

Submission ID #: 70979

Scriptwriter Name: Praveen Kumar

Project Page Link: https://review.jove.com/files_upload.php?src=21648643

Title: Sex Hormone Detection in Human Tear Film using Enzyme-linked Immunosorbent Assays

Authors and Affiliations:

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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**

- 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: 14

Number of Shots: 35

Interviews

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

INTRODUCTION:

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

- 1.1. **Nutchaarpa Puangmalai:** Our research focuses on women's health in ocular surface diseases and adaptation of ELISA for sex hormone quantification in basal tear fluid.
 - 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. **Nutchaarpa Puangmalai:** Traditional ELISAs require large sample volumes, making tear fluid analysis challenging. This protocol enables hormone or protein quantification from limited tear 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. **Anna Tichenor:** This protocol allows researchers to measure sex hormone concentration by ELISA in basal tear fluid collected by using micro-capillary tubes.
 - 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. **Anna Tichenor:** The tear film is easily accessible for ocular surface research, but limited tear volume often requires sample collection across multiple visits.
 - 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. **Anna Tichenor**: Future studies can use this protocol to investigate how tear hormone levels correlate with diseases or monitor treatment effectiveness.
 - 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.

Ethics Title Card

This research has been approved by the Institutional Review Board (IRB) at Indiana University

Protocol

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

2. Tear Film Collection and Storage

Demonstrator: Anna Tichenor

2.1. Begin by obtaining appropriate microcapillary tubes to collect tears and a 1.5-milliliter microcentrifuge tube for tear film storage [1].

2.1.1. WIDE: Talent walking into the room with microcapillary tubes placed in a rack. **Videographer:** Please obtain CU shot as well for this shot.

~~2.2. Take the rubber squeeze bulb from the microcapillary tube kit to facilitate transfer of tear fluid into a microcentrifuge tube [1].~~

~~2.2.1. Talent taking the rubber squeeze bulb out from the box for tear fluid collection.~~ **NOTE: Not filmed**

2.3. To collect the basal tears, instruct the participant to look straight ahead or slightly upward [1]. Then, gently place the microcapillary tube opening in the tear meniscus near the lateral canthus, avoiding lid and conjunctival contact to minimize reflex tearing [2]. Fill the tube with tears until it's full [3].

2.3.1. Talent is instructing the participant to look straight ahead or slightly upward.

2.3.2. Talent placing the microcapillary tube opening in the tear meniscus near the lateral canthus.

2.3.3. Close-up of the tube being filled with tears until it's full.

2.4. Occlude the hole on the rubber squeeze bulb [1], insert the microcapillary tube into the bulb [2], and place the open end into the microcentrifuge tube [3]. Gently squeeze the bulb to release the tear into the microcentrifuge tube [4]. Close the microcentrifuge tube cap to prevent evaporation [5].

2.4.1. Talent blocking the hole of the rubber squeeze bulb with a finger. **NOTE: 2.4.1 to 2.4.5 are combined in a single shot**

2.4.2. Talent inserting the microcapillary tube in the rubber squeeze bulb.

- 2.4.3. Talent placing open end of microcapillary tube in the 1.5 mL microcentrifuge tube.
- 2.4.4. Talent squeezing the bulb and tear is getting collected in 1.5 mL microcentrifuge tube.
- 2.4.5. Talent closing the cap of the microcentrifuge tube.
- 2.5. Spin the tube at 2,000 *g* for 3 to 5 seconds to collect the sample at the bottom [1]. Then, store the sample at minus 80 degrees Celsius for preserving analyte stability prior to analysis [2].
 - 2.5.1. Talent placing the tubes in a benchtop microcentrifuge device and closing the lid. *Videographer: If possible, film the setting/parameters of the device.*
 - 2.5.2. Talent placing the samples at -80 °C.

3. Estradiol Assay

Demonstrator: Nutcharpa Puangmalai

- 3.1. Obtain all required reagents and materials and let them reach room temperature [1].
 - 3.1.1. WIDE: Talent placing required reagents on the workbench.
- 3.2. Aliquot only the required amounts of ready-to-use standards and controls to minimize contamination and repeated temperature changes [1].
 - 3.2.1. Talent dispensing the reagent into a small 1.5 mL microcentrifuge tube.
- 3.3. Prepare six standard solutions labeled as number zero to five [1]. Include one control sample provided with the kit [2]. Keep the frozen samples on ice for thawing [3]. Spin down the tear samples [4] to ensure samples are at the bottom of the tube [5].
 - 3.3.1. Talent preparing standard solution in a vial labeled as No.5 and other standards labeled vials labeled from 0 to 4 are in the background
 - 3.3.2. Talent placing control sample on the workbench.
 - 3.3.3. Talent placing samples on ice.
 - 3.3.4. Talent placing the sample in spinner
 - 3.3.5. Shot of samples at bottom after spinning

- 3.4. Mix all standards, controls, and samples prior to pipetting into the wells for homogeneous distribution [1]. Add 20 microliters of each standard, control, and sample into wells [2]. Add 160 microliters of 17 beta Estradiol-HRP (*H-R-P*) Conjugate to the testing wells [3].
 - 3.4.1. Representative shot of talent pipetting a standard solution up and down.
 - 3.4.2. Talent adding 20 μL of sample into wells.
 - 3.4.3. Talent adding 160 μL of 17 β -Estradiol-HRP Conjugate to the testing wells.
- 3.5. Then, cover the plate with aluminum foil or use the provided cover foil from the kit [1] and incubate it at thirty-seven degrees Celsius for two hours [2].
 - 3.5.1. Talent covering plate with aluminum foil.
 - 3.5.2. Talent placing the covered plate in the incubator
- 3.6. In the meantime, prepare wash buffer from 10X (*ten-x*) washing solution [1].
 - 3.6.1. Talent adding deionized water to a container with 10x washing solution
- 3.7. After incubation, remove the aluminum foil from plate [1] and aspirate the liquid from the wells [2]. Wash each well three times with the wash buffer for at least 5 seconds each [3]. Add 100 microliters of TMB (*T-M-B*) substrate solution into all wells [4]. Cover the whole plate with aluminum foil and incubate at room temperature for thirty minutes [5].
 - 3.7.1. Talent removing the aluminum foil.
 - 3.7.2. Talent taking the liquid out from the wells.
 - 3.7.3. Talent adding wash buffer to wells
 - 3.7.4. Talent adding 100 μL of TMB Substrate Solution into wells.
 - 3.7.5. Talent covering wells with aluminum foil and keeping aside on the workbench.
- 3.8. Post incubation, add one hundred microliters of Stop Solution into the wells [1] and observe the reagent color change from blue to yellow [2]. Then, shake the microplate gently [3]. **NOTE: The VO is edited for the rearranged shots**
 - 3.8.1. Talent adding 100 μL of Stop Solution into all wells. **NOTE: 3.8.1. and 3.8.3 are filmed in a single shot**
 - 3.8.3. Shot of color changing from blue to yellow in plate **NOTE: This shot is moved here as per the author's request**
 - 3.8.2. Talent shaking the plate and placing back on workbench.

3.9. Measure the absorbance at 450 nanometers within 30 minutes of adding the Stop Solution [1]. Then, calculate the concentration of 17 beta Estradiol using appropriate tools [2].

3.9.1. Talent placing the plate in the spectrophotometer and closing the lid.

3.9.2. Representative shot of talent at the computer doing statistical analysis or generating a graph. NOTE: able to show a quick demo when getting results/measurement from spectrophotometer rather than showing the graph

Results

4. Results

4.1. In the present study, twenty women aged between forty-three and sixty-six years with and without dry eye disease or DED (*D-E-D*) were included [1]. The average tear film 17 beta Estradiol concentrations in older women without DED were approximately 599.3 (*five hundred-ninety-nine-point-three*) picograms per milliliter [2] and 423.6 (*four hundred-twenty-three-point-six*) picograms per milliliter in older women with DED [3].

4.1.1. LAB MEDIA: Table 2 *Video Editor: Please do not show the column called "Testosterone Concentrations (ng/mL)" as it is not included in the protocol.*

4.1.2. LAB MEDIA: Table 2 *Video editor: Please emphasize the row named "non-DED" and then the value 599.30 in it.*

4.1.3. LAB MEDIA: Table 2 *Video editor: Please emphasize the row named "DED" and then the value 423.60 in it.*

4.2. The percent coefficient of variation for estradiol measurements was 1.0% for non-DED samples [1] and 6.65% for DED samples, confirming assay reproducibility [2].

4.2.1. LAB MEDIA: Table 2 *Video Editor: Please emphasize the row named "non-DED" and then the value 1.00 in it.*

4.2.2. LAB MEDIA: Table 2 *Video Editor: Please emphasize the row named "DED" and then the value 6.65 in it.*

1. Microcapillary

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

IPA: /ˌmaɪ-kroʊ-ˈkæp-ə-lər-i/

Phonetic Spelling: my·kroh·kap·uh·lair·ee

2. Microcentrifuge

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

IPA: /ˌmaɪ-kroʊ-ˈsen-trə-ˌfyütʃ/

Phonetic Spelling: my·kroh·sen·truh·fyooj

3. Meniscus
Pronunciation link: <https://www.merriam-webster.com/dictionary/meniscus>
IPA: /məˈnɪskəs/
Phonetic Spelling: muh·nis·kuhs
4. Lateral
Pronunciation link: <https://www.merriam-webster.com/dictionary/lateral>
IPA: /ˈlæt-rəl/, /ˈla-tə-rəl/
Phonetic Spelling: lat·ruhl; la·tuh·ruhl
5. Canthus
Pronunciation link: <https://www.merriam-webster.com/dictionary/canthus>
IPA: /ˈkan-thəs/
Phonetic Spelling: kan·thuhs
6. Conjunctival
Pronunciation link: <https://www.merriam-webster.com/dictionary/conjunctiva>
IPA: /kənˌdʒʌŋ(k)ˈtaɪ-vəl/
Phonetic Spelling: kuhn·jungk·tye·vuhl
7. Occlude
Pronunciation link: <https://www.merriam-webster.com/dictionary/occlude>
IPA: /əˈkluːd/
Phonetic Spelling: uh·klood
8. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /ˈsen-trə-ˌfyūj/
Phonetic Spelling: sen·truh·fyooj
9. Analyte
Pronunciation link: <https://www.merriam-webster.com/dictionary/analyte>
IPA: /ˈænəˌlaɪt/
Phonetic Spelling: an·uh·lyte
10. Estradiol
Pronunciation link: <https://www.merriam-webster.com/dictionary/estradiol>
IPA: /ˌes-trə-ˈdī-ˌoʊl/
Phonetic Spelling: es·truh·dye·awl
11. Aliquot
Pronunciation link: <https://www.merriam-webster.com/dictionary/aliquot>
IPA: /ˈa-lə-ˌkwät/
Phonetic Spelling: al·uh·kwaht
12. Reagent
Pronunciation link: <https://www.merriam-webster.com/dictionary/reagent>
IPA: /rē-ˈā-jənt/
Phonetic Spelling: ree·ay·juhnt
13. Pipetting
Pronunciation link: <https://www.merriam-webster.com/dictionary/pipette>
IPA: /pī-ˈpet/
Phonetic Spelling: pie·pet·ing

14. Conjugate
 Pronunciation link: <https://www.merriam-webster.com/dictionary/conjugate>
 IPA: /'kän-jə-gət/, /'kän-jə-ˌgāt/
 Phonetic Spelling: kon·juh·guht; kon·juh·gate
15. Incubate
 Pronunciation link: <https://www.merriam-webster.com/dictionary/incubate>
 IPA: /'ɪŋ-kyə-ˌbāt/
 Phonetic Spelling: ing·kyuh·bate
16. Deionized
 Pronunciation link: <https://www.merriam-webster.com/dictionary/deionize>
 IPA: /dē-ˈī-ə-ˌnīz/
 Phonetic Spelling: dee·eye·uh·nyzd
17. Aspirate
 Pronunciation link: <https://www.merriam-webster.com/dictionary/aspirate>
 IPA: /'as-pə-ˌrāt/
 Phonetic Spelling: as·puh·rate
18. Substrate
 Pronunciation link: <https://www.merriam-webster.com/dictionary/substrate>
 IPA: /'səb-ˌstrāt/
 Phonetic Spelling: sub·strate
19. Microplate
 Pronunciation link: <https://www.merriam-webster.com/dictionary/microplate>
 IPA: /'maɪ-kroʊ-ˌplāt/
 Phonetic Spelling: my·kroh·plate
20. Absorbance
 Pronunciation link: <https://www.merriam-webster.com/dictionary/absorbance>
 IPA: /əb'zɔːr.bəns/
 Phonetic Spelling: ub·zor·buhns
21. Nanometer
 Pronunciation link: <https://www.merriam-webster.com/dictionary/nanometer>
 IPA: /'na-nō-ˌmē-tər/
 Phonetic Spelling: nan·oh·mee·tur
22. Spectrophotometer
 Pronunciation link: <https://www.merriam-webster.com/dictionary/spectrophotometer>
 IPA: /ˌspek-trō-fə-'tä-mə-tər/
 Phonetic Spelling: spek·troh·fuh·tom·uh·tur
23. Coefficient
 Pronunciation link: <https://www.merriam-webster.com/dictionary/coefficient>
 IPA: /ˌkō-ə-'fɪ-shənt/
 Phonetic Spelling: koh·uh·fish·uhnt
24. Reproducibility
 Pronunciation link: <https://www.merriam-webster.com/dictionary/reproducibility>
 IPA: /ˌrē-prə-ˌdū-sə-'bi-lə-tē/
 Phonetic Spelling: ree·pruh·doo·suh·bil·uh·tee

25. Picograms
Pronunciation link: <https://www.merriam-webster.com/dictionary/picogram>
IPA: /ˈpɪ-kə-gram/
Phonetic Spelling: pie·kuh·gram
26. Testosterone
Pronunciation link: <https://www.merriam-webster.com/dictionary/testosterone>
IPA: /tes-ˈtäs-tə-rŏn/
Phonetic Spelling: tes·tahs·tuh·rohn
27. Evaporation
Pronunciation link: <https://www.merriam-webster.com/dictionary/evaporation>
IPA: /i-,va-pə-ˈrā-shən/
Phonetic Spelling: ee·vap·uh·ray·shuhn
28. Stability
Pronunciation link: <https://www.merriam-webster.com/dictionary/stability>
IPA: /stə-ˈbi-lə-tē/
Phonetic Spelling: stuh·bil·uh·tee
29. Concentration
Pronunciation link: <https://www.merriam-webster.com/dictionary/concentration>
IPA: /,kän-sən-ˈtrā-shən/
Phonetic Spelling: kon·suhn·tray·shuhn
30. Thawing
Pronunciation link: <https://www.merriam-webster.com/dictionary/thaw>
IPA: /ˈθɔ:ɪŋ/
Phonetic Spelling: thaw·ing