone ash [2] and record the exact weight [3].

3.6.1. Talent placing the empty bottom of the Petri dish onto the weighing pan of an analytical balance.

3.6.2. Talent adding approximately 5 milligrams of the bone ash to the Petri dish with a micro spatula.

3.6.3. Talent recording the exact weight of the bone ash specimen.

3.7. Immediately cover the weighed ash with 3 milliliters of ultrapure water [1].

3.7.1. Talent covering the bone ash by adding 3 milliliters of the ultrapure water while the Petri dish remains on the analytical balance.

Authors: Weighing now comes before hydration

4. Diffusion Dish Assembly

- 4.1. Add 50 microliters of 0.05 normal sodium hydroxide in three drops onto the inner surface of the Petri dish lid, away from the rim to form a fluoride trap [1].
 - 4.1.1. Close up of Talent dispensing three drops of 0.05 normal sodium hydroxide onto the inner surface of the Petri dish lid away from the rim.
- 4.2. Invert the Petri dish lid without disrupting the sodium hydroxide drops [1]. Tightly seal the lid to the bottom of the Petri dish with the petrolatum [2].
 - 4.2.1. Talent inverting the Petri dish lid while keeping the sodium hydroxide drops intact.
 - 4.2.2. Talent sealing the Petri dish lid to the bottom of the Petri dish with the petrolatum.
- 4.3. Prepare a 3 normal HMDS-saturated sulfuric acid solution by mixing equal volumes of the 6 normal HMDS-saturated sulfuric acid and ultrapure water [1-TXT].
 - 4.3.1. Talent mixing equal volumes of the 6 normal hexamethyldisiloxane-saturated sulfuric acid solution and ultrapure water. **TXT: HMDS: Hexamethyldisiloxane**
- 4.4. Immediately load 3 milliliters of the prepared solution into a 10-milliliter syringe fitted with a 20-gauge needle [1]. Inject the solution through the hole in the lid into the water at the bottom of the diffusion dish [2]. Carefully withdraw the needle without splashing acid onto the inner surface of the lid [3].
 - 4.4.1. Talent loading 3 milliliters of the solution into a 10 milliliter syringe fitted with a 20-gauge needle.
 - 4.4.2. Talent injecting the solution through the hole in the Petri dish lid into the water at the bottom of the diffusion dish.
 - 4.4.3. Talent carefully withdrawing the syringe needle without splashing the acid onto the inner surface of the Petri dish lid.
- 4.5. Quickly seal the injection hole completely with a bead of petrolatum [1]. Place the diffusion dishes on a variable rocker set to a slow speed and incubate overnight at room temperature [2].
 - 4.5.1. Talent sealing the injection hole with a bead of petrolatum that completely covers the opening.
 - 4.5.2. WIDE: Talent placing the diffusion dishes on a variable rocker for overnight incubation at room temperature.

5. Fluoride Measurement with an Ion-Selective Electrode (ISE)

5.1. Before starting the measurements, set up the filled ion-selective electrode in the holder and connect it to the potentiometer [1]. ~~or ISE, in advance, [1]. Fill the double channel fluoride selective electrode with the appropriate filling solution [2], connect it to the potentiometer, and secure it in an electrode holder [3].~~

5.1.1. WIDE: Talent pointing out the assembled fluoride-selective electrode secured in its holder, the connection of the electrode to the potentiometer and the potentiometer itself. **Author's NOTE:** This was all one movement and it didn't make sense to break it up in several shots.

~~5.1.2. Talent adding the filling solution.~~

~~5.1.3. Talent connecting connecting it to the potentiometer.~~ **Author's NOTE:** Solution was added beforehand. The electrode will be filled once for the days, weeks, or months it is in continuous use. It didn't make sense to show the filling at that point.

5.2. Position an untreated Petri dish on a scissor jack beneath the sensing surface of the electrode, ensuring that the sensing surface is parallel to the bottom of the dish [1]. Raise the table of the scissor jack until the Petri dish is almost touching the electrode [2].

5.2.1. Talent positioning an untreated Petri dish on a scissor jack beneath the sensing surface of the electrode and aligning the sensing surface parallel to the bottom of the Petri dish.

5.2.2. Talent raising the table of the scissor jack until the Petri dish is almost touching the electrode.

5.3. After the incubation period, remove the diffusion dish from the rocker [1]. Carefully lift off the lid by breaking the petrolatum seal [2]. Then, invert the lid onto a flat surface without disturbing the three fluoride-trap drops [3].

5.3.1. WIDE: Talent removing the diffusion dish from the rocker after the incubation period.

5.3.2. Talent carefully lifting off the lid by breaking the petrolatum seal.

5.3.3. Talent inverting the lid onto a flat surface while keeping the three fluoride trap drops undisturbed. **Author's NOTE:** Step 5.3.3. was combined with shot 5.3.2

- 5.4. Next, use a P20 pipette to add 15 to 20 microliters of 0.2 normal acetic acid to one of the fluoride trap drops [1] and merge all three drops into a single drop without changing the pipette tip. Keep the combined sample on the inverted lid [2].
- 5.4.1. Talent adding 15 to 20 microliters of 0.2 normal acetic acid to one of the fluoride trap drops with a P20 pipette.
- 5.4.2. Talent merging all three fluoride trap drops into a single drop without changing the pipette tip. **Author's NOTE: Step 5.4.2. was integrated into step 5.4.1.**
- 5.5. Set a P100 pipette to 75 microliters [1]. Aspirate the entire volume of the fluoride trap without introducing air [2] and adjust the sample volume to exactly 75 microliters using ultrapure water. Retain the sample in the pipette tip and proceed immediately with the measurement [3].
- 5.5.1. Talent setting a P100 pipette to 75 microliters.
- 5.5.2. Talent aspirating the entire volume of the fluoride trap without introducing air
- 5.5.3. Talent adjusting the sample volume to exactly 75 microliters using ultrapure water. **Author's NOTE: 5.5.2 and 5.5.3 were combined in 1 action**
- 5.6. Dispense the sample between the sensing surface of the fluoride-selective electrode and the Petri dish [1]. Ensure that the entire sensing surface is submerged in the liquid without contacting the Petri dish [2]. Allow the reading to stabilize, then record the electrode potential in millivolts from the potentiometer display [3].
- 5.6.1. Talent dispensing the sample between the sensing surface of the fluoride-selective electrode and the Petri dish.
- 5.6.2. Close up of Talent checking that the entire sensing surface is submerged in the liquid without directly contacting the Petri dish.
- 5.6.3. Close-up of the stabilized electrode potential in millivolts on the potentiometer display.

Results

6. Results

6.1. In mice given fluoride-free drinking water, the femoral fluoride concentration was significantly lower in the adolescents [1] than in the mature mice [2].

6.1.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the open white bar under the fluoride label '-'.*

6.1.2. LAB MEDIA: Figure 4B. *Video editor: Highlight the grey bar under the fluoride label '-'.*

6.2. In contrast, exposure to 125 parts per million fluoride reversed this pattern, with significantly higher femoral fluoride concentrations in the adolescents [1] than in the mature mice [2].

6.2.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the open white bar under the fluoride label '+'.*

6.2.2. LAB MEDIA: Figure 4B. *Video editor: Highlight the grey bar under the fluoride label '+'.*

6.3. Following fluoride exposure, the adolescent mice showed a 14-fold increase in femoral fluoride concentration [1], whereas the mature mice showed a smaller 3-fold increase [2].

6.3.1. LAB MEDIA: Figure 5A. *Video editor: Highlight the open white bar under the fluoride label '+'.*

6.3.2. LAB MEDIA: Figure 5A. *Video editor: Highlight the grey bar under the fluoride label '+'.*

1. Fluoride

Pronunciation link: <https://www.merriam-webster.com/dictionary/fluoride>

IPA: /'flɒr,aɪd/

Phonetic Spelling: floor·ide

2. Murine

Pronunciation link: <https://www.merriam-webster.com/dictionary/murine>

- IPA: /'mjɔr,am/
Phonetic Spelling: myur·ine
3. Hexamethyldisiloxane
Pronunciation link: No confirmed link found
IPA: /,hɛksə,mɛθəl,daisɪ'la:kseɪn/
Phonetic Spelling: hek·suh·meth·uhl·dye·sih·lok·sayn
 4. Femora
Pronunciation link: <https://www.merriam-webster.com/dictionary/femur>
IPA: /'femərə/
Phonetic Spelling: fem·uh·ruh
 5. C57BL/6J
Pronunciation link: No confirmed link found
IPA: /,si:ˌfɪftiˈseɪənˌbi:ˈelˌsɪksˈdʒeɪ/
Phonetic Spelling: see·fif·tee·sev·uhn·bee·el·siks·jay
 6. Alumina
Pronunciation link: <https://www.merriam-webster.com/dictionary/alumina>
IPA: /əˈlu:mənə/
Phonetic Spelling: uh·loo·muh·nuh
 7. Crucible
Pronunciation link: <https://www.merriam-webster.com/dictionary/crucible>
IPA: /'kru:səbəl/
Phonetic Spelling: kroo·suh·buhl
 8. Petri
Pronunciation link: <https://www.merriam-webster.com/dictionary/Petri%20dish>
IPA: /'pi:tri/
Phonetic Spelling: pee·tree
 9. Petrolatum
Pronunciation link: <https://www.merriam-webster.com/dictionary/petrolatum>
IPA: /,petrəˈleɪtəm/, /,petrəˈla:təm/
Phonetic Spelling: peh·truh·lay·tuhm; peh·truh·laa·tuhm
 10. Microcentrifuge
Pronunciation link: No confirmed link found
IPA: /,maɪkroʊ'sentrəˌfju:dʒ/
Phonetic Spelling: my·kroh·sen·truh·fyooj
 11. Pulverized
Pronunciation link: <https://www.merriam-webster.com/dictionary/pulverize>
IPA: /'pʌlvəˌraɪzd/
Phonetic Spelling: pul·vuh·rized
 12. Porcelain
Pronunciation link: <https://www.merriam-webster.com/dictionary/porcelain>
IPA: /'pɔrsələn/
Phonetic Spelling: por·suh·luhn
 13. Microliters
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>

- IPA: /'maɪkroʊˌliːtər/
Phonetic Spelling: my·kroh·lee·ter
14. Sodium hydroxide
Pronunciation link: <https://www.merriam-webster.com/dictionary/sodium%20hydroxide>
IPA: /'soʊdiəm haɪ'drɔːksaɪd/
Phonetic Spelling: soh·dee·uhm hy·drok·side
15. HMDS
Pronunciation link: No confirmed link found
IPA: /,eɪtʃˌɛmˌdiː'ɛs/
Phonetic Spelling: aitch·em·dee·ess
16. Sulfuric acid
Pronunciation link: <https://www.merriam-webster.com/dictionary/sulfuric%20acid>
IPA: /səl'fjʊrɪk 'æsaɪd/
Phonetic Spelling: sul·fyur·ik as·id
17. Ion-selective
Pronunciation link: No confirmed link found
IPA: /'aɪən sə'lektɪv/
Phonetic Spelling: eye·uhn suh·lek·tiv
18. Electrode
Pronunciation link: <https://www.merriam-webster.com/dictionary/electrode>
IPA: /ɪ'lekˌtroʊd/
Phonetic Spelling: ih·lek·trohd
19. Potentiometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/potentiometer>
IPA: /pəˌtɛnʃi'ɑːmɪtər/
Phonetic Spelling: puh·ten·shee·aa·muh·ter
20. Acetic acid
Pronunciation link: <https://www.merriam-webster.com/dictionary/acetic%20acid>
IPA: /ə'siːtɪk 'æsaɪd/
Phonetic Spelling: uh·see·tik as·id
21. Aspirate
Pronunciation link: <https://www.merriam-webster.com/dictionary/aspirate>
IPA: /'æspəˌreɪt/
Phonetic Spelling: as·puh·rayt
22. Millivolts
Pronunciation link: <https://www.merriam-webster.com/dictionary/millivolt>
IPA: /'mɪləˌvoʊlts/
Phonetic Spelling: mil·uh·vohlts
23. Femoral
Pronunciation link: <https://www.merriam-webster.com/dictionary/femoral>
IPA: /'femərəl/
Phonetic Spelling: fem·uh·ruhl